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Correlated Response to Selection and Effects of Pre-Slaughter Environment on Meat Quality in Broilers Divergently Selected for Muscle Color

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by

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ABSTRACT

Two broiler lines divergently selected for high (HMC) and low (LMC) muscle lightness (L*) were formed from a random bred control line (RBC). After eight generations of selection, three experimental trials were conducted to investigate the correlated response to selection and effects of pre-slaughter environment on meat quality in all three lines.

In the first experiment, a total of 100 birds from each line were bled for blood chemistry analysis and were evaluated for meat quality the following day. The HMC line had higher fillet drip loss (DL), L*, heterophil to lymphocyte ratio (H:L), and platelet count (Plt), and lower postmortem muscle pH, lower red blood cell count (RBCC) and hematocrit (HCT) than the LMC line.

In the second experiment, the effects of pre-slaughter transportation on meat quality were investigated. Sixty birds from each line were selected and placed into transport (T) and non-transport (NT) groups. The T group was transported for approximately 3 h prior to processing. In both treatments, the HMC line had higher DL, L*, and b*, and lower postmortem muscle pH and a* than the LMC line. Within the HMC line, the T group had higher DL, and L*, and lower postmortem pH. Within the LMC line, the T group had higher a*, and lower L* and b*.

In the third experiment, the effects of pre-slaughter temperature (40^{o}C, 22^{o}C, 1^{o}C) on meat quality were investigated. Twenty birds from each line were selected and placed into heat stress (HS), cold stress (CS), and control groups for 2 h prior to processing. In all treatments, the HMC line had higher DL and L*, and lower muscle pH than the LMC line. In all lines, the HS group had lower DL and muscle pH than the control, whereas the CS group just had lower DL than the control, however within the LMC line the CS group had higher a*, and lower L* and b* than the control group.

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TABLE OF CONTENTS

INTRODUCTION

It is important to note that the introduction to research questions discussed in this dissertation is designed to give the reader a very broad and up to date understanding of meat quality and its effect on meat industries. The information presented has not greatly changed in the last five years, and as a result the author of this manuscript relied heavily on his master's thesis in the writing of the introduction.

Modern Poultry Selection

In 1946, the first national "Chicken of Tomorrow" contest was held to encourage meat-type broiler breeders to develop better, more efficient poultry using selective breeding techniques (Shrader, 1952). Since then, the time required to achieve market weight, and feed required to achieve a pound of meat has been cut in half through selection for increased feed conversion and yield (Havenstein et al., 1994 a,b; Anthony, 1998; Havenstein et al., 2003 a,b). Selective breeding has been reported to account for 85-90% of the differences between today's bird and the average bird 70 years ago. (Havenstein et al., 1994 a,b; Havenstein et al., 2003 a,b)

Unfortunately, these advancements resulted in unintended physiological consequences related to growth (Siegel and Dunnington, 1987). Selecting for profitable characteristics such as six week body weight and breast yield distorts a bird's ability to grow in a physiologically ideal manner by changing its resource allocation for each developmental stage. To develop properly, a bird must allocate nutritional resources for growth and development in a prioritized manner (Dunnington and Siegel, 1998; Siegel, 1999). Resources are first allocated to the high priority systems first such as neural, circulatory, and immune systems early in the growth phase, which are then shifted to developing the bird's skeletal structure. This is followed by the intestinal system for better utilization of nutrients, then muscle tissue, and finally fat deposition prior to

reproduction. The order of the developmental stages is imperative since each stage supports the developmental stage that follows it. Improper resource allocation can cause complications such as ascites (Julian, 2000), leg problems (Nestor, 1984), decreased reproduction (Siegel and Dunnington, 1985; Qureshi and Havenstein, 1994; Emmerson, 1997; Anthony, 1998; Julian, 1998; Cheema et al., 2003), increased carcass fat (Soller and Eitan, 1984; Chambers, 1990), skeletal abnormalities (Julian, 1998; Cook, 2000), atypical poultry meat (Anthony, 1998; Barbut, 1997 a,b; Barbut, 1998), and reduced immune function (Qureshi and Havenstein, 1994).

Meat Quality

One consumer-related physiological abnormality that is a recent concern for the meat industry is atypical meat quality (Froning, 1995; Barbut, 1997 a,b; Anthony, 1998; Barbut, 1998; Sosnicki et al., 1998). The quality of meat is based on its physical properties such as palatability, texture, tenderness, taste, color, pH, and water-holding capactity (WHC). In a typical processing plant, meat is generally not characterized by the aforementioned qualities but graded on an aesthetic basis such as tears, bruises, discoloration, or missing parts (Barbut, 1996). However, a growing demand for a convenient, cheap, and palatable product has shifted the market towards value-added products. The effect of a meat's physical properties on its marketability and versatility has become apparent to processors trying to utilize poor quality meat.

Conditions that affect meat quality include pale, soft and exudative meat (PSE), and dark, firm, and dry meat (DFD). PSE meat was first identified in pork, but it is also found in poultry and beef (Ludvigsen, 1953; Solomon et al., 1998). It is often characterized as meat that has decreased palatability, pale appearance, decreased pH, soft texture, decreased water holding capacity and excess water loss subsequently resulting in decreased tenderness. Dark firm and dry (DFD) or dark cutting beef (DCB) in cattle is particularly a major problem in beef, but has

been described in poultry as well. The meat is characterized as red or dark in color, sticky texture, high enzymatic activity and relatively high pH (Wulf et al., 2002; Allen et al., 1997; Allen et al., 1998).

The physiological chemical reactions that occur before and after slaughter provide insight into why these abnormal conditions deviate from ideal meat quality conditions. Typically, an unstressed animal has appropriate levels of glucose which is anaerobically metabolized into lactic acid. In the presence of oxygen, the lactic acid by-product can be further metabolized aerobically into carbon dioxide and water. In the event of exsanguination, the animal's anaerobic metabolism of glucose continues even after death. The final by-product is lactic acid that accumulates in the absence of oxygen consequently lowering the muscle pH (Lawrie, 1998). Postmortem fiber contraction occurs due to the decline in muscle pH. This is known as rigor mortis which is normally followed by the minor relaxation of the now denatured contractile proteins allowing for water absorption and retention.

The characteristics of "PSE-like" and "DFD-like" meat stem from an abnormal rate of glycolysis (pH decline or lack of decline) that is associated with harvest stress and inherent differences in metabolic rates. Animals associated with PSE meat generally have been stressed for some period prior to slaughter. This causes them to rapidly metabolize glycogen into lactic acid which is prevented from being further metabolized due to the small time frame between the introduction of the stressor and exsanguination. The rapid glycolysis and subsequent decline in pH while at relatively high carcass temperatures permanently contracts and abnormally denatures the muscle's contractile proteins. The compromised integrity of the contractile proteins greatly inhibits the muscles ability to retain water due to the fact that the majority of water found in muscle tissue is between contractile proteins. Microstructure evaluations of pork and poultry

meat have revealed distinct morphological differences between meats exhibiting PSE, normal, and DFD muscle characteristics. At one end, muscle samples classified as PSE showed extreme contraction of myofibrillar proteins which was evident by the increased number and size of interstitial spaces between muscle fibers and bundles in the endomysium and perimysium networks. Meat classified as DFD exhibited significantly smaller interstitial spaces in size and number. Normal meat was an intermediate between the two (Sijacki et al., 1991; Offer and Cousins, 1992; Barbut et al., 2005). Offer (1984) speculated that the leaky or tough qualities of raw or cooked PSE-like meat may be due to loss of water that has been deposited into the interstitial spaces due to a reduction of intracellular space. DFD is essentially the opposite of PSE and occurs when an animal has been stressed much earlier prior to slaughter. Excessive stressing hours in advance not only depletes existing glycogen reserves thus preventing the production of lactic acid after death, but also allows existing ante-mortem lactic acid to be metabolized before slaughter (Hedrick et al., 1989; Gregory, 1994; Lawrie, 1998). After slaughter, the animal's lack of lactic acid prohibits the muscle pH to drop adequately resulting in dry and dark colored meat that is prone to early spoilage. It is interesting to note, that the relatively neutral pH can potentially increase some characteristics that may be favorable to processors; increased tenderness (Barbut et al., 2005), WHC (Barbut et al., 2005; Qiao et al., 2001), marinade absorption (Barbut et al., 2005), cooked meat yield (Qiao et al., 2002; Barbut et al., 2005), and emulsification capacity (Qiao et al., 2001) have been reported for DFD meat. Unfortunately, the additional characteristics of DFD meat, atypical flavor and color, and decreased shelf-life, offset its advantages (Newton and Gill, 1981; Fletcher, 1999; Allen et al., 1998).

The Economic significance of PSE and DFD-like Meat

Although the incidence and form of atypical meat in various production animals varies, meat quality is an especially important characteristic to all meat industries. In a typical processing plant, meat is generally graded on an aesthetic basis such as tears, bruises, extreme discoloration, or missing parts (Barbut, 1996). Although this method helps processors recover acceptable portions for specific uses, it does not take the muscle properties into account which are contributing to economic losses greater than what may be apparent.

Incidence. In the turkey industry, it was reported that some U.S. based processing plants had an incidence of PSE defects in mature turkey hens that ranged from 5 to 40% (Barbut, 1996), 5 to 30% in young turkey flocks (McCurdy et al., 1996), and approximately 40% of turkey breast fillets in another set of commercial processing plants (Owens et al., 2000a). In 2005, approximately 9% of turkey carcasses in Canada were condemned for cyanosis which is a bluish discoloration of the carcass, however it was later discovered that this was a misdiagnoses and the dark color was caused by the DFD condition (Mallia et al., 2000 a, b). In broilers, 47% of breast fillets from 3 different commercial processing plants exhibited high paleness that could potentially lead to low WHC (Woelfel et al., 2002), and 0 to 28% among 7 commercial broiler flocks (Barbut, 1996) in the U.S. In Europe the occurrence of PSE-like meat was estimated to reach up to 40% in flocks during hot climate (Petracci et al., 2009). In England, the incidence of PSE in broilers was calculated to be at 20% (Wilkens et al., 2000). In the pork industry, a survey showed an incidence of PSE pork to be 24% across five EU countries (Murray and Johnson, 1998) and 13% in Canada (Warriss et al., 1998). The 1995 National Beef Quality Audit (NBQA) reported that dark cutting beef carcasses (DFD-like) result in a loss of $6.08 per animal harvested in the United States (Smith et al., 1995).

Appearance. In a whole carcass or parts market, consumers rely heavily on visual appeal of the product to judge what they think is fresh, wholesome, and flavorful (Froning, 1995). Discoloration, non-uniform coloration between or within products, or even excess water in the package may be considered to most consumers as unacceptable. These inconsistencies could cost the producer/vendor by having to sell the product at a discount price, or discard the product after expiration, resulting in the loss of product and valuable shelf space for acceptable products thereby resulting in increased opportunity costs. In northern Georgia, 16 food retail stores were examined for variation of color between breast fillets within packages of 4 breast fillets. The value of the colors were not evaluated, only the color variation within a package. Approximately, 7.1% of the 997 examined packages exhibited non-uniformity in color which is often considered a negative quality even if the majority of fillets fell within acceptable color ranges. Interestingly, certain brands showed significantly less variation within packages then others. This suggests that brands have differences in color variation, or a sorting procedure is being used to match fillets with similar colors for packaging (Fletcher, 1999).

Shelf-Life. The shelf-life of meat products is a particular concern for retailers, consumers, and processors of fresh and value-added meat products. A 2005-2006 report for annual retail losses estimated that 4.5 % of losses were due to spoilage of fresh meat, poultry and seafood (Buzby et al., 2009). These losses translate into significant economic losses considering the estimated retail value for broilers and turkeys in 2010 was $54 billion (U.S. Dept. of Agriculture, 2012). Typically, the shelf-life of a product is dependent on the time of bacterial introduction, the initial total vaiable count of bacteria (TVC) present on the product, the temperature and method of storage, and any treatments used to increase the shelf-life. The observed events leading up to spoilage begin with the introduction of spoilage bacteria. The

most common organism responsible for spoilage in poultry is P. fluorescens which can grow in refrigeration temperatures (3C) and is found on live birds and surfaces of processing facilities which are likely agents of contamination (Russell et al., 1995; Allen et al., 1998). A "Lag" phase, occurs between bacterial introduction and growth. Initially, the energy supplied for growth is in the form of glucose and other carbohydrates found on the surface of the meat (Allen et al., 1997). After surface glucose is depleted, the amino acids are then metabolized. The resulting foul and sulfurous odor is the first sign of spoilage (Ayres et al., 1950; Pooni and Mead, 1984; Russell et al., 1995). The effectiveness of spoilage causing organisms is directly related to its growth and can be affected by chemical properties of meat. Rey et al. (1976) reported a correlation between pH and rate of microbial spoilage. A common problem for DFD meat is early spoilage for several reasons. The relatively high pH of DFD meat reduces the lag time before bacterial growth occurs (Newton and Gill, 1981; Allen et al., 1998). Additionally, the depleted glucose of DFD meat initiates pre-mature amino acid metabolism resulting in early odor production. Newton and Gill (1981) reported increased odor production of dark meat at bacterial levels that were lower than normal odor producing levels. Lastly, some spoilage organisms like S. putrefaciens that cannot grow at normal meat pH levels thrive at higher DFD pH levels (Newton and Gill, 1981; Allen et al., 1998).

