EVALUATION OF DIFFERENCES IN NORMAL AND PALE BROILER BREAST MEAT THROUGH ANALYSIS OF IMPORTANT QUALITY ATTRIBUTES AND BY PROTEIN ANALYSIS

by

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(Under the Direction of Louise Wicker)

ABSTRACT

Recent research has shown that the poultry industry is facing similar problems with meat quality that resemble the pale,soft, and exudative (PSE) condition in pigs. Although the condition and its causes have been established in pork, the incidence in broiler breast meat has not been fully explained. Thus, the overall objective of this study was to investigate the occurrence of PSE in broilers by examining potential causes and quality indicators. Color and pH of boneless, skinless, broiler breast meat obtained from two commercial processing plants were measured. Production and processing factors for each sample were recorded and correlated to quality parameters. L* was heavily influenced by several factors such as grower, age, and bird weight. The research also showed an increase in L* compared to previous research, with a* having a greater correlation between the production and processing factors than L*. Color measurements, pH, and water holding capacity studies were completed to evaluate the impact selection for growth had on the quality of the meat. Results showed that the heavier birds with a higher growth rate exhibited a higher occurrence of pale birds than the lighter birds with a slower

growth rate. L* values greater than 60 were observed in 57% of birds selected for greater yield and in 26% of slower growing birds. Samples from normal and pale broiler breast fillets were analyzed using SDS-PAGE, water holding capacity and protein solubility studies. Results showed that pale broiler breast meat exhibited lower water holding capacity, protein solubility, and pH values. The presence and intensity of protein bands on SDS-PAGE were similar in water soluble and salt soluble extracts from pale and normal muscle. A peptide that migrated to the molecular weight of phosphorylase was consistently present in myofibrillar fractions of both normal and pale broiler breast fillets. Because there are no definitive solutions for eradicating the occurrence of PSE in broilers, NIRS was examined as a tool for rapidly determining water holding capacity. Results suggested that NIRS had the potential to predict water holding capacity in broiler breast meat.

INDEX WORDS: broiler growth rate, PSE, NIRS, water holding capacity, and gel electrophoresis

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A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2009

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DEDICATION

I dedicate this dissertation in loving memory of my parents, Edward and Lou Alice Samuel, who were always my biggest fans. Their undying love has propelled me to reach heights greater than I could have imagined. Their wisdom and quiet strength has taught me to endure hardness as a good soldier.

I also dedicate this work to my aunts, brothers, and sisters whose faith, love, and pride in me has encouraged me to go the distance. You all are the wind beneath my wings and I love you all.

ACKNOWLEDGEMENTS

I would like to thank all those who have helped me more than you know along this tedious journey. First of all, I would like to thank God Almighty without whom none of this would have been remotely possible. Secondly, I thank Dr. Wicker for her patience, direction, and commitment. Because of her, I am a better scholar and have learned to always dig deeper and give more than is expected. I also would like to thank the rest of my committee: Dr. Harrison for challenging me to be a better scholar and for his jokes; Dr. Park for introducing me to a new subject matter; Dr. Pringle for his shoulder and encouragement; and Dr. Shewfelt for always giving me a positive outlook, making my transition here at UGA easier, and for his willingness to help in any way. I would also like to thank Dr. Estes Reynolds who saw something in me and decided to extend his helping hand and point me in the right direction.

I would like to thank the following for their prayers and spiritual support: Believer's Christian Fellowship, Grace Fellowship, Greater Highway Church of Christ, Mt. Zion FBH, Mother Dear (Ollie Allgood), Sandra Reaves, The Bethunes, Rev. Sidney and Greta Samuel, Min. Linda Samuel, Min. Vincent and Carolyn Samuel, Marie Durant, Alma Samuel, Miriam and Lafon Wall, Deborah Fulton, Charles and Nicole Cooley, Bobby and Geneen McRae, Schreeda Johnson, The Moss Family, Aunt Hannah, and Aunt Fannie.

To the rest of you here at UGA, thanks for making my burdens lighter and my days brighter: Anne Morrison, Josh Rivner, Maruj and Ann, Enyam, Brenda, Tina, Priyadarshi, Lashkme, Lindsey, Jiyoung, Abe, Vicki, Tamar, Julie, Karen, and Solandre.

Special thanks to Mr. Scott who allowed me to focus on my work while he took on my headaches in SC. Last but not least, I am forever grateful to Dr. Linda Samuel who walked side by side with me on this journey.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

Due to an increased requirement of consumers for processed poultry products versus whole birds, geneticists have introduced new lines of birds selected for increased yield of breast as well as thigh muscle. Poultry selection has changed on a continual basis since the early 1970's. This continual change has resulted in a very large increase in growth rate of chickens and turkeys. The focus of this poultry selection has been primarily on the reduction of breeding costs while improving production efficiency. The impact of poultry selection on meat quality has been neglected (Remignon and Le Bihan-Duval, 2003).

The selection for growth rate has resulted in an alteration in the processing quality of poultry meat. This is evidenced by changes in carcass composition and meat quality such as increased muscle glycolytic activity, lower water holding capacity and higher meat hardness (Piles et al., 2000; Ramirez et al., 2004). Remignon and Le Bihan-Duval (2003) states that color and water holding capacity are frequently reported as being poorer in modern poultry flocks. These two factors are known to influence perceptions of the acceptability of the meat product although they vary with the species, muscle function, age of the animal and storage conditions (Berri, 2000). Fletcher (1999) found significant variations in breast meat lightness within five commercial broiler-processing plants which would indicate variations at the retail level also.

Barbut et al. (2008) states increased selection for growth has induced more stress on birds and has possibly resulted in an increase in pale, soft, and exudative (PSE) meat among poultry.

Thus, the objectives of this research are: (1) to examine the existence of PSE among broilers and the factors influencing it; (2) to determine the impact of growth selection on the incidence of pale meat among broilers; (3) to identify quality indicators and protein differences between normal and pale broiler breast meat; and (4) to identify a rapid method of detection of pale broiler breast meat.

History of Poultry

During the 20th century, poultry has evolved from being a secondary, small scale, and local agricultural commodity to a vertically integrated, international food products industry. During this period, the industry has grown to meet the growing demand for poultry meat driven by strong economic incentives of low cost and special market advantages (Fletcher, 2003). Per capita consumption of poultry has increased from 41.1 pounds in 1965 to 104.9 pounds in 2006 (http://www.meatami.com/). Marketing has also changed dramatically. At the turn of the last century, most poultry were either marketed live, were slaughtered at time of purchase, or were slaughtered and sent to market as New York Dressed (NYD) carcasses. NYD carcasses were the dominant market form through World War II and into the early 1950's. By the early 1950's the dominant market form in the United States shifted to the ready-to-cook (RTC) carcass. In the 1970's the market began to shift to more cut-up, deboned, and further processed products. Prior to 1970, approximately 80% of broilers were marketed as whole RTC carcasses. However, from 1975 to present the market share has changed dramatically. In 1975 approximately 60% of the broilers were marketed whole, 30% cut-up, and less than 10% as furthered processed as

compared to 15%, 50%, and35%, respectively, today. This change can be attributed to the consumer's preference for white meat as opposed to dark meat. This preference created a demand for the breast portion (Fletcher, 2003).

The American Meat Institute (2003) reported that the meat and poultry industry is the largest segment of the United States agriculture. Total meat and poultry production in 2007 reached more than 89 billion pounds in the United States. Live weight broiler production was 49.2 billion pounds with a production value of $21.5 billion (Agricultural Statistics Board, NASS, USDA). The American Meat Institute (2003) also reported that Americans are not the only people benefiting from the most bountiful supply of agricultural commodities on earth. Meat and poultry products represent America's top agricultural export and account for 8 percent of the total U.S. meat production. Meat and poultry production and consumption statistics illustrate the impressive size and scope of the industry. Bilgili (2001) reports that broiler supply and demand is expected to grow more internationally, especially for frozen whole birds, parts, paws, bone-in-leg quarters, and boneless dark meat, driven primarily by large fast-food chains. According to the U.S. Poultry & Egg Association, U.S. broiler exports totaled approximately 5.8 billion pounds in 2007, equating to approximately twelve percent of the total broiler production (http://www.poultryegg.org/economic_data/).

Haley (2001) stated that the substitution of poultry meat in place of beef by U.S. consumers is the most significant change that has occurred since 1970. Olentine (2003) agrees that poultry meat is the most popular meat today. The dramatic increase in poultry consumption over the past 50 years can be attributed to the following: economics, consumer perceptions, consumer eating habits, and marketing. Not only has poultry become inexpensive compared to other meats since WWII, but it is perceived as the healthier choice. Consumer eating habits have

resulted in a large food processing and institutional feeding system that uses portion control and cost accounting to meet consumer demands for desirable yet economical foods. Poultry has been shown to be very well adapted to these changes based on composition and economics and marketing has reflected these changes in consumer eating and purchasing patterns.