Value-Added Products. A growing demand by consumers for a convenient, affordable, and palatable product has greatly affected the way the poultry industry produces products and has shifted the market from whole bird to parts and value added products (Woelfel et al., 2002). In 1962, whole broiler products made up 83% of the market with cut-up and processed products making up only 17%. Today, whole birds only make up 11% of the market with cut-up and processed products making up 89%. An estimated 36.6 and 5.95 billion lbs. of ready to cook

chicken and turkey products were sold in 2008 and 2007 respectively. (National Chicken Council, 2013; National Turkey Federation, 2013). This shift created an increased incentive for producers to reduce the incidence of PSE meat in poultry. PSE meat can be difficult to utilize in value added products due to problems associated with emulsification, binding, and water retention properties (Barbut, 1996). Typically, formed products, utilizing PSE meat, are dry and have difficulty forming gels due to water separating from the product (Kauffman et al., 1978)

Quantitative meat quality evaluation methods

The characterization of meat as PSE or DFD can be determined by the objective measurement of its properties. Several studies have established the causes and relationships of meat properties associated with atypical meat. The most common characteristics measured to classify defective meat include color, WHC, pH, and texture.

Muscle Color. An accepted method for quantitatively measuring color is the use of a colorimeter (Barbut, 1993, 1996; McCurdy et al., 1996). The color measurement is described in units of lightness (L* value) and can be further broken down in units of redness (a*) and yellowness (b*). Although the L* value measurement is objective, the ranges used to classify meat as PSE or DFD are subjective and must be determined by the investigator. In addition, the method is non-destructive and therefore allows for repeated measures of the same fillet. The drawback of using this method is that it can produce false positives for PSE detection. Fillets considered light or dark have the potentiality of being PSE or DFD. There may be other factors influencing the color of tissue. In other words, PSE meat may be light, but not all light meat may be PSE (Northcutt et al., 1998).

Muscle pH. Several methods are used for measuring muscle pH. The two most prominent methods include the iodoacetate method and the use of pH spear probe. The

iodoacetate method which was first described by Jeacocke (1977) involves the flash freezing of the muscle samples which stops glycolysis. The samples are then ground and homogenized in an iodoacetate solution. The pH of the solution is then measured by a pH meter. This method's drawback is that it is time consuming and expensive and is generally not practical for use in a commercial breeding program. The spear probe (Dosler et al., 2007) method involves the direct measurement of pH by the insertion of the pH probe into tissue. This method is preferred in most cases due to its convenient and instant measurements. However, it may have repeatability issues. After a long period of use, it tends to have difficulty settling on a pH measurement.

Water-Holding Capacity. WHC can be determined by the drip loss, cook loss, and/or expressible moisture of a product. Drip loss is essentially the moisture content that is lost during storage. It can be measured by the weight of product before and after a certain amount of time. Cook loss is the weight loss of a product after cooking. While the majority of weight loss may be to moisture, increased cook loss can be due to high fat content of the product. Expressible moisture is simply the moisture that is extruded from a product after a certain amount of pressure is applied over time. A common protocol of expressible moisture measurement is described by Wierbicki and Deatherage (1958). Expressible moisture (EM) is negatively correlated to WHC thus when WHC is high, EM is low.

Tenderness. Measuring tenderness of a product using a sensory panel has been a widely used method for decades that provided valuable data, however it is subjective and is costly in time and resources. Three common objective methods that agree with sensory panel evaluations involve the shearing of products for measuring tenderness (Lyon and Lyon, 1990). The Allo-Kramer (AK), Warner-Bratzler (WB), and Razor Blade (RB) methods essentially involve the cooking then shearing of product (Bratzler, 1932; Palmer et al., 1965; Smith and Fletcher, 1988,

Lyon and Lyon, 1990, 1998; Cavitt et al., 2004). The total force and force/mm required to shear the product estimate tenderness. Since the AK and WB methods require further preparations before shearing, the RB method is preferred due to its ease of use (Cavitt et al., 2004).

Causes of PSE and DFD-like Meat

Causes for atypical meat can be anything that ultimately disrupts the eating quality, stability, or wholesomeness of a product. Meat quality is a trait that is subject to many factors. Currently, the contribution of each factor has not been quantified, nor is there evidence to suggest that these factors contribute equally across species exhibiting PSE and DFD-like characteristics. Factors associated with PSE and DFD-like conditions include nutrition and rearing conditions, pre-slaughter stress (Sayre et al., 1963; Owens and Sams, 2000), postmortem handling (Owens and Sams, 2000), and genetic components (Anthony, 1998).

Nutrition. Vitamin E is a common feed additive that retards lipid oxidation in feed and animals which has been indicated to reduce the incidence and defects of PSE meat by stabilizing membrane fluidity and integrity (Monahan et al., 1994). Vitamin E has been shown to prevent the occurrence of PSE meat in pork (Cheah et al., 1995), turkey (Ferket et al., 1995), and chicken (Olivo et al., 2001) and has been shown to stabilize meat color (Buckley et al., 1995), and to decrease drip loss (Jensen et al., 1997). Interestingly, vitamin E is used to prevent lipids from souring in feed and is depleted at higher rates while at high temperatures. This may be another factor contributing to the higher incidence of atypical meat quality in the summer (McCurdy et al., 1996).

Antemortem Stress. Generally, factors affecting meat quality are stress related. The type, duration, and occurrence of stressors greatly determine whether PSE or DFD-like characteristics are present. PSE meat is typically caused by an acute pre-slaughter stress, whereas

DFD is caused by chronic pre-slaughter stress (Hedrick et al., 1989; Lawrie, 1998; Owens and Sams, 2000). Due to their size, the time and effort required to process beef and pork are much larger than for poultry. The increased time requirement for mobilization and preparation of beef and pork for slaughter results in longer periods of pre-slaughter stress and may be the reason for higher reported incidences of DFD-like characteristics in beef and pork compared to poultry (Sams, 1999).

Pre-slaughter temperature has been shown to be highly correlated to the incidence of PSE and DFD meat conditions in porcine, beef, turkey, and broiler meat. Sayre et al. (1963) reported that pigs subjected to elevated temperatures shortly before slaughter had exhibited increased rates of pH decline and decreased color indicating an increased rate of glycolysis. Wismer-Pedersen (1959) and Forrest et al. (1963) reported that elevated temperatures or wide temperature fluctuations prior to slaughter increased the incidence of PSE conditions in pork. Sayre et al. (1963) reported DFD-like characteristics in pork when subjected to sudden change in temperature from warm to cold. Briskey (1963) found pigs that were cold stressed prior to slaughter exhibited increased tenderness. Lawrie et al. (1966) reported cattle cold-stressed prior to slaughter exhibited DFD-like characteristics. Froning et al. (1977) reported that turkeys which were heat stressed prior to slaughter exhibited decreased tenderness, whereas turkeys subjected to cold stress exhibited increased tenderness and DFD-like characteristics. Wood and Richards (1975) reported increased rate of postmortem glycolysis and a lengthening of the postmortem glycolysis period in heat and cold stressed broilers.

Typically, production animals are not grown and processed at the same location. Generally, these animals must be transported to processing plants which the transportation time will vary from 0.5 to 24 h depending on the species. Consequently, this can stress an animal due

to requiring the animal to stabilize itself during transportation, overcrowding or prolonging other present stressors such as heat stress (Warriss et al., 2005; Nijdam et al., 2005). Approximately 0.05 to 0.6% broilers transported for processing die, however a portion of those deaths can be attributed to ascites (Gregory, 2007).

Owens and Sams (2000) studied the effects of transportation on meat quality in a commercial line of turkeys. Eighty 21 week old male turkeys were divided into two groups. One group served as the control line with no transportation and the other was transported for approximately 3 hours and processed immediately with no rest. Significant differences were found between the groups in color and pH at 2 and 24 hours postmortem as well as differences in marination retention. The transported group exhibited lower L* values, higher pH and marination retention compared to the non-transported group. This is consistent with other studies in that longer periods of stress deplete glycogen reserves while also allowing the metabolization of the accumulated lactic acid resulting in increased DFD characteristics such as increased color, pH, and WHC (Owens and Sams, 2000).

A typical concern for animal welfare activists is the treatment and handling of livestock and poultry before slaughter in the hopes of reducing animal suffering as much as possible. Fortunately, this is a top priority for processors as well; not only to improve the public opinion of the meat industry, but also because it can significantly impact the meat quality of the animal. When animals arrive at the abattoir, it is common to permit a resting period so that excited animals have a chance to calm down. This allows for easier handling of the animal as it is about to be slaughtered, it also decreases the chances of PSE meat by extending the stress period (Gregory, 2007). However, if animals are left in confinement areas too long, processors increase the risk of DFD meat, fecal contamination of product, animal injury/muscle damage incidence.

This can be avoided by consistently confining animals at appropriate lengths of time for rest, withholding feed hours before slaughter depending on the species, and maintaining proper stocking densities to avoid fighting, crowding, or falling of animals. Unfortunately, avoiding the presence of feces on animals coming from the farm is impossible. The majority of sources of product contamination are dirty carcasses. Although its effectiveness of reducing contamination is disputed, some processors wash animals before slaughter (Swanenburg et al., 2001; Gregory, 2007), fortunately it may be an even more effective way to reduce PSE meat in summer months by cooling down the animal. Turkeys that were washed before being processed in summer heat levels showed lower incidences of PSE meat as compared to non-washed birds (Guarnieri et al., 2004).

The advancements in understanding animal behavior has enhanced the way processing facilities are designed for reducing the introduction of stressors as an animal is handled and moved into the processing area. Chutes and confinement areas used for directing and moving livestock are designed to reduce falling, slipping, trampling, reverse movement, injury due to sharp edges, excessive noise as well avoiding spatial situations that livestock find particularly stressful. In poultry, birds are taken either from transportation or holding crates and hung on shackles. A common problem for handlers is wing flapping and can be especially difficult when processing large turkeys. The duration of wing flapping is largely determined by the flightiness of the birds, lighting conditions, and method of shackling. Wing flapping can alter $pH^{15\ min}$ and increase paleness or redness of breast tissue (Berri et al., 2005; Gregory, 2007). Limb dislocation, leg fractures, and wing injuries are common if birds are not hung carefully.

In order to achieve proper exsanguination, an animal must be alive so that blood evacuation is maximized. The purpose of stunning animals before exsanguination is to deprive

them of consciousness as to avoid pain and additional stress, and the ability of voluntary movement during exsanguination. There are various methods of stunning across species. The three most common types are concussion, gas, and electrical stunning (Gregory, 2007).

Concussion stunning is used primarily in cattle and horses and consists of a forceful blow to the animals head to achieve unconsciousness. It can be carried out using a captive bolt gun, or percussion bolt gun; they differ in that the former penetrates the cranial cavity resulting in higher incidences of irreversible stunning and even death in some cases. A disadvantage of this stun method is that it is mostly limited to cattle since pigs and poultry react to it violently increasing the rate of postmortem glycolysis as well as difficulty of bolt sticking in hogs makes it impractical. Additionally, appropriate maintenance of the gun is required to accomplish correct placement and velocity (up to 47 m/s) of the blow as to achieve a proper stun (Gregory, 2007).

Electrical stunning can be used for most animals, but is especially common in pigs and poultry. The electrical charge can be delivered to either the animal's body so that cardiac arrest follows or only to its head which is reversible and requires the animal to be immediately killed to avoid regain of consciousness. Unfortunately, this method is known for producing meat quality issues (Skarovsky and Sams, 1999). Application of electrodes anywhere else besides the head can result in lower ultimate pH for the respective muscle. Additionally, it may produce blood splash or blood speckle in muscle tissue (Gregory, 2007)

Gas stunning is also used primarily in pigs and poultry. Carbon dioxide is the most widely used gas for stunning. If used at high enough concentrations, animals can be incapacitated before excessive struggle can take place. This has its advantages over electrical stunning for several reasons. Animals do not need to be restrained and can be stunned as a group consequently reducing stress levels (Gregory, 2007). Meat plants using electrical stunning

methods have shown to have a higher incidence of PSE meat (von Zwiegbergk et al., 1989; Gregory, 2007). Slower rates of muscle glycolysis and an incidence decrease of blood splash has been reported with gas stunning compared to electrical stunning (Channon et al., 2002; Gregory, 2007). Unfortunately, in swine production gas stunning is only effective in controlling PSE meat in individuals that are *NN* or *Nn* for the halothane gene (Channon et al. 2000; Gregory, 2007). O_2 has been shown to increase the severity of PSE meat in *nn* individuals by inducing more physical activity (Troeger, 1990; Troeger and Woltersdorf, 1991; Gregory, 2007).

Postmortem handling. Harvesting an animal is just the first step in the complex process of converting muscle tissue into meat. The postmortem handling of carcasses is one of the most critical factors affecting the potential quality of meat. The objective for processors is to produce meat at a profitable rate while satisfactorily sustaining desirable textural and chemical properties of the eventual product.

In poultry, scalding is performed simply for cleaning and feather removal through carcass submersion in 55 °C turbulated water, however if done improperly quality issues may arise. If the scalder is not heated up enough or is allowed to cool, carcasses may be left with excess feathers and possibly tears, blemishes, or broken bones after entering the feather picker. If, the scalding temperature is too high, the incidence of skin blemishes and cooked appearance increase (Dickens et al., 1999). Scalding can also be a source of increased carcass temperatures that may increase the incidence of PSE (deFemery and Pool, 1960; Anadon, 2002). Additionally, fecal matter that remains in the scalder can be a source of contamination to other carcasses. Mulder et al. (1978) reported that carcasses entering the scalder after carcasses contaminated with marker organism exhibited marker organism cross-contamination (Mulder et al., 1978; Dickens et al., 1999). Lillard et al. (1973) reported that scalder contaminants that entered the circulatory system

could spread to the circulatory system, internal organs and perhaps the whole carcass (Lillard et al., 1973; Dickens et al., 1999).

PSE type meat can be caused by a rapid decline in pH while carcass temperatures are still high, but can be minimized if carcasses are properly chilled (Offer, 1991). McKee and Sams (1998) found that turkey carcasses chilled at 40ºC exhibited increased ATP and glycogen depletion, decreased pH, increased L* values at .25, .5, 1, 2 and 4 hours postmortem, increased drip loss, cook loss, shear force, and decreased sarcomere length when compared to turkeys chilled at 20ºC and 0ºC (McKee and Sams, 1998). In two different studies, Alvarado and Sams (2002, 2004) found turkey carcasses chilled at 30ºC and deboned at 60 min postmortem exhibited increased L* values (60 min), increased drip loss (24 hr.), 60 min cook loss, 24 hr. cook loss, decreased gel strength (60 min), and decreased ultimate pH when compared to 60 min deboned turkey carcasses chilled at 20ºC, 10ºC, 0ºC. They also found that 105 min deboned turkey carcasses chilled at 30ºC exhibited increased drip loss (24 hr.), cook loss (24 hr.), expressible moisture (24 hr.), decreased gel strength (24 hr.), and ultimate pH when compared to 105 min deboned turkey carcasses chilled at 20ºC, 10ºC, 0ºC (Alvarado and Sams, 2002: Alvarado and Sams, 2004). Rathgeber et al. (1999) found turkey carcasses that were delayed before chilling exhibited higher L*, a*, and b* values, and decreased protein extractability and cook yield versus turkey carcasses that were chilled immediately (Rathgeber, et al., 1999).

The time at which a carcass is deboned relative to slaughter can also affect the quality of meat. A 1992 study investigated the effects of 0, 1 and 24 h post-chill deboning times on breast tenderness. Deboning breast meat immediately after slaughter resulted in the toughest breast meat, followed by 1 hour deboning time. Deboning breast meat at 24 hours post-chill resulted in the tenderest meat (Lyon and Lyon, 1992). A similar 2005 study found comparable results.

Among other quality traits, tenderness was measured on breast tissue that had been deboned at 0.25, 1.5, 3.0, 6, and 24 h postmortem. The shear values for each deboning time were consistent with the previous study. Breasts deboned at 0.25 h postmortem were the least tender and breasts deboned at 24 h postmortem were the tenderest (Cavitt et al., 2005).

Genetic component. The genetic component associated with atypical meat is one of the most difficult contributing factors to quantify especially across species since there are many environmental conditions that can affect muscle properties. There seems to be an underlining genetic component interacting with environmental factors resulting in susceptibility or resistance to atypical meat across species. Unfortunately, in many cases, a large proportion of the genetic risk factors associated with modern day broiler diseases are critical to economically important traits.

In the 1960's, physicians in Australia began noticing the effects of anesthesia on certain patients which consisted of increased muscle rigidity, heart rate, oxygen consumption, carbon dioxide production, and body temperature. If not treated quickly, tissue and brain damage or even death may have occurred (Denborough et al., 1962). This adverse reaction to certain drugs was named Malignant Hyperthermia (MH). A short time after the discovery of MH in humans, a similar condition was found to be present in boars injected with 100 mg of suxamethonium chloride (Hall et al., 1966). Meanwhile, researchers noticed that pigs exposed to physical stress exhibited extreme muscle rigidity and would collapse and die (Topel et al., 1968). This condition was named Porcine Stress Syndrome (PSS) and was a result of improper intense selection of carcass leanness, and growth (Gregory, 2007). The connection between MH and PSS wasn't made until the use of halothane gas as a screening method for PSS susceptible pigs was discovered. The underlining factor in PSS and MH in humans is the result of abnormal

calcium homeostasis (Nelson, 1983; Mickelson et al., 1986; Strasburg and Chiang, 2009) and is caused in pigs by a single point mutation of the porcine ryanodine receptor (RYR) resulting in a substitution of cysteine for arginine at amino acid residue 615 (Fujii et al., 1991; Strasburg and Chiang, 2009). This mutation produces a faulty RYR channel which is responsible for the storage and release of calcium in storage compartments to sarcoplasmic fluid (Fujii et al., 1991; Owens et al., 2009). The mutant RYR tends to leak or fails to store calcium completely (MacLennan, 2000). Although, no RYR mutation has been identified in poultry, the use of halothane gas on broilers and turkeys has revealed anesthetic sensitive individuals. Unfortunately, it fails to identify individuals susceptible to the PSE condition since sensitive birds did not exhibit greater tendency to form PSE meat (Wheeler et al., 1999; Owens et al., 2000b,c; Cavitt et al., 2002).

In pigs, the Porcine Stress Syndrome (PSS) syndrome has served as the primary genetic model used for detection of PSE susceptible pigs (Houde et al., 1993). In response to stress, pigs affected by PSS exhibit hyper-metabolism resulting in rapid production of lactic acid and the formation of MH. The combination of acidic meat and elevated temperatures at slaughter increases the incidence of PSE meat. There have been several studies that have suggested the presence of a genetic predisposition to atypical meat in poultry by showing variation in traits associated with PSE among different breeds, flocks, and sexes. McCurdy et al. (1996) used L* value measurements of turkey breasts from several flocks to predict the incidence of PSE meat and found as low as 1% incidence of PSE in one flock and 29% in another (McCurdy et al., 1996). Gardzielewska et al. (1995) reported differences in pH decline in 5 commercial chicken lines (Gardzielewska et al., 1995). Musa et al. (2006) measured muscle quality characteristics in both male and females in two breeds of chickens Anka and Rugao and found significant

differences in color, pH, and tenderness between the two breeds and significant differences in tenderness between males and females among the two breeds (Musa et al., 2006). As of now though, no one has been able to characterize specific genetic causes for PSE in poultry.

Although there has not been a specific "PSE" gene identified in poultry like there has been in swine, there are still plenty of genetic factors that can contribute to PSE-like meat. These genetic factors, however, may have been accumulated in poultry through intense selection for body weight and growth as the halothane gene was for the swine industry. The size of a muscle is determined by its fiber number and fiber size. The eventual size of poultry muscle is determined by two developmental stages; one stage occurring in the embryo, and the other post-hatch. The first stage primarily consists of proliferation, differentiation, and maturation of muscle fibers (hyperplasia). The number of fibers present is determined by this stage and no increase in fiber number occurs after this stage is complete. Post-hatch, muscle size is determined by the growth of the fibers that are present (muscle hypertrophy). As of now, selection for growth is largely phenotypic and unfortunately fails to utilize specific methods (hypertrophy or hyperplasia) for optimal growth selection. Sire selection for yield typically occurs 6-8 weeks post-hatch. Most of the selection emphasis is focused on hypertrophic growth.

A British United Turkeys study found that most of the advancements in selection for growth were due to post-hatch muscle fiber growth when comparing unimproved and improved lines of turkeys. The number and size of muscle fibers can greatly affect the eventual quality of the product. On one end of the spectrum tissue that compromises of many muscle fibers risks decreased tenderness due to increased connective tissue between muscle fibers. Muscle tissue comprised of large white fibers risk several PSE characteristics. Large white fibers contain higher amounts of water that can potentially be lost as drip loss, higher amounts of stored

glycogen potentially resulting in decreased ultimate pH. Additionally, large white fiber sizes can lead to muscle damage during harvesting resulting in increased protein denaturation. The same study conducted histological examinations of muscles in the unimproved and improved lines. The improved lines showed increased fiber size as well as an increase in 4 atypical fibers: 1) Large round fibers that are associated with a loss of calcium homeostasis and if permanently damaged can result in decreased tenderness. 2) Necrotic fibers that, if not repaired, will be replaced by fatty tissue resulting in higher cook loss. 3) Small angular fibers associated with muscle degeneration. 4) Lastly, central core fibers that were lacking mitochondrial activity which indicated those fibers were becoming more glycolytic which can result through inadequate supply of oxygen to those fibers. Similar studies showed similar results (Smith, 1963; Dransfield and Sosnicki, 1999; Velleman et al., 2003). A selection program would benefit by finding a good balance between muscle fiber size and number and implementing it in its growth selection.

Besides contributing to the physiological defects responsible for producing PSE meat, intense selection for breast yield and growth may also be contributing to PSE meat by mechanically complicating the chilling and rigor mortis process. A common problem for turkey producers is the inability to drop the core carcass temperature quickly during chilling. This results in the formation of rigor mortis while the carcass is at relatively high temperatures increasing the rate of pH decline and perhaps the incidence of PSE (Khan, 1971).

Synthesis

Poor muscle quality is a problem that is affecting the poultry industry and is a result of a combination of decades of intense selection for economically important traits and environmental factors. While integrators are beginning to see the importance of meat quality, selection practices remain the same. New meat quality issues are slowly forming and will not disappear as

selection for growth rate, breast yield, and feed conversion continue. Effective methods have been identified for the detection and quantification of poor meat quality traits, as well as what environmental conditions are more likely to affect meat quality; however little is known about the genetic component of meat quality. Selection for muscle color over eight generations has resulted in muscle quality lines that have diverged for PSE and DFD-like characteristics. The purpose of this thesis was to characterize the concomitant physiological changes that have occurred with divergence. A better understanding of the physiological mechanisms resulting in PSE and DFD-like meat will better identify ways to improve meat quality on the primary breeder level.

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Effects of selection on blood chemistry in broilers divergently selected for muscle color

ABSTRACT

Two conditions known to affect meat quality are pale, soft, and exudative meat (PSE) and dark, firm and dry meat (DFD). The purpose of this study was to elucidate potential factors contributing to muscle color and their association with the PSE or DFD condition through blood chemistry analysis of lines selected for high (HMC) and low (LCM) breast muscle lightness (L*) and compared with a random-bred control line (RBC). At six weeks of age, two replications of approximately fifty birds from each line were bled for blood chemistry analysis and were processed the following day. At process, measurements were collected for muscle pH at 15 min, 4 h, and 24 h postmortem, and muscle color at 4 and 24 h postmortem, and percent fillet drip loss (DL) at 24 h. The HMC line was highest for 4 and 24 h L*, and the random-bred control line (RBC) was higher than the LMC line. Similar results were observed for DL. The HMC line had the highest DL, and the RBC line was higher than the LMC line. The LMC and RBC lines were higher than the HMC line for initial pH (15 min). For 4 and 24 h pH, the LMC line was highest, and the RBC line was higher than the HMC line. The HMC line had the highest heterophil to lymphocyte ratio (H:L), and the RBC line was higher than the LMC line. The LMC line had the highest red blood cell count (RBCC), and the RBC line was higher than the HMC line. The RBC line had the highest blood hemoglobin levels (Hgb). The LMC line had the highest hematocrit levels (HCT). These results demonstrate that selection for muscle color has resulted in changes in blood chemistry that may be correlated with PSE and DFD syndromes.

INTRODUCTION

PSE meat results from a high rate of decline in postmortem pH while carcass temperatures are still relatively high resulting in protein denaturation and subsequent loss of water-holding capacity (WHC) (Owens and Sams, 2000; Qiao et al., 2001). Factors associated with PSE and DFD-like conditions include nutrition (Buckley et al., 1995; Jensen et al., 1997; Olivio et al., 2001) rearing conditions (Sayre et al., 1963), pre-slaughter stress and behavior (Owens and Sams, 2000; Petracci et al, 2001; Debut et al., 2003; Warriss et al., 2005), stunning method (Skarovsky and Sams, 1999; Gregory, 2007) postmortem handling (McKee and Sams, 1997, 1998; Owens and Sams, 2000; Alvarado and Sams, 2002:2004), and genetic components (Le Bihan-Duval et al., 2001; Debut et al., 2003; Harford et al., 2014).

The muscle color lines used in this study have been selected and diverged for high and low breast fillet 24 h L*; as a result, divergence has been achieved in PSE and DFD-like characteristics such as muscle pH and drip loss (Harford et al., 2014). However the mechanisms that contribute to muscle color that are both associated and not associated with PSE and DFD-like meat are not fully understood. The link between pre-slaughter stress or stress susceptibility and poor meat quality such as PSE and DFD-like meat has been extensively documented (Hedrick et al., 1989; McPhee and Trout, 1995; Lawrie, 1998; Owens and Sams, 2000; Petracci et al., 2001). Stress factors such as heat and crating have been associated with increased heterophil to lymphocyte ratios (Gross and Siegel, 1983; Huff et al., 2005; Yalcin et al., 2003; Aksit et al., 2006). Aksit et al. (2006) observed a negative correlation (-0.56) between H:L and muscle pH, and a positive correlation (0.67) between H:L and muscle color (L*) in broilers.

Predominantly, muscle color is closely linked with PSE and DFD-like meat, however there is some evidence to suggest that there are other factors that may contribute to muscle color

that are unrelated to PSE and DFD-like meat. Significant line mean color differences were achieved between selected lines almost immediately after their subpopulations were formed. However, DL and pH differences did not begin to occur until the fourth or fifth generation. The delayed correlated response to selection for L* in the other PSE/DFD linked traits suggests that the preceding selection response in L*, potentially, involved other sources of muscle color variation, perhaps larger or more heritable, that are not directly associated with the metabolic mechanisms responsible for producing PSE or DFD-like meat. For example, residual heme proteins such as hemoglobin and myoglobin as well as cytochrome c have been shown to affect the color of fresh poultry meat (Froning et al., 1968; Pikul et al., 1986; Kranen et al., 1999). Total pigment, myoglobin, and iron content have been shown to be highly correlated to muscle lightness and redness in broilers (Boulianne and King, 1995; Berri et al., 2001). Blood hemoglobin, hematocrit, and plasma iron have been shown to influence muscle color in veal (Miltenburg et al., 1992; Lagoda et al., 2002). Miltenburg et al. (1992) observed a negative correlation between L* and muscle iron (-0.63) and hematin (-0.69), and a positive correlation between redness (a*) and muscle iron (0.48) and hematin (0.66) in veal muscle. Divergent selection for muscle color has resulted in PSE and DFD-like meat (Harford et al., 2014), however selection may have also resulted in changes of physiological mechanisms that effect color but are not associated with the PSE and DFD-like syndrome. The lines used in this study serve as a model to identify and understand potential mechanisms that contribute to muscle color.