Conversion of Muscle to Meat

Before defining meat quality, the conversion of muscle to meat must be examined. There are several reactions that occur in this process that fall under one of the following: biochemical, molecular, physiological, and structural.

Biochemical

After slaughter, myofibrillar contraction takes place. In order for contraction to take place, high-energy phosphate compounds must be synthesized and degraded. These phosphate compounds present are adenosine triphosphate (ATP), adenosine diphosphate (ADP), and creatine phosphate (CP). They provide the required energy sources for muscle contraction. Contraction is brought on when the ATP is converted to ADP and the actin and myosin forms a rigor or atomyosin bond. These bonds are only broken when there is ATP present. When they are broken, additional contraction occurs. Myofibrillar ATP is produced from glycolysis and is necessary for postmortem or anaerobic muscle contraction. In postmortem contraction, lactic acid accumulates and reduces the localized pH. Thus, muscle is converted to meat. The pH will continue to lower until all ATP is depleted or the contractile proteins can no longer function (Berg, 2001). Greaser (1986) states that the length of time until rigor is completed varies with

species, muscle, fiber type, holding temperature, rate of glycolysis and the extent of struggle at the time of death.

Although postmortem changes that occur during the conversion of muscle to meat are similar between avian and mammalian species, the rate of glycolysis and rigor mortis occurs at a significantly faster rate in poultry (Addis, 1986; Grey and Jones, 1977; Grey et al., 1974). Within poultry, the completion of rigor occurs in approximately 1 hour (Dransfield and Sosnicki, 1999) and Ma and Addis (1973) reported that the pH decline in turkey breast muscle can be faster than that found in the most severe cases of PSE pork muscle. Dransfield and Sosnicki (1999) associated the differences with the higher content of white muscle fiber found in poultry. White muscles fibers are anaerobic (Wiskus et al., 1976).

Physiological

Muscle fiber types are identified by differences in metabolic activity and are classified as either fast-twitch or slow-twitch (Foegeding, 1996). Fast-twitch muscles are considered the white fiber muscles and require a rapid source of energy utilizing glycolysis as its predominant pathway. Slow-twitch muscles are associated with an oxidative metabolism which requires higher amounts of myoglobin for oxygen storage (Foegeding, 1996). For example, legs and thighs are the muscles used for locomotion and they have greater aerobic endurance, more myoglobin, and higher concentration of mitochondria. They are also darker in color. The breast muscle is higher in glycogen, but lower in mitochondria and myoglobin. Thus, the breast meat is lighter in color and more suited for short bursts of power.

Structural

Protein, fat, and water are the three main components that contribute to the overall flavor, texture and palatability of meat. Protein plays a significant role in the contraction of muscles and subsequently influences the tenderness of the meat and its ability to retain moisture. Water in the muscle also influences the retention of moisture because it bonds to the molecules in the muscle and can get trapped between the thick and thin filaments. Expulsion of water occurs as the space between the two filaments is reduced. The last component, fat, contributes to the unique flavor, juiciness, and perception of tenderness (Berg, 2001).

Pre-slaughter Handling and Stress Factors

Much research has been done on the study of stress and its relation to the quality of broiler meat. Stress occurs when the birds are exposed to an external stimuli or unsettling circumstances that are adverse or disruptive. As a result, there is a cascade of reactions that take place in the broilers. The physiological response to stress is controlled by the autonomic nervous system which consists of the sympathetic and para sympathetic nervous systems. The combined systems balance the response and recovery from the stress exerted on the broiler (Berg, 2001).

While sympathetic nervous system mediates fight, fright, and flight, the parasympathetic nervous system mediates calm, vegetative activities. The parasympathetic nervous system promotes growth, energy storage, digestion, absorption and tissue repair. The sympathetic nervous system is responsible for speeding up the heart rate while the parasympathetic works to slow it down. While the sympathetic moves energy from storage, the parasympathetic works in the opposite direction. The two components of the nervous system work in balance of each other (Berg, 2001).

The sympathetic nervous system is associated with vigilance, arousal, activation, and mobilization and can be characterized as the physiological response of fight, fright, and flight. When stimulated by stress, the sympathetic nervous system rapidly generates energy while simultaneously inhibiting energy storage, digestion, and immune function. The heart rate and blood pressure rises along with increased muscular tension. This is caused by the epinephrine released from the adrenal medulla in response to the activation of the sympathetic nervous system. The epinephrine is also responsible for the stimulation of glycogenolysis and lipolysis which mobilizes glucose. With the increase in blood flow, lactic acid is removed and heat is dissipated. An increase in the temperature of the muscles causes the following: increases the rate of shortening, increases contractile force, increases maximum tension, and enhances Ca^{2+} sensitivity of the contractile proteins. These are the responses caused by the sympathetic nervous system and are in direct contrast to the parasympathetic nervous system (Berg, 2001).

It is evident that there are a host of reactions taking place when the broilers are exposed to stress or external stimuli that is disruptive or adverse. Bramwell (2000) list key stressors as follows:

1. Transportation or hauling- Chickens are transported from the farm to the processing facility. This process can be one of the leading causes of stress in commercial birds.
2. Environmental temperatures-The chickens can become stressed when exposed to extreme conditions in the weather. Extremely hot and cold temperatures can contribute to stress.
3. Feed and water- Stress can result from temporary shortages, especially during warm or hot weather.
4. Changes in feeds or feeding methods – Stress may be induced when there are changes in the daily routine. This can be prevented if proper precautions are taken.

5. Poor nutrition- Inadequate diets can result in unhealthy chickens.
6. Overcrowding- Stress takes place when the birds have to fight to eat or drink. Lack of food and water is not conducive to weight gains and production.
7. Physical disturbances- Excessive noise or aggravated activity causes the birds to be nervous. The stress is heightened when the disturbance is sporadic, sudden and without warning.

Meat Quality Attributes

Although quality is the consumer's perspective, it can also be defined in market terms by the attributes by which similar products can be differentiated for purposes of price or preference (Fletcher, 2003). For broiler meat, those attributes would be tenderness, juiciness, color, and flavor which may be heavily influenced by changes that occur during the conversion process of muscle to meat (Aberle et al., 2001). Thus, it is critical that these factors be controlled to provide the best quality of the meat.

Color

The primary heme pigments found in poultry muscle include myoglobin, hemoglobin, and cytochrome c (Lawrie, 1998). Myoglobin, which is in the sarcoplasmic fraction, is the principal heme pigment found in poultry meat that contributes most to meat color (Froning, 1995). Hemoglobin is the blood pigment which, along with myoglobin, complex with oxygen in the metabolic system of the live animal. Hemoglobin acts as a carrier of oxygen in the blood, and myoglobin serves as a mechanism for oxygen storage in the muscle cell (Lawrie, 1998). The appearance of poultry meat is largely influenced by the quantity of myoglobin, the chemical state

of myoglobin and the chemical and physical state of other meat components (Lawrie, 1998). Nevertheless, in a well bled bird, 20 to 30% of the hemoglobin is still present, and it may play a significant role in defining color (Froning, 1995). Overall, color problems may relate to age of the bird, preslaughter stress, slaughter procedures, endpoint cooking temperatures, additives, both intentional and unintentional, and other further processing parameters (Froning, 1995). Conforth (1995) states that color is the most important attribute by which consumers determine the product acceptability or meat quality. If a product does not look good or is not appealing to the consumer eye, the consumer is not going to buy it. If the consumer does not buy the meat, then all other attributes are useless. The appearance of the meat is synonymous with color (Conforth, 1995). In order to understand the causes of color defects in poultry, one must first understand the origin of meat color. As reported in Ahn and Maurer (1989), meat color is a result of the concentration of heme pigments, reactions of the pigments with gaseous elements or compounds, and the structural properties of muscle protein. The most important meat pigment is myoglobin (Mb). The amount of Mb in meat can vary greatly by kind of animal, age, sex, and muscle types within a carcass (Fronin, et al., 1968; Ginger et al., 1954; Nocito et al., 1973; Rickansrud and Henrickson, 1967). Fleming et al. (1960) reported that in a well bled beef rib eye muscle, as much as 95% or more of the pigment is accounted for by Mb. Ahn and Maurer (1989) reported that the combined hemoglobin (Hb) and Mb in turkey breast and leg meat were 0.75 mg/g and 2.65 mg/g meat, respectively. The amount of Hb can vary depending on bleed out, slaughter method, and preslaughter conditions.

Tenderness

Maltin et al., (2003) states that tenderness is a very important element of eating quality and that variations in tenderness affect the decision to re-purchase. The most important factors affecting tenderness are those involved in postmortem: temperature, sarcomere length and proteolysis (Maltin et al., 2003). Several researchers have associated tenderness of meat with the breakdown of myofibrillar proteins affected by the presence of calcium-dependent proteases or calpains (Boehm et al., 1998; Claeys et al., 2001; Geesink, et al., 2000; Huff-Lonergan, et al., 1996; Koohmaraie, 1996; Morton et al., 1999; Ouali, 1990). Hwang et al. (2003) also found that rapid cooling of meat with a relatively high pH resulted in tougher meat due to contraction of the sarcomeres and by altering the calpain activity. Slaughter and processing also have an impact on the tenderness of the meat (Fletcher, 2003). Whether or not poultry meat is tender also depends upon the rate and extent of the chemical and physical changes discussed earlier in conversion of muscle to meat. After an animal is slaughtered, there is no source of oxygen or nutrients being supplied to the muscles. Thus, the muscles run out of energy and began to contract and become stiff (rigor mortis). Eventually the muscles will relax and cause the meat to be tender when cooked. This process of rigor mortis can be affected by ante mortem stress. Birds that struggle before or during slaughter expend their energy quicker and cause rigor mortis to set in faster than normal. This increases the toughness of the meat. Also, Fletcher (2002) states that if meat is deboned before completion of rigor mortis, the meat will be tough due to the contraction of muscle fibers and the shortening of the muscle. Young et al. (1996) found that longer stunning times resulted in greater shear values. Papinaho and Fletcher (1996) also reported that longer stunning times resulted in tougher meat.

Tenderness can also be affected by breed and strain. Certain breeds and strains have larger muscles (Fletcher, 2003). Lawrie (1998) stated that within a breed tenderness is heritable to an extent of over 60 percent. Age and maturity also affect tenderness due to the collagen content and the change in muscles (red fibers change to white fibers). An increase in age results in a decrease in tenderness due to the fact that younger animals have less cross-bonding in their connective tissue (Lawrie, 1998). Aberle et al. (2001) states that larger bundles of muscle fibers and large amounts of perimysial connective tissue surrounding primary and secondary bundles are associated with coarse-textured meat. Lawrie (1998) also states that species is one of the most general factors affecting tenderness. Cattle were found to be tougher than sheep and pig due to its larger size. In addition to species, sex was also determined to be a factor. Tougher meat was associated with male animals (Lawrie, 1998). However, caponization of male birds was found to make the meat tender (Fletcher, 2003). Miguel et al. (2008) found that capon meat showed a higher fat content than that of cocks, making it juicier and less fibrous. In addition, castration resulted in more tender thigh and drumstick meat.

Juiciness

Juiciness is also known as the sensation of moisture that occurs at early mastication of the meat. It is indirectly related to texture and flavor and highly correlates to fat and water content (Fletcher, 2003). Juiciness also contributes to mouth feel because it keeps the mouth moist during chewing due to the rapid release of meat fluid (Lawrie, 1998). The biggest factor contributing to juiciness is the pH of the meat. The pH of the meat correlates to the water holding capacity of the meat. Low pH meat results in excessive cook loss. Yield is reduced and the meat is dryer. Higher pH meat delivers a better cooking yield as well as a juicier product

(Fletcher, 2003). The pH of the meat is directly related to the changes occurring in the conversion of meat to muscle.

Flavor

Lindsay (1996) states that the term "flavor" has evolved to a usage that implies an overall integrated perception of all of the contributing senses (smell, taste, sight, feeling, and sound) at the time food consumption. Fletcher (2003) defines the components that make up flavor as taste, aroma, body (similar to texture), and mouthfeel. The purpose of flavor is to deliver the expected flavor and avoid any distinct off-flavor defect. Smell or odor is thought to be the most important of them all (Lawrie, 1998). Northcutt (1997) states that flavor is the least important of the quality attributes unless an off-flavor defect is present. The factors that affect flavor are minimal during production and processing. Other factors include bird strain, diet, environmental conditions (litter, ventilation, etc.), scalding temperatures, chilling, product packaging, and storage. However, the impact of these factors is not great enough for the consumer to identify (Northcutt, 2000).

In summary, the quality of broiler meat is affected by several factors. However, it is important to understand all physical and chemical changes that occur during the conversion of meat to muscle. The understanding of this process further enables one to understand how conditions prior to slaughter as well as during the production process impact the overall quality of the end product.

Water Holding Capacity

Huff-Lonergan and Lonergan (2005) state that unacceptable water-holding capacity costs the meat industry millions of dollars annually. Aberle et al. (2001) describes water holding capacity as the ability to retain naturally occurring or added water during application of external forces such as cutting, heating, grinding, or pressing. Muscle tissue consists of approximately 75% water, 20% protein, 5% fat, 1% carbohydrates, and 1.5% vitamins and minerals (Huff-Lonergan and Lonergan, 2005). Belitz et al. (2004) states that approximately 5% of the water found in muscle is bound by hydrophilic groups on the proteins and the other 95% is held by capillary forces between the thick and thin filaments. Some of the water in muscle is also found in free form and is expelled during even the mildest form of applications. The ability of meat to retain water is influenced by several factors. Those factors include production of lactic acid, loss of ATP, onset of rigor mortis, and changes of cell structure associated with proteolytic enzyme activity (Aberle et al., 2001; Huff-Lonergan and Lonergan, 2005). Many of the quality attributes of meat such as color, texture, and juiciness are influenced by water-holding capacity (Aberle et al., 2001).

As postmortem changes occur, there is an increase in lactic acid which causes a reduction in the pH of the meat. As the pH approaches the isoelectric point (pI of myosin is approximately 5.4) of the myofibrillar proteins, the net charge of the proteins becomes zero. With a net charge of zero, maximum attraction between oppositely charged residues minimize the amount of water that can be held and attracted by the protein (Aberle et al., 2001; Huff-Lonergan and Lonergan, 2005). With a net charge effect, the end result is a more closely packed structure with reduced space within the myofibril.

In addition to the drop in pH, changes associated with the onset of rigor mortis are also responsible for water-holding capacity (Aberle et al., 2001). With the breakdown of ATP and the formation of actomyosin during rigor mortis, a steric effect occurs where there is a reduction in the space of the myofibrils. Water is forced fromthe intracellular spaces to the extramyofibrillar spaces where the fluid can be more easily expelled (Huff-Lonergan and Lonergan, 2005). The reduction in space is also attributed to the shortening of the sarcomere as Honikel et al. (1986) has reported that there is a linear increase in drip loss as sarcomeres decrease in length. In addition, the shrinkage of the cell has also been associated to the proteolysis or degradation of the cytoskeletal proteins by calpain proteinases. Huff-Lonergan and Lonergan (2005) reports that reduced degradation of proteins like desmin, results in increased shrinkage and thus, more drip loss. However, proteolysis is accompanied by increased oxidation of myofibrillar proteins (Martinaud et al., 1997) which may lead to inactivation or modification of calpain activity (Huff-Lonergan and Lonergan, 2005). Guttmann and Johnson (1998) found that oxidation of myofibrillar proteins inhibits proteolysis by μ-calpain.

Pale, Soft, and Exudative Broiler Meat

The success of the poultry industry has been fueled by the ability to economically produce acceptable products. This accomplishment has been achieved through high selection intensities, shorter generation intervals and reduced environmental influences. The result is reduced slaughter age and increased body weight at specific ages. Muscle yields and feed conversion have also improved. Unfortunately, genetic progress has resulted in a reduction of meat quality associated with muscle texture and flavor (Anthony, 1998). Recent research has shown that the poultry industry is facing similar problems with meat quality that resemble the

PSE condition in pigs (Barbut, 1997; Owens et al., 2000; Woelfel et al., 2002) In contrast to pigs, the genetic basis of meat quality alterations in poultry is not fully established (von Lengerken, 2002).

Pale, soft, and exudative breast meat purportedly results from a rapid postmortem pH decline while carcass temperatures are still warm. The resulting protein denaturation leads to pale color, decrease in water-holding capacity, and excessive yield losses (Alvarado and Sams, 2003). PSE incidence in broilers and turkeys has been reported to range from 5 up to 50% in commercial plants (Barbut, 1996; Barbut, 1997; McCurdy et al., 1996; Owens et al., 2000; Woelfel et al., 2002; Woelfel and Sams, 2001) PSE is one of the major contributors to decreased water holding capacity and can cost the poultry industry millions of dollars annually (Pietrzak et al., 1997).