MATERIALS AND METHODS

Animals

The HMC line selected for high 24 h L*, and the LMC line selected for low 24 h L* were formed from the RBC line. After eight generations of selection for muscle color, the HMC, RBC, and LMC lines were bled for blood chemistry analysis and were processed for characterization of meat quality. Specific details on the selection process were outlined in Harford et al. (2014).

A random-bred control line (RBC) and two broiler lines divergently selected for 24 h muscle color were evaluated for this study. The study consists of two hatches (two replications), birds were reared under normal industry broiler conditions and were provided ad libitum consumption of water and a corn-soybean based broiler starter and grower ration that was formulated to meet or exceed National Research Council (NRC, 1994) requirements. The day before slaughter, fifty straight run birds at six weeks of age of each line were bled for blood chemistry analysis. Twelve hours prior to processing, feed was removed but water access was continued.

Blood Sample Collection and Analysis

Birds were bled using venipuncture into EDTA-coated tubes. All lines were bled at the same time which was 1 d prior to slaughter. Total red blood cell counts (RBCC) and the numbers and proportions of heterophils (Het), lymphocytes (Lym), hemoglobin (Hgb), and hematocrit (HCT) were determined using a Cell-Dyn 3500 blood analysis system (Abbott Cel-Dyn 3500R, Abbott Laboratories Philippines Inc., Mandaluyong, Philippines) which uses electronic impedance and laser light scattering and has been standardized for analysis of chicken

blood. Heterophil/lymphocyte ratios (H:L) were calculated by dividing the number of heterophils in 1 mL of peripheral blood by the number of lymphocytes (Huff et al., 2005).

Slaughter

Birds were briefly transported approximately 300 yards in coops with 6 birds per coop. Upon arrival to the plant, birds were weighed and hung on a shackle line and processed using in-line commercial equipment. Birds were electrically stunned (11V, 11 mA, 10), manually exsanguinated (severed left carotid artery and jugular vein), bled (1.5 min), scalded (55°C, 2 min) and picked with the use of in-line commercial de-feathering equipment.

Meat Quality

Birds were eviscerated and immediately evaluated for 15 min postmortem muscle pH and then placed in a chill tank for 4 h (1°C). At 4 h postmortem, fillets and tenders were deboned and weighed. Fillets were bagged in individual zip lock bags and refrigerated until 24 h postmortem at which time they were towel dried and re-weighed for evaluation of drip loss. Muscle pH and L* were recorded on the dorsal side of breast fillets using the Testo 205 pH probe (Testo 205 pH Probe, Testo Limited, Hampshire, UK) and Minolta CR-400 colorimeter (Minolta Chroma Meter CR-400, Minolta Italia S.p.A., Milano, Italy) at 4 and 24 h postmortem. The pH probe and colorimeter were both calibrated according to manufacturer's specifications at the beginning of every time of use.

Statistical Analysis

A one-way analysis of variance (anova) test was performed on data using the SAS software package (SAS Institute, 1988). Least squares means were generated in the model for main effects. Line and hatch were used as fixed effects. No line by hatch interactions were observed. Multi-comparisons were made using the Tukey adjustment in the GLM procedure of

SAS. A P-value level of 0.05 was used to determine level of significance. Pearson correlations were performed on pooled data for all traits. Degree of correlation significance (P- value) was indicated with number of asterisks (p*≤ 0.05; p**≤ 0.009; p***≤0.0009).

RESULTS AND DISCUSSION

Meat Quality Characterization of Lines

The muscle color results for each line were similar to those observed in previous studies (Harford et al., 2014) and can be found in Table 1. For 4 h and 24 h L*, the HMC line was highest, and the RBC line was higher than the LMC line. For 4 h and 24 h a*, the LMC line was highest, and the RBC line was higher than the HMC line. The fillet yellowness of the lines differed from the other color components as the line rank changed from 4 h to 24 h. For 4 h b*, the RBC line was highest and the LMC line was lowest. For 24 h b*, the HMC line was highest, and the RBC line was higher than the LMC line. Postmortem fillet pH and fillet drip loss results also mirrored previous studies (Harford et al., 2014) and can be found in Table 2. For initial pH, the LMC and RBC lines were higher than the HMC line. For 4 and 24 h pH, the LMC line was highest, and the RBC line was higher than the HMC line. The HMC line had the highest drip loss, and the RBC line was higher than the LMC line. These results demonstrate that the HMC line is more PSE-like, and the LMC line is more DFD-like respective to the RBC line.

Blood Parameter Correlated Response to Selection

Presently, there is both indirect and direct evidence that shows the components and metabolites of blood play a key role in the determination of muscle color of meat animals. Pre-slaughter stress in broilers has been independently shown to impact blood H:L ratio (Gross and Siegel, 1983; Yalcin et al., 2003; Huff et al., 2005; Aksit et al., 2006) and muscle color (Hedrick et al., 1989; McPhee and Trout, 1995; Lawrie, 1998; Owens and Sams, 2000; Petracci et al.,

2001; Harford et al., 2014). Unfortunately the direct correlation between H:L ratio and muscle color has not been as extensively studied. Zhang et al. (2009) observed no changes in muscle color or H:L ratio between unstressed and transport stressed groups of broilers. However, Aksit et al. (2006) observed significant correlations between H:L ratio and pH (-0.557) and L* (0.665) in temperature stressed birds. The HMC, RBC, and LMC lines showed differences for H:L ratio under minimal stress associated with bleeding and handling (Table 3). The HMC line was highest for H:L ratio, whereas the LMC line was lowest. The RBC line, although intermediate, was not significantly different than the HMC and LMC lines. It is unclear at this point whether the variation observed for H:L ratio is directly related to variation for muscle color between these lines, however the heritability of H:L ratio has been shown to be highly heritable (0.59) (Campo and Davila, 2002). If H:L ratio is factoring into selection for muscle color, then line to line differences in H:L ratio should be expected over time. Greater sensitivity to transportation stress in the HMC line than the RBC or LMC lines for PSE-like meat was observed in chapter II. It was postulated that selection for increased 24 h L* in the HMC line could have resulted in hyper-reactivity to stress as a result of lowered stress thresholds, or the basal level of stress related mechanisms and steroids such as corticosterone have been elevated, or the metabolic rates of glycogen and lactate have increased or decreased respectively. Either of these scenarios would result in hypersensitivity to stress and increased incidences of stress related conditions like PSE-like meat. As previously mentioned, H:L ratio is an indicator of stress levels, the higher H:L ratio observed in the HMC line while unstressed suggests that the HMC line is relatively more susceptible to stress. While the differences observed for H:L ratio between the selected lines cannot be proven to be the complete or even a significant cause for differences in L* between

selected lines, it is likely that it contributes to some degree to the PSE-like characteristics of the HMC line. Re-measuring H:L ratios in future generations could offer more insight.

The proper exsanguination of meat animals has been shown to greatly affect meat quality. Residual blood and heme proteins cause increased oxidation resulting in increased darkness, redness, rancidity, and reduction of shelf-life (Alvarado et al., 2007). The findings of Alvarado et al. (2007) suggested that even proper bleeding resulted in little blood removal from breast tissue. However, it was also reported that un-bled birds had greater residual breast hemoglobin and fillet redness. Mohamed and Mohamed (2012) observed that imperfectly-bled birds had higher ultimate pH, redness, yellowness, and lower L* than perfectly-bled birds. It is possible that the variation in color between the HMC and LMC lines is in part due to variation in bleed out effectiveness, or perhaps variation in blood parameters resulting in variation of residual components of blood such as red blood cells, hemoglobin, or plasma iron left in the breast tissue.

Variation in blood hemoglobin, hematocrit, and plasma iron has been shown to influence muscle color in veal (Miltenburg et al., 1992; Lagoda et al., 2002). Blood parameter means for all three lines can be found in Table 3. The HMC line was highest in blood platelets. For RBCC, the LMC line was highest and the HMC line was lowest. Unexpectedly, the RBC line had the highest hemoglobin levels; however the LMC line was numerically higher than the HMC line. The LMC line was highest in hematocrit. Red blood cell count, hematocrit, and hemoglobin levels together are strong indicators of oxygen carrying capacity which dictates the rate at which tissue can be supplied oxygen. There are two potential ways that increased oxygen carrying capacity could impact muscle color. First, it's possible that the increased oxygen supply to muscle tissue increases the likelihood of residual blood and myoglobin oxidation. When exposed to oxygen, blood and muscle tissue will turn from a purplish color to bright red. If so,

decreased L* and increased a* should be expected. The other possibility is that the increased oxygen carrying capacity allows the LMC line to aerobically metabolize accumulated muscle lactate at a faster rate than the HMC line. If so, this would produce a lower muscle lactate concentration, glycolytic potential and rate of pH decline at the time of slaughter which in turn would produce a darker muscle color. This is supported by results outlined in chapter II where a more detailed explanation will be given. Although small in magnitude, mean line differences are significant and fit the pattern that was anticipated.

Meat Quality and Blood Parameter Correlations

When data of all three lines are pooled, significant correlations are observed between muscle color, drip loss, pH, and blood parameters (Table 4 & 5). The H:L ratio was moderately positively correlated to drip loss and L*, and is negatively correlated to pH and redness. This is supported by the fact that H:L has been shown to be a good indicator of stress (Gross and Siegel, 1983; Yalcin et al., 2003; Huff et al., 2005; Aksit et al., 2006). The RBCC was slightly correlated to yellowness. Hemoglobin was moderately negatively correlated to L* and hematocrit was moderately negatively correlated to L*, and positively correlated to pH. Unexpectedly, blood platelet level appeared to have the strongest correlations to meat quality traits. It was strongly positively correlated to L* and drip loss, and negatively correlated to redness and pH. Blood platelets primary function is to aid in clotting and reduce blood loss. It is unclear how variation in blood platelet levels could impact meat quality traits, however elevated platelet counts have been associated with iron-deficiency anemia (Koury and Rhodes, 2012). Iron-deficiency anemia results in reduced oxygen supply to tissues, which could impact lactate metabolism. The reduced Hgb of the HMC line relative to the RBC line supports this potentiality.

Synthesis

Correlated response to selection for muscle color appears to include some blood chemistry traits. The extent to which these concomitant changes affect muscle color is not fully known. However, line differences for these correlated traits fit the pattern expected if they were to contribute an active role in muscle color determination. Substantial divergence has been achieved in these lines for muscle color, which has helped identify potential sources of muscle color variation. It is worth understanding that these lines are purely selected on color, and not directly for PSE or DFD-like meat. The fact that rapid change occurred in PSE and DFD-like qualities relatively soon after respective lines were formed reflects the significant relationship between postmortem glycolytic activity and muscle color. It is for this reason that relatively small potential sources of color variation can be difficult to detect. However slight, these small sources of variation will accumulate gradually and over time will collectively and progressively become more important for selection for muscle color as the major genes associated with PSE/DFD-like meat become fixed within the selected populations. Consequently, sufficient identification and understanding of secondary sources of color variation will require continued selection and series of experimental observations. It is through this methodical and gradual process that a true comprehensive understanding of muscle color and corresponding meat quality traits can be achieved.

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Table 1. Muscle color values at 4 h and 24 h postmortem for 3 broiler lines divergently selected for muscle color[1,2]

	HMC	RBC	LMC
L* 4 h	53.35 ± 0.37a	47.32 ± 0.25b	43.82 ± 0.29c
L* 24 h	54.21 ± 0.31a	49.65 ± 0.21b	46.71 ± 0.24c
a* 4 h	3.12 ± 0.11c	3.81 0.07b	4.92 ± 0.08a
a* 24 h	2.99 ± 0.14c	3.99 0.09b	4.88 ± 0.10a
b* 4 h	4.20 ± 0.19ab	4.34 ± 0.12a	3.81 ± 0.14b
b* 24 h	5.43 ± 0.18a	4.46 ± 0.12b	4.00 ± 0.14c

[1]HMC=high L* line, RBC=random bred control line, LMC=low L* line, L*=lightness, a*=redness, b*=yellowness.
[2]Least-Squares Means within different letters are significantly different ($P<0.05$).

Table 2. Postmortem muscle pH and fillet drip loss (%) for 3 broiler lines divergently selected for muscle color[1,2]

	HMC	RBC	LMC
pH 15 min	6.17 ± 0.02b	6.27 ± 0.02a	6.28 ± 0.02a
pH 4 h	5.67 ± 0.02c	5.87 ± 0.02b	6.09 ± 0.02a
pH 24 h	5.65 ± 0.02c	5.89 ± 0.02b	6.14 ± 0.02a
Drip Loss (%)	2.27 ± 0.09a	1.28 ± 0.06b	0.88 ± 0.07c

[1]HMC=high L* line, RBC=random bred control line, LMC=low L* line.
[2]Least-Squares Means within different letters are significantly different ($P<0.05$).

Table 3. H:L ratio, red blood cell, protein, and platelet counts for 3 broilers lines divergently selected for muscle color[1,2]

	HMC	RBC	LMC
H:L	1.77 ± 0.18a	1.55 ± 0.17ab	1.05 ± 0.17b
RBCC	2.65 ± 0.02b	2.67 ± 0.02ab	2.73 ± 0.02a
Hgb	7.47 ± 0.05b	7.78 ± 0.05a	7.62 ± 0.05ab
HCT	62.21 ± 0.49b	62.40 ± 0.48b	64.15 ± 0.49a
Plt	9.47 ± 0.23a	7.86 ± 0.22b	8.21 ± 0.22b

[1]HMC=high L* line, RBC=random bred control line, LMC=low L* line, H:L=heterophil lymphocyte ratio, RBCC=red blood cell count, Hgb=hemoglobin, HCT=hematocrit, Plt=platelets.
[2]Least-Squares Means within different letters are significantly different ($P<0.05$).

Table 4. Correlations among muscle color and blood parameters for pooled data of 3 broiler lines divergently selected for muscle color.[1,2]

	4 h L*	24 h L*	4 h a*	24 h a*	4 h b*	24 h b*
H:L	0.22**	0.25**	-0.25**	-0.22**	0.02	0.06
RBCC	-0.05	-0.08	-0.01	-0.02	0.17*	0.05
Hgb	-0.22**	-0.23**	0.13	0.13	0.17*	-0.03
HCT	-0.19*	-0.21*	0.07	0.11	0.12	0.01
Plt	0.36***	0.35***	-0.24**	-0.27**	-0.05	0.18*

[1]RBCC=red blood cell count, Hgb=hemoglobin, HCT=hematocrit, Plt=platelets.
[2]Significance level is indicated by * (P*≤ 0.05; P**≤ 0.009; P***≤0.0009).

Table 5. Correlations among postmortem fillet drip loss, muscle pH and blood parameters for pooled data of 3 broiler lines divergently selected for muscle color.[1,2]

	Drip Loss (%)	pH 15 min	pH 4 h	pH 24 h
H:L	0.22**	-0.11	-0.25**	-0.22**
RBCC	0.07	0.13	0.02	0.04
Hgb	-0.04	0.15	0.12	0.09
HCT	-0.09	0.21*	0.14	0.17*
Plt	0.24**	-0.20*	-0.30***	-0.32***

[1]RBCC=red blood cell count, Hgb=hemoglobin, HCT=hematocrit, Plt=platelets.
[2]Significance level is indicated by * (P*≤ 0.05; P**≤ 0.009; P***≤0.0009).

APPENDIX

UNIVERSITYOF

ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Nicholas Anthony
FROM: Craig N. Coon, Chairman Institutional Animal Care And Use Comittee

DATE: May 23, 2012
SUBJECT: IACUC PROTOCOL APPROVAL
Expiration date: April 6, 2015

The Institutional Animal Care and Use Committee (IACUC) has APPROVED Protocol #12038- "GENERAL REARING OF SELECTED CHICKEN AND QUAIL POPULATIONS ". You may begin this study immediately.

The IACUC encourages you to make sure that you are also in compliance with other UAF committees such as Biosafety, Toxic Substances and/or Radiation Safety if your project has components that fall under their purview.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes in the protocol during the research, please notify the IACUC in writing [Modification Request form] prior to initiating the changes. If the study period is expected to extend beyond 04-06-2015, you must submit a new protocol. 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 for research involving animal subjects.

ene/car

cc: Animal Welfare Veterinarian

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Effects of pre-slaughter transportation on meat quality in broiler lines divergently selected for muscle color

ABSTRACT

After eight generations of divergent selection for muscle color in broilers, muscle quality parameters were investigated in 3 broiler lines that were transported or non-transported. The High (HMC) and Low (LMC) lines were selected for high and low 24 h L* (lightness) respectively, and were formed from their random bred control line (RBC). Two replications of approximately sixty birds from each line were equally and randomly separated into transported (T) and non-transported (NT) groups. The transported group were cooped and transported for approximately 3 h prior to processing, whereas the non-transported group were not cooped until moments before slaughter. Measurements were collected for muscle pH at 15 min, 4 h, and 24 h postmortem, muscle color at 4 and 24 h postmortem, and fillet drip loss at 24 h postmortem (DL). A line by treatment interaction was present for all traits measured with the exception of 15 min pH. The pattern of line differences for DL, muscle color and pH were consistent with patterns observed historically in both the NT and T treatment groups. Subjecting the RBC line to transport stress resulted in only a decrease in 24 h L* and increase in 4 and 24 h a*. Similarly, the T group for the LMC line exhibited decreased L* and b*, and increased a* at 4 and 24 h postmortem. In contrast, the HMC line exhibited increased DL and 4 and 24 h L*, and decreased 4 and 24 h muscle pH postmortem. When subjected to transportation stress, the HMC line selected for high L* became more PSE-like, the LMC line selected for low L* became more DFD-like, and the RBC changed very little. These results suggest that greater cumulative improvements could be made through stabilizing genetic selection for muscle quality traits.

INTRODUCTION

Meat quality is a complex threshold trait that is subject to many factors. Causes for atypical meat can be anything that ultimately disrupts the eating quality, stability, or wholesomeness of a product. Two conditions known for affecting meat quality are pale, soft, and exudative (PSE) and dark, firm and dry meat (DFD). PSE meat is defined by a high rate of decline in postmortem pH while carcass temperatures are still relatively high resulting in protein denaturation and subsequent loss of water-holding capacity (WHC) (Owens and Sams, 2000; Qiao et al., 2001). Factors associated with PSE and DFD-like conditions include nutrition (Buckley et al., 1995; Jensen et al., 1997; Olivio et al., 2001) rearing conditions (Sayre et al., 1963), pre-slaughter stress and behavior (Owens and Sams, 2000; Petracci et al, 2001; Debut et al., 2003; Warriss et al., 2005), stunning method (Skarovsky and Sams, 1999; Gregory, 2007) postmortem handling (McKee and Sams, 1998; Owens and Sams, 2000; Alvarado and Sams, 2002; Alvarado and Sams, 2004), and genetic components (Le Bihan-Duval et al., 2001; Le Bihan-Duval et al., 2003; Debut et al., 2003; Harford et al., 2014).

Generally, physiological factors affecting meat quality are stress related. The type, duration, and occurrence of stressors greatly determine whether PSE or DFD-like characteristics are present. PSE meat is typically caused by an acute pre-slaughter stress that increases the rate of glycolysis at the time of slaughter, whereas DFD is caused by chronic pre-slaughter stress that depletes glucose prior to slaughter (Hedrick et al., 1989; McPhee and Trout, 1995; Lawrie, 1998; Owens and Sams, 2000; Petracci et al., 2001). Strong to moderate heritabilities for muscle color, postmortem pH, and drip loss have been estimated under experimental and commercial conditions (Le Bihan-Duval et al., 2001; Le Bihan-Duval, 2003; Harford et al., 2014). The muscle color lines used in this study have been selected and diverged for high and low 24 h L*;

as a result, divergence has been achieved in PSE and DFD-like characteristics such as muscle pH and drip loss (Harford et al., 2014). It remains unclear what mechanisms have been changed by selection and how these strains would respond to pre-slaughter stress such as transportation. The purpose of this study was to compare the effects of transportation stress and genetic selection for PSE/DFD conditions on meat quality and to determine if an environment by genotype interaction exists.

MATERIALS AND METHODS

With the transition from whole bird to further processed and ready to eat products, more attention has been focused towards product quality. Recently, a metabolic syndrome known as PSE-like (pale, soft, exudative) poultry meat has been impacting the broiler industry. PSE-like meat is associated with increased muscle lightness (L*), decreased water holding capacity, decreased tenderness, and the inability to form gels in further processed products. All of which have significant economic impacts on the broiler industry. An increase in muscle lightness, L*, is a key visual indicator for breast fillets exhibiting the PSE-like condition. Lines were tested for 24 h postmortem muscle color using a Minolta CR-400 Colorimeter (Minolta Chroma Meter CR-400, Minolta Italia S.p.A., Milano, Italy) on a random bred broiler population processed at 56 days of age (Petracci et al., 2004). Based on those results, divergent selection was applied for muscle color in order to develop divergent muscle color lines and determine the impact on color characteristics and product quality. However, evaluation of muscle color is a terminal measure; therefore, a sufficient number of progeny must be tested to produce sufficient sire information for effective selection. The following methods were developed to address the issues associated with selection for a terminal trait.

Animals

A random bred control line (RBC) and two broiler lines divergently selected for 24 h muscle color were evaluated for this study. The study consists of two hatches (two replications), birds were reared under normal industry broiler conditions and were provided ad libitum consumption of water and a corn-soybean based broiler starter and grower ration that was formulated to meet or exceed National Research Council (NRC) (1994) requirements. At six weeks of age, sixty straight run birds of each line per treatment group were processed for the purpose of evaluating the effects of transportation stress on meat quality. The day before processing, birds were assigned to transported (T) or non-transported treatment (NT) groups. 12 h prior to processing, feed was removed but water access was continued.

Transport Stress & Slaughter

On the day of processing (July, 2012), transported birds were cooped (3 birds per line) and were placed on a flatbed truck and transported for 3 h prior to processing in a mixture of rural, city, and interstate traffic. Non-transported birds were cooped moments prior to the arrival of transported birds at the processing plant. Both treatment groups were processed at the same time across 4 batches.

Upon arrival to the plant, birds were weighed, and hung on a shackle line and processed using in-line commercial equipment. Birds were electrically stunned (11V, 11 mA, 10), manually exsanguinated (severed left carotid artery and jugular vein), bled (1.5 min), scalded (55°C, 2 min) and picked with the use of in-line commercial de-feathering equipment.

Meat Quality Measurements

Birds were eviscerated and evaluated for 15 min postmortem muscle pH and then placed in a chill tank for 4 h (1°C). At 4 h postmortem, fillets were deboned, weighed, and bagged in

individual zip lock bags and refrigerated until 24 h postmortem at which time they were towel dried and re-weighed for evaluation of drip loss. Muscle pH and L* were recorded on the interior left (pH) and right (color) breast fillets using the Testo 205 pH probe (Testo 205 pH Probe, Testo Limited, Hampshire, UK) and Minolta colorimeter at 4 and 24 h postmortem.

Statistical Analysis

A two-way analysis of variance (anova) test was performed on data using the SAS software package (SAS Institute, 1988). Least squares means were generated in the model for main effects. Line, treatment, and hatch were used as fixed effects. Multi-comparions were made using the Tukey adjustment in the GLM procedure of SAS. A P-value level of 0.05 was used to determine level of significance.

RESULTS AND DISCUSSION

As previously mentioned, the HMC line selected for high 24 h L* and the LMC line selected for low 24 h L* were formed from the RBC line. The results of this study characterize the response to selection under transported and non-transported conditions. Muscle color measured in units of lightness (L*) is of interest not just for its consumer appeal, but because it is highly correlated with muscle pH and drip loss and is a strong indicator of PSE and DFD-like meat due to its high accuracy and repeatability of measurement (Fletcher, 1999; Le Bihan-Duval, 2001). For these reasons, muscle quality lines were created through selection for muscle color with the expectation that indirect selection for other correlated muscle quality traits would result in PSE and DFD-like meat respectively. After eight generations of selection the HMC, RBC and LMC lines were split into transported (T) and non-transported (NT) groups and evaluated for muscle color. Multi-comparisons were made for each line by treatment group when main effect interactions were present. As previously mentioned, hatch effect was included in the model.

Slight hatch differences were detected for most traits. In addition, two-way hatch interactions were observed for some traits, however the observed interactions involving hatch were not of a qualitative nature but rather quantitative and only arose as a result of the magnitude or significance of mean differences. Since line by trait comparisons were directionally consistent for both hatches, traits with hatch interactions will not be dissected for the sake of clarity. A treatment by line interaction existed for all traits with the exception of muscle pH at 15 min postmortem. For the sake of clarity, line comparisons will be made within treatment groups (Table 1), and treatment comparisons will be made within lines (Table 2).

Fillet Drip Loss (%) and Muscle pH

One characteristic of reduced protein functionality that is associated with PSE-like meat is increased fillet drip loss. In PSE-like meat, contractile proteins lose their ability to bind to water molecules; bound water is converted to free water and is more easily lost during the aging, processing and cooking. For both the NT and T groups, the HMC line had the greatest drip loss (Table 1). In the NT group, the RBC line had greater drip loss than the LMC line. Although approaching significance, no differences were detected between the RBC and LMC lines in the T group. Similarly, no differences were observed between the NT and T groups within the RBC and LMC lines. However, in the HMC line, the T group had greater drip loss than the NT group (Table 2).

During anaerobic respiration, stored glycogen is metabolized and converted into lactic acid and ATP. The lactic acid that is produced is further metabolized for the production of additional ATP through aerobic respiration. After death, glycogen continues to be metabolized anaerobically however aerobic respiration is no longer possible thus lactic acid is not metabolized. This results in an accumulation of lactic acid thereby reducing muscle pH over

time (Holm and Fletcher, 1997; McKee and Sams, 1997; Lawrie, 1998). PSE-like meat occurs when the pH decline occurs too rapidly or severely, whereas DFD-like meat occurs when the pH decline is too gradual or mild. Muscle pH has thus shown to be a good method for monitoring postmortem metabolism and predicting PSE/DFD-like meat.

Muscle pH was recorded at 15 min, 4 h, and 24 h postmortem for all three lines in NT and T groups. Shortly after death, lines had already begun showing differences for initial pH (15 min) with the HMC line exhibiting significantly lower muscle pH than the LMC and RBC lines (Table 1). No differences were detected between the NT and T groups for any of the lines (Table 2). At 4 h postmortem, muscle pH was greatest in the LMC line, and the RBC line was greater than the HMC line for both the NT and T groups (Table 1). No differences were detected between the NT and T groups for 4 h pH in the RBC and LMC lines, however the T group was lower than the NT group in the HMC line (Table 2). Similar results were observed at 24h postmortem (Tables 1 & 2).

Muscle Color

As previously mentioned, when the rate or extent of postmortem pH decline is too severe, denaturation of contractile proteins occurs. When protein functionality is lost, bound water is forced out between contractile proteins on to the surface as free water. The resulting free water is more easily lost during the aging and cooking, but also increases the degree of light reflectance resulting in a pale appearance. The opposite occurs in DFD-like meat.