The first reports of a PSE-like condition in poultry were reported in the early 1970's (Aberle et al., 1971; Vanderstoep and Richards, 1974). Only within the last decade has more research focusing on the PSE condition in poultry emerged (Barbut et al., 2008). Vanderstoep and Richards (1974) reported that the postmortem changes in the fast glycolyzing muscles of turkeys were similar to the rapid pH drop leading to reduced protein functionality seen in PSE pork. This phenomenom was not studied until 15-20 years later where Sante et al., (1991) examined the differences between fast- and slow-growing breeds of turkeys. Afterward, a series of studies by other researchers were conducted to determine the occurrence of PSE meat in turkeys and broilers. The following table shows the estimated occurrence, species, L*, and researchers.

Table 1.1. Reported incidence of pale, soft, and exudative meat in broilers and turkeys

Researcher	Poultry Type	L*	Occurrence (%)
Barbut 1996	turkeys	L>50	18-34
McCurdy et al 1996	turkeys	L>50.5	5-30
Barbut 1997	turkeys	L>49	0-28
Wilkins et al 2000	broilers	L>56	~25
Petracci et al 2004	broilers	L>56	2.7-15.5

From these studies, it was determined that pale meat showed correlations with water holding capacity, pH, color, and texture. In addition, higher incidences of pale, soft, and exudative meat were observed during the summer months, indicating that heat stress was a factor (Barbut et al., 2008).

Once occurrences of PSE meat in poultry were reported, researchers began to investigate the causes to see if they were related to genetic differences like those found in pork. One of the major contributors to the development of PSE in pork has been the Porcine Stress Syndrome which is linked to a single point mutation in the sarcoplasmic reticulum (SR) calcium release channel ryanodine receptor gene (RYR1) (Barbut et al., 2008). The RYR1 gene is commonly called the Halothane gene because the use of HAL-1843® has allowed for the detection of this mutation in pork. This gene has subsequently provided pig breeders with a way to limit the incidence of PSE in pork (Barbut et al., 2008)

Pietrzak et al. (1997) examined the effect of rapid rigor mortis processes on protein functionality in domestic turkeys using a combination of biochemical, meat quality, microscopic and gel electrophoresis techniques. Results showed that phosphorylase became tightly associated with the myofibrils in muscles from the PSE group and that less myosin could be solubilized from PSE muscle than from normal muscle in turkeys. They concluded that the similar rapid postmortem glycolysis in pigs and turkeys suggests that there may be a defect in either or both of the turkey skeletal muscle ryanodine receptors. Thus, genetic testing would be beneficial. Wang

et al. (1999) examined the differences in skeletal muscle calcium channel ryanodine binding activity between genetically unimproved and commercial turkey populations through use of SDS PAGE. Results suggested that altered SR calcium channel protein activity, or altered channel regulation, may be associated with the increased incidence of PSE meat from turkeys selected for growth characteristics. These findings were in agreement with Mickelson et al. (1988). They found that purified SR from genetically defined stress-susceptible pigs bound RYR three times more than normal pigs. In spite of the similarities shown here to pigs, Owens et al. (2000b) showed that halothane response was a limited predictor of PSE meat in turkeys and there was not a higher incidence of PSE meat in halothane sensitive birds when slaughtered at 20 weeks of age. However, Chiang et al. (2004) did report that a homozygous *αRYR-II (a genomic DNA allele)* genotype of turkey exhibited a significantly higher postmortem pH15 and a better water-holding capacity than the *α-RYR-I* genotype.

Currently, the poultry industry has not identified a reliable genetic marker responsible for PSE meat but continues to search for indicators to minimize production losses resulting from PSE meat. Therefore, several hypotheses should be taken into consideration for future research into the PSE condition within broilers (Barbut et al., 2008). Boulianne and King (1995) found that color characteristics (*L, a*, and *b*), pH, total pigment, myoglobin, and iron concentrations were all significantly different in pale meat when compared with normal chicken breast meat. Dransfield and Sosnicki (1999) suggested that higher growth rates may induce morphological abnormalities, larger fiber diameters and a higher proportion of glycolytic fibers, and a lower proteolytic potential in the muscles; and subsequently increase the occurrence of paler color, reduced water holding capacity, and poorer quality of further processed products. Additionally,

preslaughter environmental conditions have shown to be major contributors to the increased incidence of PSE meat in poultry (Berri et al., 2005; Debut et al., 2005; McKee and Sams, 1997).

Methods for Rapid Detection of PSE Meat

The meat industry has seen increased interest in rapid screening techniques used to determine quality characteristics (Geesink et al., 2003). Spectroscopy has become a more desirable method for analyzing qualitative characteristics in food due to a decrease in instrument prices, an improvement in equipment design, and an improvement in data-analysis methodology such as chemometrics (Brondum et al., 2000). Brondom et al. (2000) lists the main advantages of using spectroscopic measurements as rapid data acquisition, the possibilities for simultaneous determination of several quality parameters and the ability to replace expensive and slower reference techniques such as centrifugation for WHC and Warner-Bratzler shear tests for measuring tenderness. Monin (1998) considered near infrared spectroscopy (NIRS) to be one of the most promising of these techniques for large-scale meat quality evaluation.

The use of NIRS as a qualitative and quantitative measurement tool dates back to the 1950's (Benson, 1993). Several applications have been applied to the meat industry. Research involving NIRS and meat include fat and moisture contents in emulsions of meat products (Ben-Gera and Norris, 1968; Iwamoto et al., 1981; Kruggel et al., 1981; Lanza, 1983); fat, moisture, and protein contents (Berzaghi et al., 2005; Isaksson et al., 1995; Kadim et al., 2005; Renden et al., 1986; Valdes and Summers, 1986); fat depths and softness (Swatland, 1995); and meat texture and tenderness (Liu et al., 2004; Lyon et al., 2001; Muellenet et al., 2004; Park et al., 2001). NIRS has also been utilized to evaluate pigment content in meat (Chen and Massie, 1993; Chen et al., 1994; Chen et al., 1996b; Chen et al., 1996a; Mitsumoto et al., 1991). In a review of

NIRS for on/in-line monitoring of quality in foods and beverages, Huang et al. (2008) concluded that NIRS analysis is capable of rapid assessment of fat, water, protein, and other parameters in meat. In addition, Cozzolino and Murray (2004) used NIRS to correctly classify 80% of their meat samples according to the muscle species (beef, lamb, pork, or chicken). Muellenet et al. (2004) suggested that near infrared reflectance could be used to predict poultry meat texture and to classify muscles according to tenderness levels. Park et al. (1998) used NIRS to predict Warner-Bratzler shear force values of beef longissimus steaks.

More recent applications involved the determination of water binding characteristics, water holding capacity and drip loss in fresh pork (Forest et al., 2000). Forrest et al. (2000) developed a partial least squares regression (PLSR) - based prediction model for drip loss from pork longissimus. The model showed strong correlation between early post mortem NIR spectra and measured drip loss during post rigor storage. Results indicated that the technology could be used to predict drip loss 24 h after slaughter. Geesink et al. (2003) and Hoving-Bolink et al., (2005) also studied NIRS as a predictor of pork quality. Although Hoving-Bolink et al. (2005) found NIRS to be a better predictor of intra-muscular fat than of drip loss, Geesink et al. (2003) results showed the direct opposite. Their results indicated that NIRS does enable the classification of pork longissimus muscles with a superior or inferior water-holding capacity. Overall, NIRS has shown great potential as a tool for rapid assessment of meat quality.

In conclusion, the existence of pale, soft and exudative meat and its causes has been established in pork while the incidence in broiler meat has not been fully explained. There are several factors that influence meat quality and the purpose of this research is to investigate the incidence of a PSE-like meat in broilers and examine changes in proteins that may be responsible for the condition. In addition, a tool for rapid assessment of meat quality will be evaluated in an

effort to provide a short term solution to minimize yield losses resulting from this condition of a PSE-like meat.

REFERENCES

Aberle, E. D., J. C. Forrest, D. E. Gerrard, and E. W. Mills. 2001. Principles of Meat Science. pp 83-116. Fourth ed. Kendall/Hunt Publishing Company, Dubuque, Iowa.

Aberle, E. D., W.J. Stadelman, G.L. Zachariah, & C.G. Haugh. 1971. Impedance of turkey msucle: relation to post mortem metabolites and tenderness. Poult. Sci. 50:746-749.

Addis, P. B. 1986. Poultry muscle as food. Pages 371-404 in Muscle as Food. P. J. Bechtel ed. Academic Press, New York, N.Y.

Agricultural Statistical Board. 2008. Poultry-Production and Value 2007 Summary. N. A. S. S. USDA. http://www.nass.usda.gov/ accessed 2008.

Ahn, D. U., and A. Maurer. 1989. Effects of sodium chloride, phosphate, and dextrose on the heat stability of purified myoglobin, hemoglobin, and cytochrome c. Poult. Sci. 68:1218-1225.

Alvarado, C. Z. and A.R.. Sams. 2003. Injection marination strategies for remediation of pale, exudative broiler breast meat. Poultry Sci. 82:1332-1336.

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

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

Barbut, S. 1997. Occurrence of pale soft exudative meat in mature turkey hens. Br. Poult. Sci. 38:74-77.

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

Belitz, H. D., W. Grosch, and P. Schieberle. 2004. Meat. Pages 566-618 *in:* Food Chemistry. Springer-Verlag, Germany.

Ben-Gera, I., and K. H. Norris. 1968. Direct spectrophotometric determination of fat and moisture in meat products. J. Food Sci. 33:64.

Benson, I. B. 1993. Compositional analysis using near infrared absorption analysis. Pages 121-166. *in* Instrumentation and Sensors for the Food Industry. E. Kress-Rogers ed. Butterworth-Heinemann Ltd.

Berg, E. P. 2001. Influence of stress on composition and quality of meat, poultry and meat products.http://www.fass.org/fass01/pdf/Berg.pdf, University of Missouri. Accessed 2004.

Berri, C. 2000. Variability of sensory and processing qualities of poultry meat. World's Poult. Sci. J. 56:209-224.

Berri, C., M. Debut, V. Sante-Lhoutellier, C. Arnould, B. Boutten, N. Sellier, E. Baeza, N. Jehl, Y. Jego, M. J. Duclos, and E. Le Bihan-Duval. 2005. Variations in chicken breast meat quality: implications of struggle and muscle glycogen content at death. Br. Poult. Sci. 46:572-579.

Berzaghi, P., A. Zotte, L. M. Jansson, and I. Andrighetto. 2005. Near-infrared reflectance spectroscopy as a method to predict chemical composition of breast meat and discriminate between different n-3 feeding sources. Poult. Sci. 84:128-136.

Bilgili, S. F. 2001. Poultry Products and Processing in the International Market Place. http://www.fass.org/fass01/pdfs/Bilgili.pdf, Auburn, AL. Accessed 2008.

Boehm, M. L., T. L. Kendall, V. F. Thompson, and D. E. Goll. 1998. Changes in the calpains

and calpastatin during postmortem storage of bovine muscle. J. Anim. Sci. 76:2415-2434.

Boulianne, M. and A.J. Knight. 1995. Biochemical and color characteristics of skinless boneless pale chicken meat. Poultry Sci.74:1693-1695.

Bramwell, R. K. 2000. Stress, What Is It? in Broiler Tip. March 2000. University of Georgia, Athens, Ga.

Brondum, J., L. Munck, P. Henckel, A. Karlsson, E. Tornberg, and S. B. Engelsen. 2000. Prediction of water-holding capacity and composition of porcine meat by comparative spectroscopy. Meat Sci. 55:177-185.

Chen, Y. R., and D.R. Massie. 1993. Visible/near-infrared reflectance and interacctance spectroscopy for detection of abnormal poultry carcasses. Trans. ASAE 36:863-869.

Chen, Y. R., B. Park, and R.W. Huffman. 1994. Instrument inspection of poultry carcasses. In ASAE-Paper No. 94-6026 St. Joseph, MI.

Chen, Y. R., B. Park, M. Nguyen, and R.W. Huffman. 1996b. Instrumental system for on-line inspection of poultry carcasses. SPIE 2786:121-129.

Chen, Y. R., R.W. Huffman, and B. Park. 1996a. Changes in the visible/near-infrared spectra of chicken carcasses in storage. J. Food Proc. Engineering 19:121-134.

Chiang, W., C. P. Allison, J. E. Linz, and G. M. Strasburg. 2004. Identification of two [alpha]RYR alleles and characterization of [alpha]RYR transcript variants in turkey skeletal muscle. Gene 330:177-184.

Claeys, E., S. De Smet, D. Demeyer, R. Geers, and N. Buys. 2001. Effect of rate of pH decline on muscle enzyme activities in two pig lines. Meat Sci. 57:257-263.

Conforth, D. P. 1995. Color- its basis and importance. Pages 34-75 in Quality Attributes and

Their Measurement *in:* Meat , Poultry, and Fish Products: Advances in Meat Research A. M. Pearson, and T. R. Dutson eds. Springer, London.

Debut, M., C. Berri, C. Arnould, D. Guemene, V. Sante-Lhoutellier, N. Sellier, E. Baeza, N. Jehl, Y. Jego, C. Beaumont, and E. Le Bihan-Duval. 2005. Behavioural and physiological responses of three chicken breeds to pre-slaughter shackling and acute heat stress. Br. Poult. Sci. 46:527-535.

Dransfield, E., and A. A. Sosnicki. 1999. Relationship between muscle growth and poultry meat quality. Poult. Sci. 78:743-746.

Fleming, H. P., T. N. Blumer, and H. B. Craig. 1960. Quantitative estimations of myoglobin and hemoglobin in beef muscle extracts. J. Anim. Sci. 19:1164-1171.

Fletcher, D. L. 1999. Broiler breast meat color variation, pH, and texture. Poult. Sci. 78:1323-1327

Fletcher, D. L. 2003. Poultry processing and products technology.University of Georgia, Athens, GA.

Foegeding, E. A., T.C. Lanier, and H.O. Hultin. 1996. Characteristics of edible muscle tissue. Pages 879-942 in Food Chemistry. O. R. Fennema ed. CRC Press: Taylor & Francis Group, Boca Raton, FL.

Froning, G. W. 1995. Color of poultry meat. Poultry and Avian Biology Reviews 6:83-93.

Froning, G. W., J. Daddario, and T. E. Hartung. 1968. Color and myoglobin concentration in turkey meat as affected by age, sex and strain. Poult. Sci. 47:1827-1835.

Geesink, G. H., A. D. Bekhit, and R. Bickerstaffe. 2000. Rigor temperature and meat quality characteristics of lamb longissimus muscle. J. Anim. Sci. 78:2842-2848.

Geesink, G. H., F. H. Schreutelkamp, R. Frankhuizen, H. W. Vedder, N. M. Faber, R. W.

Kranen, and M. A. Gerritzen. 2003. Prediction of pork quality attributes from near infrared reflectance spectra. Meat Sci. 65:661-668.

Ginger, I. D., G. D. Wilson, and B. S. Schweigert. 1954. Biochemistry of myoglobin. Quantitative determination in beef and pork muscle. J. Agric. Food Chem. 2:1037-1040.

Greaser, M. L. 1986. Conversion of Muscle to Meat. Pages 37-102 in Muscle as Food. P. J. Bechtel ed. Academic Press, New York, N.Y.

Grey, T. C., and J. M. Jones. 1977. The influence of factory processing on the development of rigor in the breast muscle of broilers. Bri. Poult. Sci. 18:671-674.

Grey, T. C., J. M. Jones, and D. S. Robinson. 1974. The influence of death struggle on the rate of glycolysis in chicken breast muscle. J. Sci. Food Agri. 25(1):57-66.

Guttmann, R. P., and G. V. Johnson. 1998. Oxidative stress inhibits calpain activity in situ. J. Biol. Chem. 273:13331-13338.

Haley, M. 2001. Changing Consumer Demand for Meat: The U.S. Example, 1970-2000. In Changing Structure of Global Food Consumption and Trade/WRS-01-1. http://www.ers.usda.gov/publications/wrs011g.pdf. Accessed 3/23/04

Honikel, K. O., C. J. Kim, R. Hamm, and P. Roncales. 1986. Sarcomere shortening of prerigor muscles and its influence on drip loss. Meat Sci. 16:267-282.

http://www.meatami.com/ American Meat Institute. Accessed 4/7/04.

Huff-Lonergan, E., and S. M. Lonergan. 2005. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Sci. 71:194-204.

Huff-Lonergan, E., T. Mitsuhashi, D. D. Beekman, F. C. Parrish, Jr., D. G. Olson, and R. M.

Robson. 1996. Proteolysis of specific muscle structural proteins by mu-calpain at low pH and temperature is similar to degradation in postmortem bovine muscle. J. Anim. Sci. 74:993-1008.

Hwang, I. H., C. E. Devine, and D. L. Hopkins. 2003. The biochemical and physical effects of electrical stimulation on beef and sheep meat tenderness. Meat Sci. 65:677-691.