For both the NT and T groups, the HMC line had the greatest 4 h L*, and the RBC line was greater than the LMC line (Table 1). Within the RBC line no differences were observed between the NT and T groups; however the T group had a greater 4 h L* than the NT group in the HMC line, but had a lower 4 h L* in the LMC line (Table 2). Similar results were observed

for 24 h L*. The HMC line had the greatest 24 h L*, and the RBC line was greater than the LMC line in both the NT and T groups (Table 1). The T group had greater 24 h L* in the HMC line, but was lower in the RBC and LMC lines (Table 2). For 4 and 24 h redness (a*), the LMC line was the greatest, and the RBC line was greater than the HMC line in both the NT and T groups (Table 1). No differences were observed between the NT and T groups for 4 and 24 h a* in the HMC line, but the T group had greater 4 and 24 h a* in the RBC and LMC lines (Table 2). For 4 h yellowness (b*), the LMC line was greater than the RBC line in the NT group, but the HMC line was greater than the RBC line which was greater than LMC line in the T group (Table 1). No differences were observed between the NT and T groups for 4 h b* in the HMC and RBC lines, but the T group was lower than the NT group in the LMC line (Table 2). Similar results were observed for 24 h b*. The HMC line was greater than both the RBC and LMC line in the NT group. In the T group, the HMC line was greatest, and the RBC line was greater than the LMC line for 24 h b* (Table 1). No differences were observed for 24 h b* between the NT and T groups for the HMC and RBC lines, but the T group was lower than the NT group in the LMC line.

Synthesis

Previous studies in broilers, turkeys and pigs have shown varying degrees of increased DFD-like meat incidence when animals have been exposed to transportation stress (Geers et al., 1994; McPhee and Trout, 1995; Owens and Sams, 2000). Two possible reasons transportation stress tends to produce DFD-like meat is that it occurs over prolonged periods of time and the moving environment forces the animal to physically respond in order to maintain balance resulting in energy expenditure (Warriss et al., 1993; Dadgar et al.,2012). Those two characteristics of transportation stress could certainly increase the likelihood of DFD-like meat if

available glucose and glycogen reserves are depleted and resulting muscle lactate concentrations have been metabolized enough to reduce glycolytic potential at slaughter. With that in mind, it was not unexpected to observe variation in the severity of response to transportation stress between lines. Under normal conditions, the LMC line relative to the RBC line has a lower rate of pH decline and higher ultimate pH (24 h) which suggests decreased glycolytic potential. This characteristic of the LMC line suggests it would be particularly susceptible to the effects of transportation stress. What was unexpected was how the HMC line became more PSE-like when exposed to transportation stress. The T group of the HMC line had greater drip loss and paleness, and lower 4 and 24 h pH. This differs, in part, from previous studies linking DFD-like meat to transportation stress (Geers et al., 1994; McPhee and Trout, 1995; Owens and Sams, 2000).

During periods of stress, quick bursts in energy supply are favored as it allows an animal to more effectively respond to stress often the result of sudden changes in environment. Thus anaerobic respiration is a valuable survival mechanism as it can quickly produce energy by converting glucose into lactic acid and ATP. A likely explanation for asymmetrical response to pre-slaughter stress is that selection for muscle color has changed mechanisms involved in anaerobic respiration. PSE meat is typically caused by an acute pre-slaughter stress that increases glycolytic potential at the time of slaughter, whereas DFD is caused by chronic pre-slaughter stress that increases glycolytic activity but to the point of glucose depletion prior to slaughter (Hedrick et al., 1989; McPhee and Trout, 1995; Lawrie, 1998; Owens and Sams, 2000; Petracci et al., 2001). When transported, the HMC line became more PSE-like, whereas the LMC line became more DFD-like. Perhaps selection for muscle color has changed the enzymatic activity and substrate (glycogen) levels differently for each line. Zhang et al. (2009)

outlines a comprehensive list of possible mechanisms that could explain the qualitative difference between the HMC and LMC lines under transportation stress.

Briefly, the conversion of glucose, glycogen, and glucose-6-phosphate into lactate is a spontaneous reaction that will occur even after death. Ultimately, it is the muscle lactate concentrations that determines the pH decline observed in rigor development. Glycolytic potential can be defined as the amount of glucose, glycogen, glucose-6-phosphate, and lactate muscle concentration at the time of death. Thus glycolytic potential is a good indicator of what the eventual pH decline will be. Consequently, any changes in the components of glycolytic potential will affect postmortem decline.

Corticosterone is a stress hormone primarily responsible for increasing blood glucose levels. Transportation stress has been shown to increase plasma corticosterone concentration in broilers (Freeman et al., 1984; Kannan et al., 1997; Zhang et al., 2009). Zhang et al. (2009) observed that, among the transport stressed groups, broilers given a short period of stress and recovery time had the highest levels of plasma corticosterone levels whereas broilers given long periods of stress and recovery time had the lowest levels. It was concluded that the increased stress and recovery times allowed birds to acclimate to the stressors before slaughter. Perhaps, the HMC line has more difficulty acclimating to stressors resulting in prolonged periods of heightened stress and gluconeogenesis resulting in increased glycolytic potential at the time of slaughter. This could possibly explain results observed in chapter I where elevated levels of H:L were detected in the HMC line while under relatively low stress conditions.

Transportation stress has been shown to elevate plasma glucose in the short-term due to break down of glycogen in the liver (Kent and Ewbank, 1983, 1986; Mayes, 1996; Zhang et al., 2009). However prolonged periods of transportation stress can exhaust hepatic glycogen

reserves resulting in plasma glucose depletion and subsequent decreased glycolytic potential (Zhang et al., 2009). If substrate availability is depleted before death, then the extent of postmortem pH decline would be less. Selection for L* in the HMC line could have resulted in increased hepatic glycogen reserves thereby maintaining normal or even elevated blood glucose levels for longer periods of stress. Perhaps the 3 h transportation stress administered was not great enough to deplete glycogen reserves in the HMC line, but was enough to stimulate an increase in catalytic activity and glycolytic potential at the time of death. A follow-up study comparing the lines subjected to pre-slaughter stress and resting time for multiple lengths of time could confirm this.

As previously mentioned, aerobic respiration is a slower yet more efficient way of producing energy by converting the lactate by-product of anaerobic respiration into ATP. Glycogen is also stored in skeletal muscle tissue for energy production. It has been shown to deplete during extended periods of transportation stress (Zhang et al., 2009). The resulting accumulation of the lactate by-product would decrease ultimate pH and produce PSE-like meat. However if periods of stress or recovery are long enough, muscle lactate concentrations can be reduced through aerobic respiration. Variation in metabolic rate of muscle lactate could affect glycolytic potential and subsequent postmortem pH. Perhaps the HMC line metabolizes accumulated muscle lactate concentrations slower than the LMC line. If the HMC line had higher levels of accumulated muscle lactate right at slaughter, the HMC line should exhibit in an immediate drop in postmortem muscle pH. This is supported by the immediate separation of the HMC line from the RBC and LMC lines in both treatment groups for initial pH. Additionally, it was observed in chapter I that the LMC line had higher levels of RBCC, Hgb (numerically), and HCT compared to the HMC line. Collectively, the primary function of RBCC, Hgb, and HCT is

to supply tissue with oxygen for the purpose of aerobic respiration. In an attempt to maintain required tissue oxygen levels, broilers have been shown to exhibit increased levels of HCT when reared under hypoxic conditions. Perhaps the increased levels of RBCC, Hgb (numerically), and HCT observed in the LMC line increases the metabolic rate of accumulated muscle lactate concentrations. Such an advantage would reduce the glycolytic potential as lactate concentration is a component of it.

Heat stress that was accompanied by transportation stress could also have played a role in meat quality determination for the transport group. This study was conducted in July of 2012, the hottest month in recorded history (NOAA, 2012). Previous research has shown that temperature stressors can play a large role in the determination of meat quality. In pigs, increased quality defects in pork have been reported in summer and fall months (Wismer-Pedersen, 1959; Forrest et al., 1963). Elevated temperatures immediately prior to slaughter in pigs have been reported to increase muscle glycolysis and increase pale appearance (Sayre et al., 1963). In poultry, increased rates of glycolysis and PSE-like meat incidence, decreased muscle pH and tenderness have occurred after birds were subjected to elevated temperatures (Wood and Richards, 1975; Froning et al., 1978; Holm and Fletcher, 1997; McKee and Sams, 1997; Mckee and Sams, 1998; Petracci et al., 2001). The aforementioned studies suggest that DFD-like meat is typically associated with transportation stress mainly due to the length of time involved in transportation, and heat stress is associated with PSE-like meat. Perhaps selection for muscle color has moved the selected lines in terms of susceptibility or resistance to these stressors resulting in either defect respective to line.

The genetic predisposition for meat quality, at least in these experimental populations, appeared to dictate the directional response to transportation stress whereas the transportation

stress appeared to only affect the intensity of the response. The random-bred control line practically responded no differently in either treatment group whereas the selected lines worsened in their respective defects for certain traits. It was surprising that little difference was seen between treatment groups for the RBC line. It was expected that the response to transportation stress in the RBC line would be similar to what has been observed in previous broiler studies. As previously mentioned, the random-bred population was formed from 7 male and 6 female commercial lines in the early 1990's. As a result, this population and its subpopulations are not remotely as close to the physiological limits for growth rate and yield as today's commercial broiler. This could explain why slight differences were observed between treatment groups for the RBC line, which in turn would suggest that the differences observed within the HMC and LMC lines are a direct result of selection for muscle color. The HMC line appeared to exhibit decreased muscle pH, and increased drip loss and L* when transported, whereas the LMC line exhibited decreased L* and b*, and increased a*. It is probable that some metabolic change has occurred between the selected lines that has changed the time frame at which acute stress transitions to chronic stress. The LMC line entering the chronic phase earlier, and the HMC line later. It is interesting to note that the affected traits of the selected lines were not consistent with the exception of L*. This asymmetry raises the question of whether continued selection is all that is needed for consistency in which traits are affected for each population or whether the current set of respective affected traits are specific to the underlying mechanisms responsible for the predisposition of that population. The latter would suggest distinct sets of genes and mechanisms between selected lines are responsible for the observed differences in muscle color. Furthermore, it is unclear if the intensifying effect of pre-slaughter stress on meat quality will increase as divergent selection for muscle color continues in

progressive generations or whether it will diminish as the respective populations begin to reach their biological limits for meat quality traits associated with the PSE and DFD-like conditions. The results of this study suggest that improvement in meat quality can be made through stabilization selection as it not only increases uniformity for the selected trait, but may also result in more stress-tolerant populations thereby minimizing meat quality defects associated with multiple types of pre-slaughter stress.

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Table 1. Meat quality line comparisons of 3 broiler lines within non-transported and transported treatments[1,2]

	NT			T		
	HMC	RBC	LMC	HMC	RBC	LMC
Drip Loss (%)	2.54 ± 0.11a	1.29 ± 0.09b	0.93 ± 0.09c	2.99 ± 0.10a	1.13 ± 0.10b	0.85 ± 0.10b
pH 15 min	6.21 ± 0.02b	6.34 ± 0.02a	6.35 ± 0.02a	6.16 ± 0.02b	6.34 ± 0.02a	6.35 ± 0.02a
pH 4 h	5.60 ± 0.02c	5.85 ± 0.02b	6.06 ± 0.02a	5.50 ± 0.02c	5.81 ± 0.02b	6.08 ± 0.02a
pH 24 h	5.52 ± 0.02c	5.78 ± 0.02b	6.02 ± 0.02a	5.45 ± 0.02c	5.75 ± 0.02b	5.99 ± 0.02a
4 h L*	52.33 ± 0.31a	46.83 ± 0.24b	44.08 ± 0.25c	53.47 ± 0.27a	46.52 ± 0.29b	42.50 ± 0.28c
24 h L*	54.07 ± 0.27a	49.62 ± 0.21b	46.88 ± 0.22c	54.80 ± 0.24a	48.92 ± 0.25b	45.70 ± 0.24c
4 h a*	3.52 ± 0.11c	4.17 ± 0.09b	5.41 ± 0.09a	3.65 ± 0.10c	4.50 ± 0.10b	5.98 ± 0.10a
24 h a*	3.34 ± 0.12c	3.92 ± 0.09b	5.06 ± 0.09a	3.26 ± 0.10c	4.29 ± 0.11b	5.65 ± 0.11a
4 h b*	4.17 ± 0.16ab	3.97 ± 0.12b	4.43 ± 0.13a	4.44 ± 0.14a	4.25 ± 0.15b	3.83 ± 0.14c
24 h b*	5.00 ± 0.18a	3.92 ± 0.14b	3.97 ± 0.15b	5.39 ± 0.16a	4.17 ± 0.17b	3.47 ± 0.16c

[1]Three lines of birds (LMC, RBC, HMC) were separated into non-transported (NT) and transported (T) groups and evaluated for meat quality traits. Comparisons are made within treatment.
[2]Means and standard errors with different letters are significantly different ($P<0.05$).