Isaksson, T., G. Togersen, A. Iversen, and K. I. Hildrum. 1995. Non-destructive determination of fat, moisture and protein in salmon fillets by use of near-infrared diffuse spectroscopy. J. Sci. Food Agri. 69:95-100.

Iwamoto, M., K.H. Norris, and S. Kimura. 1981. Rapid prediction of chemical compositions for wheat, soybean, pork, and fresh potatoes by near-infrared spectrophotometric analysis. J. Japanese Soc. Food Sci. and Technology 28:85-90.

Kadim, I. T., O. Mahgoub, W. Al-Marzooqi, and K. Annamalai. 2005. Prediction of crude protein, extractable fat, calcium and phosphorus contents of broiler chicken carcasses using near-infrared reflectance spectroscopy. Asian-Australian J. Anim. Sci. 18:1036-1040.

Koohmaraie, M. 1996. Biochemical factors regulating the toughening and tenderisation of meat. Meat Sci. 43:S193-S201.

Kruggel, W. G., R.A. Field, M.L. Riley, H.D. Radloff, and K.M. Horton. 1981. Near-infrared reflectance determination of fat, protein, and moisture in fresh meat. J. Asso. Off. Anal. Chem. 64:692-696.

Lanza, E. 1983. Determination of moisture, protein, fat, and calories in raw pork and beef by near infrared spectroscopy. J. Food Sci. 48:471-474.

Lawrie, R. A. 1998. Lawrie's Meat Science. Pages 212-254. Sixth ed. Woodhead Publishing Ltd,

Cambridge, England.

Lindsay, R. 1996. Flavor. Pages 723-762 in Food Chemistry. O. R. Fennema ed. CRC Press: Taylor & Francis Group, Boca Raton, FL.

Liu, Y., B. G. Lyon, W. R. Windham, C. E. Lyon, and E. M. Savage. 2004. Prediction of physical, color, and sensory characteristics of broiler breasts by visible/near infrared reflectance spectroscopy. Poult. Sci. 83:1467-1474.

Lyon, B. G., W. R. Windham, C. E. Lyon, and F. E. Barton. 2001. Sensory characteristics and near-infrared spectroscopy of broiler breast meat from various chill-storage regimes. J. Food Qual. 24:435-452.

Ma, R. T.-I., and P. B. Addis. 1973. The association of struggle during exsanguination to glycolysis, protein solubility and shear in turkey pectoralis muscle. J. Food Sci. 38:995-997.

Maltin, C., D. Balcerzak, R. Tilley, and M. Delday. 2003. Determinants of meat quality: tenderness. Proc. Nutrition Society 62:337-347.

Martinaud, A., Y. Mercier, P. Marinova, C. Tassy, P. Gatellier, and M. Renerre. 1997. Comparison of Oxidative Processes on Myofibrillar Proteins from Beef during Maturation and by Different Model Oxidation Systems. J. Agri. Food Chem. 45:2481-2487.

McCurdy, R. D., S. Barbut, and M. Quinton. 1996. Seasonal effect on pale soft exudative (PSE) occurrence in young turkey breast meat. Food Res. Int. 29:363-366.

McKee, S. R., and A. R. Sams. 1997. The effect of seasonal heat stress on rigor development and the incidence of pale, exudative turkey meat. Poult. Sci. 76:1616-1620.

Mickelson, J. R., E. M. Gallant, L. A. Litterer, K. M. Johnson, W. E. Rempel, and C. F. Louis.

1988. Abnormal sarcoplasmic reticulum ryanodine receptor in malignant hyperthermia. J. Biol. Chem. 263:9310-9315.

Miguel JA, J. Ciria, B.Asenjo & J.L. Calvo. 2008. Effect of caponisation on growth and on carcass and meat characteristics in Castellana Negra in native Spanish chickens. Animal 2(2):305-311.

Mitsumoto, M., S. Maeda, and T. Mitsuhashi. 1991. Near-infrared spectroscopy determination of physical and chemical characteristics in beef cuts. J. Food Sci. 56:1493-1496.

Monin, G. 1998. Recent methods for predicting quality of whole meat. Meat Sci. 49:S231-S243.

Morton, J. D., R. Bickerstaffe, M. P. Kent, E. Dransfield, and G. M. Keeley. 1999. Calpain-calpastatin and toughness in M. longissimus from electrically stimulated lamb and beef carcasses. Meat Sci. 52:71-79.

Muellenet, J. F., E. Jonville, D. Grezes, and C. M. Ownes. 2004. Prediction of the texture of cooked poultry pectoralis major muscles by near-infrared refelctance analysis of raw meat. J. Texture Studies 35:573-585.

Nocito, J. S., B. H. Bayne, M. P. Penfield, and B. H. Meyer. 1973. Myoglobin Content and Color of Raw Pork Loin Roasts as Affected by Freezing at Two Rates. J. Anim. Sci. 37:1339-1343.

Northcutt, J. K.1997. Factors affecting poultry meat quality. In Processing Tip. University of Georgia, Cooperative Extension, Athens, GA.

Northcutt, J. K.2000. Preslaughter stress may affect poultry meat quality. in Processing Tip.University of Georgia, Cooperative Extension, Athens, GA.

Olentine, C. September 2003. Careful what you ask for. Watt Poultry USA.

Ouali, A. 1990. Meat tenderisation: Possible causes and mechanisms. J. Muscle Foods 1:129-153.

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

Owens, C. M., N. S. Matthews, and A. R. Sam. 2000b. The use of halothane gas to identify turkeys prone to developing pale, exudative meat when transported before slaughter. Poult. Sci. 79:789-795.

Papinaho, P. A., and D. L. Fletcher. 1996. The effects of stunning amperage and deboning time on early rigor development and breast meat quality of broilers. Poult. Sci. 75:672-676.

Park, B., Y. R. Chen, W. R. Hruschka, S. D. Shackelford, and M. Koohmaraie. 2001. Principal component regression of near-Infrared reflectance spectra for beef tenderness prediction. Trans. ASAE 44:609-615.

Pietrzak, M., Greaser, M.L., and Sosnicki, A.A. 1997. Effect of rapid rigor mortis processes on protein functionality in pectoralis major muscle of domestic turkeys. J. Anim. Sci. 75:2106-2116.

Piles, M., A. Blasco, and M. Pla. 2000. The effect of selection for growth rate on carcass composition and meat characteristics of rabbits. Meat Sci. 54:347-355.

Ramirez, J. A., M. A. Oliver, M. Pla, L. Guerrero, B. Arino, A. Blasco, M. Pascual, and M. Gil. 2004. Effect of selection for growth rate on biochemical, quality and texture characteristics of meat from rabbits. Meat Sci. 67:617-624.

Remignon, H., and E. Le Bihan-Duval. 2003. Meat quality problems associated with selection for increased production. Pages 53-66 in Poultry Genetics, Breeding and Biotechnology. Muir, W.M. and S.E., Aggrey eds. CAB International.

Renden, J. A., S.S. Oates, and R.B. Reed. 1986. Determination of body fat and moisture in dwarf

hens with near-infrared reflectance spectroscopy. Poultry Sci. 65:1539-1541.

Rickansrud, D. A., and R.L.Henrickson. 1967. Total pigments and myoglobin concentration in four bovine muscles. J. Food Sci. 32:57-61.

Sante, V., G. Bielicki, M. Renerre, and A. Lacourt. 1991. *Post mortem* evolution in the *Pectoralis superficialis* muscle from two turkey breeds: Relationship between pH and colour changes. Proc. 37th International Congress of Meat and Technology Kulmbach, Germany.

Swatland, H. J. 1995. On-line evaluation of meat. Technomic Publishing Company, Inc., Lancaster, PA.

Valdes, E. V., and J. D. Summers. 1986. Determination of crude protein and fat in carcass and breast muscle samples of poultry by near-infrared reflectance spectroscopy. Poult. Sci. 65:485-490.

Vanderstoep, J. and J. F. Richards. 1974. Post mortem, glycolytic and physical changes in turkey breast muscle. Can. Inst. Food. & Sci. Techn. J. 7:120-125.

von Lengerken, G., Maak, S., and Wicke, M. 2002. Muscle metabolism and meat quality of pigs and poultry. Veterinarija IR Zootechnika 20:82-85.

Wang, L. J., T.M.Byrem, J. Zarosley, A.M. Booren, and G.M. Strasburg. 1999. Skeletal muscle calcium channel ryanodine binding activity in genetically unimproved and commercial turkey populations. Poult. Sci. 78:792-797.

Wiskus, K. J., P. B. Addis, and R. Ma. 1976. Distribution of βR, βR and αW fibers in turkey muscles. Poult. Sci. 55:562-566.

Woelfel, R. L., C. M. Owens, E. M. Hirschler, R. Martinez-Dawson, and A. R. Sams. 2002. The

characterization and incidence of pale, soft, and exudative broiler meat in a commercial processing plant. Poult. Sci. 81:579-584.

Woelfel, R. L., and A. R. Sams. 2001. Marination performance of pale broiler breast meat. Poult. Sci. 80:1519-1522.

Young LL, J.K. Northcutt, and C.E. Lyon. 1996. Effect of stunning time and polyphosphates on quality of cooked chicken breast meat. Poult. Sci. 75:677-681.

CHAPTER 2

THE RELATIONSHIP BETWEEN PRODUCTION AND PROCESSING FACTORS ON COLOR CHANGES IN BROILER BREAST MEAT AND THE IMPLICATIONS ON QUALITY

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SUMMARY

Color and pH of boneless, skinless, broiler breast meat obtained from two commercial processing plants were measured. Production and processing factors for each sample were recorded and correlated to quality parameters. Water holding capacity was analyzed for quality implications. A mean difference in L* in birds with different growth rates was observed with a higher occurrence of paler birds in those selected for greater breast yield. L* was heavily influenced by several factors such as grower, age, and bird weight. The research also shows an increase in L* compared to previous research, with a* having a greater correlation between the production and processing factors than the L*.

INTRODUCTION

The success of the poultry industry has been fueled by the ability to economically produce acceptable products. The result is reduced slaughter age and increased body weight at specific ages (Anthony 1998). However, this selection for growth rate has resulted in an alteration in the processing quality of poultry meat. This is evidenced by changes in carcass composition and meat quality such as increase of muscle glycolytic activity, lowering of water holding capacity and higher meat hardness (Piles *et al.,* 2000; Ramirez *et al.,* 2004). Le Bihan-Duval *et al.*, (1999) stated that breast meat from chickens selected for increased breast yield exhibited a paler color. This pale color can be an indication of pale, soft, and exudative meat, which is caused by a rapid postmortem pH decline at warm carcass temperatures. The resulting protein denaturation leads to a pale color and a decrease in water-holding capacity, causing excessive yield losses. PSE incidence in broilers and turkeys has been reported to range from 5 up to 50% in commercial plants (Barbut, 1996; McCurdy *et al.,* 1996; Barbut, 1997; Owens *et al.,* 2000; Woelfel and Sams 2001; Woelfel *et al,*. 2002)

Much research has been done on the study of stress and its relation to the quality of broiler meat. Aberle *et al.* (2001) defined stress as a general expression referring to physiological adjustments, such as changes in heart rate, respiration rate, body temperature, and blood pressure that occur during the exposure of the animal to adverse conditions (Aberle *et al.,* 2001).

Therefore, the objectives of this research were to look at several production and processing factors that may influence color changes in broiler breast meat and to determine the implications on the quality of the breast meat. In addition, this research compares the occurrence of pale broiler breast meat in two commercial processing plants with different growth rate selections.

METHODS

Boneless, skinless, broiler breast meat was obtained from two commercial processing plants. Broilers from the first processor were approximately 42 days old averaging 4.2 lbs (0.101 lbs/day) while those from the second plant averaged 7.4 lbs at approximately 57 days (0.130 lbs/day). Samples were collected of every fifth bird during several processing runs scheduled over several months. In order to eliminate variation due to overscalding, L* was determined on the dorsal and ventral pectoralis major using a Minolta Chroma Meter CR-310, Osaka, Japan. Ventral surface measurements were used for all analysis. The pH measurements were acquired through use of a spear tip pH meter (Hannah Instruments, Van Nuys, CA) designed for meat samples. All measurements were taken 24 hours postmortem. Water holding capacity (WHC) measurements were done in triplicate as follows. An aliquot of 10 g was mixed with 16 ml of 0.6 M NaCl and then incubated for 30 min at 4ºC. Afterwards samples were centrifuged @ 7,000 g. WHC was defined as the portion of fluid retained by the sample (Barbut, 1993). The data was analyzed using SAS software (SAS Institute, Inc., Version 9.0, Cary, N.C.). A multiple linear regression was conducted to examine the individual effects of each production or processing factor on L*, a*, and b*. Pearson Correlation Coefficients were also determined to show the extent of each factor on the L*, a*, and b*.

RESULTS AND DISCUSSION

The average L* for the larger birds were slightly higher than the smaller birds. Although there were little differences in the averages, a histogram of the measurements showed significant differences (see Table 2.1). The larger birds exhibited a much higher frequency of pale birds than the smaller birds. L* > 60 was found in 57% or 26% of breast meat from larger birds or smaller birds, respectively (Table 2.1). Previous research showed that L* for poultry breast meat was on average significantly less than 60 but widely variable. The L* ranged from 43.1 (classified as dark, firm and dry meat) to 58.9 (classified as pale, soft, and exudative meat) (Fletcher, 1999; Woelfel and Sams 2001; Bianchi *et al*., 2005).

The present study shows a significant increase in higher L* and is consistent with literature suggesting that selection for growth has resulted in a paler meat (Le Bihan-Duval *et al.*, 1999). Although a higher occurrence of paler birds was confirmed, the corresponding ultimate pH values did not show extremely high correlations with the lightness values. Pearson's correlations for pH and L* were -0.51 and -0.27 for the large and small birds, respectively. Le Bihan-Duval *et al.*, (1999) found a similar correlation value of -0.59 in their study on the effect of selection for increased carcass quality and estimates of genetic parameters. The rate of pH decline has been known to vary not only between genetic lines within broilers but also between individual birds (Dransfield and Sosnicki, 1999). The Pearson' correlation coefficient between water holding capacity and L* was determined to be -0.35. While previous research showed higher correlations of L* with pH and water holding capacity, their sample selection involved subjective preselection prior to objective analysis. The present study did not utilize subjective preselection for pale breasts but used a random sample.

Several ante mortem, or pre-slaughter, factors have been identified as stressors of broilers: transportation or hauling of the birds from the farm to the processing facility; extreme

conditions in the weather; shortages of feed and water, especially during warm or hot weather; changes in the daily routine, such as changes in feeds or feeding methods; overcrowding, where birds have to fight to eat or drink; and physical disturbances in which excessive noise or aggravated activity causes the birds to become nervous. Although any of these conditions may induce stress, they differ in their effects because the response that any one environmental condition produces depends on the species, weight, age, sex, inherent stress resistance, and emotional state of the animal. Because unpredictable emotional responses are elicited, highly variable responses are produced in the muscles. The changes that occur in the muscles are responsible for the ultimate properties of the meat depending upon the duration or severity of the stress as well as the level of the animal's stress resistance at the time of death (Aberle *et al.,* 2001). Stress factors studied in this research and Pearson Correlation coefficients with L*, a*, and b* are summarized in Table 2.2.

CONCLUSIONS

L* has been associated with water holding capacity. Our results suggest that the bird weight, grower, and age of the broiler had the greatest influence on L* as seen in Table 2.2. Pearson correlations also indicate that a* shows a greater correlation with the factors studied in this research. Correlations between water holding capacity and L* and correlations between pH and L* were weak. These findings indicate the reason for the increase in L* in older birds at heavier weights may be a result of reduced total pigment, myoglobin and iron concentration versus an increased incidence of pale, soft and exudative meat.

REFERENCES

Aberle, E. D., Forrest, J.C., Gerrard, D.E., and Mills, E.W. (2001). *Principles of Meat Science*. Dubuque, Iowa, Kendall/Hunt Publishing Company.

Anthony, N. B. (1998). *Journal of Muscle Foods,* **9**: 25-33.

Barbut, S. (1993). *Food Research International,* **26**(1): 39-43.

Barbut, S. (1996). *Canadian Journal of Animal Science,* **76**: 455-457.

Barbut, S. (1997). *British Poultry Science,* **38**: 74-77.

Bianchi, M., Fletcher, D.L., and Smith, D.P. (2005). *Poultry Science*, **84**: 803-808.

Dransfield, E. and Sosnicki, A.A. (1999). *Poultry Science* **78**: 743-746.

Fletcher, D. L. (1999). *Poultry Science,* **78**: 1323-1327.

Le Bihan-Duval, E., Millet, N.,and Remignon, H. (1999). *Poultry Sci*ence, **78**(6): 822-826.

McCurdy, R. D., Barbut, S., and Quinton, M. (1996). *Food Research International*, **29**(3-4): 363-366.

Owens, C. M., Hirschler, E. M., McKee, S. R., Martinez-Dawson, R., and Sams, A. R. (2000). *Poultry Science*, **79**(4): 553-558.