Table 2. Meat quality treatment comparisons of non-transported and transported groups within 3 broiler lines[1,2]

	HMC		RBC		LMC	
	NT	T	NT	T	NT	T
Drip Loss (%)	2.54 ± 0.11b	2.99 ± 0.10a	1.29 ± 0.09	1.13 ± 0.10	0.93 ± 0.09	0.85 ± 0.10
pH 15 min	6.21 ± 0.02	6.16 ± 0.02	6.34 ± 0.02	6.34 ± 0.02	6.35 ± 0.02	6.35 ± 0.02
pH 4 h	5.60 ± 0.02a	5.50 ± 0.02b	5.85 ± 0.02	5.81 ± 0.02	6.06 ± 0.02	6.08 ± 0.02
pH 24 h	5.52 ± 0.02a	5.45 ± 0.02b	5.78 ± 0.02	5.75 ± 0.02	6.02 ± 0.02	5.99 ± 0.02
4 h L*	52.33 ± 0.31b	53.47 ± 0.27a	46.83 ± 0.24	46.52 ± 0.29	44.08 ± 0.25a	42.50 ± 0.28b
24 h L*	54.07 ± 0.27b	54.80 ± 0.24a	49.62 ± 0.21a	48.92 ± 0.25b	46.88 ± 0.22a	45.70 ± 0.24b
4 h a*	3.52 ± 0.11	3.65 ± 0.10	4.17 ± 0.09b	4.50 ± 0.10a	5.41 ± 0.09b	5.98 ± 0.10a
24 h a*	3.34 ± 0.12	3.26 ± 0.10	3.92 ± 0.09b	4.29 ± 0.11a	5.06 ± 0.09b	5.65 ± 0.11a
4 h b*	4.17 ± 0.16	4.44 ± 0.14	3.97 ± 0.12	4.25 ± 0.15	4.43 ± 0.13a	3.83 ± 0.14b
24 h b*	5.00 ± 0.18	5.39 ± 0.16	3.92 ± 0.14	4.17 ± 0.17	3.97 ± 0.15a	3.47 ± 0.16b

[1]Three lines of birds (LMC, RBC, HMC) were separated into non-transported (NT) and transported (T) groups and evaluated for meat quality traits. Comparisons are made within line.

[2]Means with different letters are significantly different ($P<0.05$).

APPENDIX

UNIVERSITYOF

ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Nicholas Anthony
FROM: Craig N. Coon, Chairman Institutional Animal Care And Use Comittee

DATE: May 23, 2012
SUBJECT: IACUC PROTOCOL APPROVAL
Expiration date: April 6, 2015

The Institutional Animal Care and Use Committee (IACUC) has APPROVED Protocol #12038- "GENERAL REARING OF SELECTED CHICKEN AND QUAIL POPULATIONS ". You may begin this study immediately.

The IACUC encourages you to make sure that you are also in compliance with other UAF committees such as Biosafety, Toxic Substances and/or Radiation Safety if your project has components that fall under their purview.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes in the protocol during the research, please notify the IACUC in writing [Modification Request form] prior to initiating the changes. If the study period is expected to extend beyond 04-06-2015, you must submit a new protocol. 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 for research involving animal subjects.

ene/car

cc: Animal Welfare Veterinarian

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Effects of pre-slaughter temperature on meat quality in broiler lines divergently selected for muscle color

ABSTRACT

Muscle quality parameters were investigated in 3 broiler lines that were subjected to cold (1°C), ambient (22°C), and hot (40°C) conditions for 2 h. The high (HMC) and low (LMC) lines were selected for high and low 24 h L* (lightness) respectively, and were formed from their random bred control line (RBC). Two replications of ten birds from each line were equally and randomly separated into control, heat stress (HS), and cold stress (CS) groups and were held at respective temperatures for approximately 2 h prior to processing. Measurements were collected for muscle pH at 15 min, 4 h, and 24 h postmortem, muscle color at 4 and 24 h postmortem, and fillet drip loss at 24 h postmortem (DL).

No line by treatment interactions were observed for DL or muscle pH. The HMC line had the greatest DL, no differences were observed between the RBC and LMC line. HS and CS treatment groups had reduced drip loss compared to the control group. For all muscle pH measurement times, the LMC line had the highest pH, and the HMC line had the lowest pH. No treatment differences were observed at 15 min pH, however the HS group had lower pH at 4 and 24 h.

A line by treatment interaction was present for all components of muscle color. For all treatment groups, the HMC line was highest for L* and lowest for a*, whereas the LMC line was lowest for L* and highest for a*. No significant line differences were observed for b* in the control group. In the HS treatment group, the HMC line was highest for b*. In the CS treatment group, the HMC line was highest for b*, and the LMC line was lowest. No treatment differences were observed for muscle color in the HMC and RBC lines. For the LMC line, the CS group had lower L* and b* and higher a*, whereas the HS group only had higher a* compared to the control group.

INTRODUCTION

For poultry growers and producers, strict environmental control is one of the most critical components of successful live production and plant operations. Conditions that are high priorities for producers can include lighting, air quality and ventilation, relative humidity, temperature, stocking density, litter quality, and bio-security. Failure to control any one of these can result in not just decreased animal health, welfare, or performance, but also result in product quality related issues such as footpad dermatitis, breast, wing, and hock burns, and carcass blemishes such as toenail scratches on legs of birds with inadequate feather coverage. Perhaps the most critical condition, but also the most difficult and expensive to control, is environmental temperature.

Two meat quality defects that is observed in both poultry and pigs that has been linked to pre-slaughter temperature is pale, soft, and exudative meat (PSE) and dark, firm, and dry meat (DFD). PSE meat is defined by a high rate of decline in postmortem pH while carcass temperatures are still relatively high resulting in protein denaturation and subsequent increased paleness and drip loss (DL) (Owens and Sams, 2000; Qiao et al., 2001). DFD meat occurs when postmortem pH decline is not rapid or severe enough for normal rigor development. The quality defects of PSE and DFD-like meat specifically involve changes in meat color, texture, water retention, and shelf-life that result from disruptions in postmortem chemical reactions that are essential to the rigor development and aging process of meat.

Generally, physiological factors affecting meat quality are stress related. The type, duration, and occurrence of stressors greatly determine whether PSE or DFD-like characteristics are present. PSE meat is typically caused by an acute pre-slaughter stress that increases the rate of glycolysis at the time of slaughter, whereas DFD is caused by chronic pre-slaughter stress that

increases the rate of glycolysis but to the point of glucose depletion prior to slaughter (Hedrick et al., 1989; McPhee and Trout, 1995; Lawrie, 1998; Owens and Sams, 2000; Petracci et al., 2001). It has been shown in swine, turkeys, and broilers that animals grown during summer seasons have higher incidences of PSE meat than in winter seasons (Santos et al., 1994; Mcurdy et al., 1996; Mckee and Sams, 1997; Petracci et al., 2004). Acute pre-slaughter heat stress has also been shown to increase the incidence of PSE meat in poultry (Northcutt et al., 1994; Holm and Fletcher, 1997; Aksit et al., 2006; Zhang et al., 2012). In contrast, other studies have shown that poultry exposed to cold temperatures prior to slaughter exhibit increased incidences of DFD meat (Froning et al., 1978; Babji et al., 1982; Holm and Fletcher, 1997; Bianchi et al., 2006; Dadgar et al., 2010; Dadgar et al., 2011).

It remains unclear what mechanisms have been changed in the HMC and LMC lines through selection for muscle color, and how the selected lines would respond to pre-slaughter stress such as environmental temperature. The purpose of this study was to compare the effects of pre-slaughter temperature and genetic selection for PSE/DFD conditions on meat quality and to determine if an environment by genotype interaction exists.

MATERIALS AND METHODS

With the transition from whole bird to further processed and ready to eat products, more attention has been focused towards product quality. Recently, a metabolic syndrome known as PSE-like (pale, soft, exudative) poultry meat has been impacting the broiler industry. PSE-like meat is associated with increased muscle color (L*), decreased water holding capacity, decreased tenderness, and the inability to form gels in further processed products. All of which have significant economic impact on the broiler industry. An increase in muscle color, L*, is a key visual indicator for breast fillets exhibiting PSE-like meat. Lines were tested for 24 h

postmortem muscle color using a Minolta CR-400 Colorimeter (Minolta Chroma Meter CR-400, Minolta Italia S.p.A., Milano, Italy) on a random bred broiler population processed at 56 days of age (Petracci et al., 2004). Based on those results, divergent selection was applied for muscle color in order to investigate the effects of selection for muscle color on product quality. However, evaluation of muscle color is a terminal measure; therefore, progeny must be tested from each sire in order to obtain sufficient information to make appropriate selection decisions in these lines. The following methods were developed to address the issues associated with selection for an invasive trait.

Animals

A random bred control line (RBC) and two broiler lines divergently selected for 24 h postmortem muscle color were evaluated for this study. The study consists of two hatches (two replications), birds were reared under normal industry conditions and were provided ad libitum consumption of water and a corn-soybean based broiler starter and grower ration that was formulated to meet or exceed National Research Council (NRC) (1994) requirements. At six weeks of age, twenty straight run birds of each line per treatment group were processed for the purpose of evaluating the effects of pre-slaughter temperature stress on meat quality. The day before processing, birds were assigned to control, heat stress (HS), and cold stress (CS) groups. 12 h prior to processing, feed was removed but water access was continued.

Temperature Stress & Slaughter

On the day of slaughter, birds were randomly selected and placed in one of the three experimental groups (control, HS, and CS). Treatment groups were held in respective pre-slaughter environments for 2 h prior to slaughter. HS birds were moved from rearing pens and placed in pre-heated pens at 40^{o}C (104^{o}F) and were given free access to water. CS birds were

placed 4 to a coop and placed in a walk-in refrigerator held at 0.5°C (33°F). Dividers were placed into CS coops so that birds were not able to huddle for warmth. Due to limitations in simulating treatment environments, the effect of cooping is confounded with cold stress. However, it was expected that limiting 4 birds per coop would alleviate the confounding cooping effects. Control birds were grouped and placed into the same pen and remained in the same house they were grown in. Temperature was maintained between 21-22°C (70-72°F). After 2 h of exposure to respective treatment temperatures, all treatment groups were sent to the plant for processing.

Upon arrival to the plant, birds were weighed, and hung on a shackle line and processed using in-line commercial equipment. Birds were electrically stunned (11V, 11 mA, 10), manually cut (severed left carotid artery and jugular vein), bled (1.5 min), scalded (55°C, 2 min) and picked with the use of in-line commercial de-feathering equipment. Equal numbers of birds from each treatment group were processed over 3 batches.

Meat Quality Measurements

Birds were eviscerated and evaluated for 15 min post-mortem muscle pH and then placed in a chill tank for 4 h (1°C). At 4 h post-mortem, fillets and tenders were deboned and weighed. Fillets were bagged in individual zip lock bags and refrigerated until 24 h postmortem at which time they were towel dried and reweighed for evaluation of drip loss. Muscle pH and L* value (lightness) were recorded on the left (pH) and right (color) breast fillets using the Testo 205 pH probe (Testo 205 pH Probe, Testo Limited, Hampshire, UK) and Minolta colorimeter at 4 and 24 h post mortem.

Statistical Analysis

The data in this trial were analyzed by the general linear model procedure of SAS software (SAS Institute, 1988). Means were separated using Least-Square means (LS means) with the Tukey adjustment. The model used in the analysis included line, treatment, and hatch effects. Batch effects were not initially detected; as a result batch was removed from the analysis.

RESULTS AND DISCUSSION

As previously mentioned, the HMC line selected for high 24 h L* and the LMC line selected for low 24 h L* were formed from the RBC line. The results of this study characterize the response to selection under controlled, hot, and cold pre-slaughter conditions.

Fillet Drip Loss (%) and Muscle pH

No line by treatment interactions were observed for DL or muscle pH. Means and standard errors for lines and treatment groups can be found on table 1. Consistent with observations made in chapters I and II, the HMC line had the highest DL. Although approaching significance, no differences were observed between the RBC and LMC line. The control group had the highest drip loss, no differences were observed between the HS and CS groups. For muscle pH, the LMC line was the highest and the HMC line was the lowest at 15 min, 4 h, and 24 h postmortem. No treatment differences were observed for 15 min pH, however the HS group was lowest for 4 and 24 h pH.

Muscle Color (L, a*, b*)*

A line by treatment interaction was observed for all muscle color values. Within-treatment line differences will be presented first and can be found on table 2. Within-line treatment differences will follow and are shown on table 3. All treatment groups were fairly

consistent for line differences, slight departures were observed for b* between treatment groups (Table 2). For all treatment groups, the HMC line was highest for L* and lowest for a*, whereas the LMC line was lowest for L* and highest for a* (Table 2). A slight departure from previous trials, no significant line differences were observed for b* in the control group. The HS and CS groups were similar in that the HMC line was highest for b*. However no differences were observed between the RBC and LMC line in the HS group whereas the LMC line was lowest in the CS group (Table 2).

No treatment differences were observed for muscle color in the HMC and RBC lines (Table 3). Only treatments within the LMC line appeared to differ. For 4 and 24 h L*, the CS group had the lowest L*, however no differences were observed between the HS and control groups. For 4 and 24 h a*, the HS and CS groups were higher than the control group, but did not differ from each other (Table 3). Similar to L*, the CS group had the lowest 4 and 24 h b*, but no differences were observed between the HS and control groups (Table 3).

Similar to results observed in chapters I and II, the pattern of line differences for DL, muscle pH, and muscle color were consistent with historic observations of these lines. Pre-slaughter temperature did not appear to have a qualitative effect on line differences as was also true for transportation stress (Chapter II). As expected the HMC line had the greatest DL. This has been consistently observed since approximately generations four or five. While the LMC line has never shown greater drip loss than the RBC line, significant differences between the two lines have not been as consistent despite large mean differences with the RBC line being the greater of the two. Slaughtering at heavier weights or measuring DL past 24 h postmortem may be needed to observe consistent significant differences between the RBC and LMC line. It is also possible that the initial population of birds used to create the LMC line already had

relatively low DL compared to other populations, so the ceiling for improvement in DL for the LMC line may already be reached. Future generations may be able to shed light on this matter. As expected the CS group had lower drip loss than the control. This is consistent with previous studies which demonstrated exposure to cold temperatures improved water holding capacity in turkeys and broilers (Lee et al., 1976; Froning et al., 1978; Babji et al., 1982, Holm and Fletcher, 1997; Bianchi et al., 2006; Dadgar et al., 2010; Dadgar et al., 2011). Unexpectedly, the HS also exhibited lower DL than the control. Broilers greatly depend on their ability to reduce elevated body temperatures by panting. Although access to water was available, it is possible that a large amount of moisture was lost through this process which could have reduced the amount of water available to be lost in the form of fillet drip loss. This is supported by previous studies in which a greater amount of live shrink was observed in broilers exposed to elevated pre-slaughter temperatures (Holm and Fletcher, 1997; Petracci et al., 2001). However, the effect of elevated temperatures on DL was not consistent across those same studies. It appears that the live shrink and PSE-like characteristics produced by heat stress could have counteractive effects in relation to fillet drip loss. As expected the HS group had lower 4 and 24 h pH and is consistent with previous studies (Northcutt et al., 1994; Holm and Fletcher, 1997; Aksit et al., 2006; Zhang et al., 2012). Although the CS group had higher means, cold exposure did not appear to affect muscle pH. Perhaps differences would have been detected if a larger sample size was used. It may also be possible that exposure to 1^{o}C temperatures may not have been sufficient to affect muscle pH in these lines. Dadgar et al. (2011) exposed birds to temperatures ranging from 22^{o}C to -17^{o}C and did not observe a change in ultimate pH until –ll^{o}C.

Surprisingly, no treatment differences were observed for muscle color in the HMC and RBC lines. However, differences were observed in the LMC line. Within the LMC line, an

increase in a* was observed in the HS group relative to the control. This has also been observed in a previous heat stress study involving broilers (Aksit et al., 2006). Also within the LMC line, the CS group relative to the control had lower L* and b*, and higher a*. This is also consistent with previous studies involving cold stress. (Dadgar et al., 2010; Dadgar et al., 2011). Although consistent patterns were emerging for line differences, heat stress appeared to have no detectable effect on any of the 3 lines with the exception of a* in the LMC line. The LMC line has a genetic tendency to express higher a* relative to the HMC and RBC lines which might explain why it was not observed in the HS groups of the HMC and RBC lines. Unlike previous studies where crating was used in addition to elevated temperatures, the HS group was exposed to elevated temperatures in a pen environment. This might have allowed birds to adequately lower body temperatures enough that muscle color differences were not detected. As a result, a sample size of ten birds may not have been adequate enough to detect what appeared to be large differences between the HS and control groups. With the exception of the LMC line, cold stress also did not appear to effect muscle color. Unfortunately, the coldest temperature that could be achieved for this study was only 1^{o}C. Perhaps colder temperatures would have produced more detectable results. What is believed to be the result of founder effect and not the response to selection for L*, the founding sub-population of the LMC line by chance happened to have a lower body weight mean compared to the base population. This has resulted in a consistent reduced body weight, relative to the HMC and RBC lines, by approximately 200g and has not increased since the formation of the lines. As a result the LMC birds used in this study were also approximately 200g lighter than the others. Perhaps these lighter weights could explain why the LMC line was more susceptible to the effects of cold stress at 1^{o}C.

Synthesis

Although mostly non-significant, it was very interesting that a consistent pattern appeared in this study similar to what was observed in chapter II. Strictly on the basis of numerical mean comparisons and not truly statistical comparisons, heat stress "appeared" to produce PSE-like effects on all three lines. Cold stress "appeared" to produce DFD-like effects on the RBC and LMC lines. In contrast, cold stress appeared to decrease muscle pH and increase L* and b* relative to the control. It was observed in chapter II that transportation stress produced a significant increase in PSE-like meat for the HMC line. It was postulated that the time required to deplete the HMC line of available substrate had been increased relative to the RBC and LMC line resulting in more PSE-like meat. A similar effect could be possible for the HMC line under cold stress and is supported by the conclusions of Lee et al. (1976). Lee et al. postulated that the observed increase in ultimate pH of cold stress birds was due to a decrease in substrate availability for postmortem metabolism. It stands to reason that an increase in total substrate availability in the HMC line could produce more PSE-like meat for a specific length of time of cold stress time that would normally create DFD-like meat in the RBC or LMC lines. Perhaps similar results could have been achieved in the HMC line with pre-slaughter cold stress if the temperature had been much lower thereby producing a more intense response.

With the exception of anecdotal evidence, temperature stress did not appear to produce any qualitative differences between the HMC, RBC and LMC lines in this study as transportation stress did appear in chapter II. The potential line differences in metabolic processes that were postulated to have occurred through the selection for L* will require more direct measurement of muscle substrates and metabolites before any specific conclusions can be made.

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Table 1. Comparisons of postmortem fillet drip loss (%) and muscle pH for line and treatment groups[1,2]

	Line			Treatment		
	HMC	RBC	LMC	HS	Control	CS
Drip Loss (%)	2.36 ± 0.14a	1.35 ± 0.13b	1.03 ± 0.13b	1.39 ± 0.11b	2.02 ± 0.14a	1.33 ± 0.15b
pH 15 min	5.99 ± 0.01c	6.10 ± 0.01b	6.21 ± 0.01a	6.08 ± 0.01	6.09 ± 0.02	6.13 ± 0.02
pH 4 h	5.57 ± 0.02c	5.84 ± 0.02b	6.08 ± 0.02a	5.76 ± 0.01b	5.84 ± 0.02a	5.89 ± 0.02a
pH 24 h	5.58 ± 0.02c	5.87 ± 0.01b	6.12 ± 0.01a	5.80 ± 0.01b	5.86 ± 0.02a	5.90 ± 0.02a

[1]Three lines of birds (HMC, RBC, LMC) were separated into heat stressed (HS), control, and cold stress (CS) treatment groups and evaluated for meat quality traits.

[2]No line by treatment interactions were present. Comparisons are made by line and treatment. Means with different letters are significantly different ($P<0.05$).

Table 2. Line comparisons for muscle color within heat stressed, control, and cold stressed groups[1,2]

	HS			Control		
	HMC	RBC	LMC	HMC	RBC	LMC
4 h L*	54.82 ± 0.48a	47.60 ± 0.42b	45.34 ± 0.41c	52.97 ± 0.55a	47.45 ± 0.58b	44.97 ± 0.58c
24 h L*	55.17 ± 0.47a	48.27 ± 0.42b	46.15 ± 0.41c	53.22 ± 0.54a	48.19 ± 0.57b	46.33 ± 0.57c
4 h a*	4.50 ± 0.17c	6.31 ± 0.15b	7.83 ± 0.15a	4.68 ± 0.20c	6.10 ± 0.20b	7.00 ± 0.20a
24 h a*	4.49 ± 0.17c	6.27 ± 0.15b	7.85 ± 0.15a	4.77 ± 0.20c	6.20 ± 0.21b	7.03 ± 0.21a
4 h b*	2.57 ± 0.23a	1.14 ± 0.20b	1.42 ± 0.20b	1.77 ± 0.26	1.04 ± 0.28	1.20 ± 0.28
24 h b*	2.94 ± 0.23a	1.48 ± 0.21b	1.95 ± 0.20b	1.89 ± 0.27	1.34 ± 0.28	1.75 ± 0.28

[1]Three lines of birds (LMC, RBC, HMC) were separated into heat stressed (HS), control, and cold stress (CS) treatment groups and evaluated for meat quality traits. Comparisons are made within treatment.
[2]Means with different letters are significantly different ($P<0.05$).

Table 2. (Cont.) Line comparisons for muscle color within heat stressed, control, and cold stressed groups[1,2]

	CS		
	HMC	RBC	LMC
4 h L*	53.80 ± 0.59a	45.94 ± 0.58b	42.42± 0.58c
24 h L*	54.10 ± 0.58a	46.65 ± 0.57b	43.42 ± 0.57c
4 h a*	5.07 ± 0.21c	6.20 ± 0.20b	8.24 ± 0.20a
24 h a*	5.08 ± 0.21c	6.36 ± 0.21b	8.32 ± 0.21a
4 h b*	2.14 ± 0.28a	0.79 ± 0.28b	0.24 ± 0.28c
24 h b*	2.34 ± 0.29a	0.97 ± 0.28b	0.56 ± 0.28c

[1]Three lines of birds (LMC, RBC, HMC) were separated into heat stressed (HS), control, and cold stress (CS) treatment groups and evaluated for meat quality traits. Comparisons are made within treatment.
[2]Means with different letters are significantly different ($P<0.05$).

Table 3. Treatment comparisons for muscle color within the HMC, RBC, and LMC lines[1,2]

	HMC			RBC		
	HS	Control	CS	HS	Control	CS
4 h L*	54.82 ± 0.48	52.97 ± 0.55	53.80 ± 0.59	47.60 ± 0.42	47.45 ± 0.58	45.94 ± 0.58
24 h L*	55.17 ± 0.47	53.22 ± 0.54	54.10 ± 0.58	48.27 ± 0.42	48.19 ± 0.57	46.65 ± 0.57
4 h a*	4.50 ± 0.17	4.68 ± 0.20	5.07 ± 0.21	6.31 ± 0.15	6.10 ± 0.20	6.20 ± 0.20
24 h a*	4.49 ± 0.17	4.77 ± 0.20	5.08 ± 0.21	6.27 ± 0.15	6.20 ± 0.21	6.36 ± 0.21
4 h b*	2.57 ± 0.23	1.77 ± 0.26	2.14 ± 0.28	1.14 ± 0.20	1.04 ± 0.28	0.79 ± 0.28
24 h b*	2.94 ± 0.23	1.89 ± 0.27	2.34 ± 0.29	1.48 ± 0.21	1.34 ± 0.28	0.97 ± 0.28

[1]Three lines of birds (LMC, RBC, HMC) were separated into heat stressed (HS), control, and cold stress (CS) treatment groups and evaluated for meat quality traits. Comparisons are made within line.

[2]Means with different letters are significantly different ($P<0.05$).

Table 3. (Cont.) Treatment comparisons for muscle color within the HMC, RBC, and LMC lines[1,2]

	LMC		
	HS	Control	CS
4 h L*	45.34 ± 0.41a	44.97 ± 0.58a	42.42± 0.58b
24 h L*	46.15 ± 0.41a	46.33 ± 0.57a	43.42 ± 0.57b
4 h a*	7.83 ± 0.15a	7.00 ± 0.20b	8.24 ± 0.20a
24 h a*	7.85 ± 0.15a	7.03 ± 0.21b	8.32 ± 0.21a
4 h b*	1.42 ± 0.20a	1.20 ± 0.28a	0.24 ± 0.28b
24 h b*	1.95 ± 0.20a	1.75 ± 0.28a	0.56 ± 0.28b

[1]Three lines of birds (LMC, RBC, HMC) were separated into heat stressed (HS), control, and cold stress (CS) treatment groups and evaluated for meat quality traits. Comparisons are made within line.
[2]Means with different letters are significantly different ($P<0.05$).

APPENDIX

UNIVERSITYOF

ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Nicholas Anthony
FROM: Craig N. Coon, Chairman Institutional Animal Care And Use Comittee

DATE: may 23, 2012
SUBJECT: IACUC PROTOCOL APPROVAL
Expiration date: April 6, 2015

The Institutional Animal Care and Use Committee (IACUC) has APPROVED Protocol #12038- "GENERAL REARING OF SELECTED CHICKEN AND QUAIL POPULATIONS ". You may begin this study immediately.

The IACUC encourages you to make sure that you are also in compliance with other UAF committees such as Biosafety, Toxic Substances and/or Radiation Safety if your project has components that fall under their purview.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes in the protocol during the research, please notify the IACUC in writing [Modification Request form] prior to initiating the changes. If the study period is expected to extend beyond 04-06-2015, you must submit a new protocol. 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 for research involving animal subjects.

ene/car

cc: Animal Welfare Veterinarian

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CONCLUSION

Poor muscle quality is a problem that is affecting the poultry industry and is a result of a combination of decades of intense selection for economically important traits and environmental factors. While integrators are beginning to see the importance of meat quality, selection practices remain the same. New meat quality issues are slowly forming and will not disappear as selection for growth rate, breast yield, and feed conversion continue. Effective methods have been identified for the detection and quantification of poor meat quality traits, as well as what environmental conditions are more likely to affect meat quality; however little is known about the genetic component of meat quality. Selection for muscle color over eight generations has resulted in muscle quality lines that have diverged for PSE and DFD-like characteristics. The purpose of this thesis was to characterize the concomitant physiological changes that have occurred with divergence. A better understanding of the physiological mechanisms resulting in PSE and DFD-like meat will better identify ways to improve meat quality on the primary breeder level.

Correlated response to selection for muscle color appears to include some blood chemistry traits. The extent to which these concomitant changes affect muscle color is not fully known. However, line differences for these correlated traits fit the pattern expected if they were to contribute an active role in muscle color determination. Substantial divergence has been achieved in these lines for muscle color, which has helped identify potential sources of muscle color variation. Sufficient identification and understanding of secondary sources of color variation will require continued selection and series of experimental observations.

Previous studies in broilers, turkeys and pigs have shown varying degrees of increased DFD-like meat incidence when animals have been exposed to transportation stress (Geers et al.,

1994; McPhee and Trout, 1995; Owens and Sams, 2000). Two possible reasons transportation stress tends to produce DFD-like meat is that it occurs over prolonged periods of time and the moving environment forces the animal to physically respond in order to maintain balance resulting in energy expenditure (Warriss et al., 1993; Dadgar et al.,2012). With that in mind, it was not unexpected to observe variation in the severity of response to transportation stress between lines. What was unexpected was how the HMC line became more PSE-like when exposed to transportation stress. It appears that selection for muscle color has changed the relationship between the duration of stress and the response to stress.

Temperature stress did not appear to produce any qualitative differences between the HMC, RBC and LMC lines in this study as transportation stress did. The potential line differences in metabolic processes that were postulated to have occurred through the selection for L* will require more direct measurement of muscle substrates and metabolites before any specific conclusions can be made. The results of this study suggest that improvement in meat quality can be made through stabilization selection as it not only increases uniformity for the selected trait, but may also result in more stress-tolerant populations thereby minimizing meat quality defects associated with multiple types of pre-slaughter stress.