Piles, M., Blasco, A., and Pla, M. (2000). *Meat Science*, **54**(4): 347-355.

Ramirez, J. A., Oliver, M. A., Pla, M., Guerrero, L., Arino, B., Blasco, A., Pascual, M., and Gil, M. (2004). *Meat Science*, **67**(4): 617-624.

Woelfel, R. L., Owens, C. M., Hirschler, E. M., Martinez-Dawson, R., and Sams, A. R. (2002). *Poultry Science,* **81**(4): 579-584.

Woelfel, R. L. and A. R. Sams (2001). *Poultry Science*, **80**(10): 1519-1522.

Table 2.1. Frequency of L* among broilers with different growth rates.

average weight 4.2 lbs	*L**	*Frequency*	*Cumulative %*
	48	*0*	0.00%
	50	0	0.00%
	52	1	0.34%
	54	6	2.38%
	56	36	14.63%
	58	88	44.56%
	60	86	73.81%
	62	60	94.22%
	64	13	98.64%
	66	4	100.00%
	68	0	100.00%
	70	0	100.00%
	>70	0	100.00%
	N=	294	

average weight 7.4 lbs	*L**	*Frequency*	*Cumulative %*
	48	0	0.00%
	50	1	0.25%
	52	1	0.51%
	54	4	1.53%
	56	17	5.85%
	58	45	17.30%
	60	100	42.75%
	62	115	72.01%
	64	79	92.11%
	66	23	97.96%
	68	7	99.75%
	70	0	99.75%
	>70	1	100.00%
	N=	393	

Table 2.2. Pearson Correlation Values between Processing and Production Factors and L*, a*, and b* of dorsal and ventral measurements for broilers. Significance levels are below coefficients.

	Top (Dorsi)			Bottom (ventral)		
Factor	L*	a	b	L*	a	b
Age	0.55	-0.64	-0.15	0.34	-0.46	-0.40
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Weight	0.56	-0.67	-0.15	0.34	-0.50	-0.39
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Yard Time	0.37	-0.45	-0.18	0.21	-0.32	-0.42
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
FWT	0.35	-0.34	-0.12	0.21	-0.14	0.33
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0002	<0.0001
Birds/Truck	-0.51	0.66	0.13	-0.29	0.49	0.35
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
DOA's	0.34	0.44	0.14	-0.14	0.36	0.35
	<0.0001	<0.0001	<0.0005	<0.0003	<0.0001	<0.0001
pH	-0.30	0.08	-0.12	-0.39	0.04	-0.22
	<0.0001	<0.0295	<0.0017	<0.0001	<0.2845	<0.0001
Grower	0.55	-0.65	-0.15	0.34	-0.48	-0.41
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Month	0.48	-0.58	-0.14	0.32	-0.52	-0.35
	<0.0001	<0.0001	<0.0003	<0.0001	<0.0001	<0.0001
Catch Crew	0.46	-0.47	-0.10	0.33	-0.42	-0.32
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

CHAPTER 3

THE IMPACT OF GROWTH RATE ON THE OCCURRENCE OF PALE BIRDS WITHIN TWO COMMERCIAL PROCESSING PLANTS

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ABSTRACT

The occurrence of pale broiler breast meat was examined within two commercial processing plants and 10 different growers. Birds from the first processing plant had an average weight of 3.36 kg, with a growth rate of 59 g/day, while the second processing plant had an average weight of 1.93 kg, with a growth rate of 46 g/ day. To evaluate the effect of growth rate on meat quality, water holding capacity (WHC), pH, and dorsal and ventral color measurements were made. Results showed that the heavier birds with a higher growth rate exhibited a higher occurrence of pale birds than the lighter birds with a slower growth rate. L* greater than 60 was observed in 57% of birds selected for greater yield and 26% of slower growing birds. A one way design analysis showed significant differences ($\alpha = 0.001$) between the mean L* of the different growers involved in the study. Pearson's correlation coefficients between pH and L* were -0.51 and -0.27 for the faster growing birds and slower growing birds, respectively. The Pearson's correlation coefficients between water holding capacity and L* and pH was determined to be -0.35 and 0.42, respectively. Results also showed that there was a higher correlation between production factors (age, weight, and grower) and a* than between L* and production factors for both dorsal and ventral surface measurements.

(Keywords: broiler growth rate, pale, soft, and exudative, water holding capacity)

INTRODUCTION

Due to an increased demand for portioned and processed poultry products, the focus of poultry breeding for the past 30 years has been to increase growth rate of chickens and turkeys (Remignon and Le Bihan-Duval, 2003). With a concentration on growth rate and muscle mass in meat lines, the production time to raise a 1.3-kg chicken has halved to 5 wk in less than 30 years (Dransfield and Sosnicki, 1999). More attention has been given to the breast muscle which is the most valuable part of the chicken (Le Bihan-Duval et al., 1998). Berri et al. (2001) reported that breast meat yield has increased from approximately 12% to 19% over the past 30 years. The focus of this poultry selection for increased breast yield has been primarily on the reduction of breeding costs while improving production efficiency. The impact of poultry selection on meat quality has been neglected (Remignon and Le Bihan-Duval, 2003).

Selection for growth rate has resulted in an alteration in the processing quality of poultry meat as evidenced by changes in carcass composition and meat quality such as increased muscle glycolytic activity, lower water holding capacity and higher meat hardness (Piles et al., 2000; Ramirez et al., 2004). Remignon and Le Bihan-Duval (2003) states that color and water holding capacity are frequently reported as being poorer in modern poultry flocks. These two factors influence perceptions of the acceptability of meat products although they vary with the species, muscle function, age of the animal and storage conditions (Berri, 2000). Fletcher (1999) found significant variations in breast meat lightness within five commercial broiler-processing plants which would indicate variations at the retail level also. Le Bihan-Duval et al. (1999) stated that breast meat from chickens selected for increased breast yield exhibited a paler color. Baeza et al. (1997) found the same characteristics in duck meat selected for growth and meat yield while Sante et al. (1991) found similar results working with turkey. This pale color has been associated

with the incidence of pale, soft, and exudative meat (PSE). Pale, soft, and exudative breast meat is caused by a rapid postmortem pH decline at higher carcass temperatures and results in protein denaturation and decreased water-holding capacity (WHC).The resulting protein denaturation and decreased WHC leads to paler meat and excessive yield losses (Alvarado and Sams, 2003). Research shows that PSE among broilers and turkeys is reported as high as 50% in commercial plants (Barbut, 1996; Barbut, 1997; McCurdy et al., 1996; Owens et al., 2000; Woelfel et al., 2002; Woelfel and Sams, 2001). Pietrzak (1997) identifies PSE as one of the major contributors to decreased water holding capacity which costs the poultry industry millions of dollars annually.

Remignon and Le Bihan-Duval (2003) state that qualitative and quantitative characteristics of muscle fibers heavily influence the quality of the meat which in turn may influence the color. Dransfield and Sosnicki (1999) reported that fibers become glycolytic (fast twitch, glycolytic or type IIB) with increasing growth rate. Higher concentrations of type IIB or white fibers translate into light-colored meat, with low fat and myoglobin. Dark meat has a higher percentage of red fibers (type I or type IIA) and contain more myoglobin and fat due to a higher oxidative metabolism (Remignon and Le Bihan-Duval 2003).

Although it has been determined that selection for growth rate has increased the occurrence of pale breast meat, previous research on broiler breast meat has failed to show the magnitude of the difference within commercial plants differing significantly in growth rate selections. The objective of this research is to compare the occurrence of pale broiler breast meat and its effect on quality in two commercial processing plants with different growth rate selections.

MATERIALS AND METHODS

Sample Collection

Boneless, skinless, broiler breast meat was obtained from two commercial processing plants. The first set of samples (n=295) collected were approximately 42 days old averaging 1.93 kg while samples (n=397) from the second plant averaged 3.36 kg at approximately 57 days old. Broiler birds were collected during the months of September, October, and November over a two year period from 10 different growers. The samples from broilers averaging 1.93 kg were taken from six growers and the samples averaging 3.36 kg were selected from 4 different growers. Every fifth bird was tagged on the evisceration line and collected at the exit of the chiller. Birds were then placed on ice and held in a cooler for 24 hours before being hand deboned by plant personnel. After color and pH measurements at 24 hours postmortem, samples were placed on ice in coolers and transported back to the university lab for water holding capacity analysis.

Color and pH Measurements

L*, a*, and b* were measured at the medial point of the dorsal and ventral surfaces of the pectoralis major using a Minolta Chroma Meter CR-310 (Osaka, Japan). The pH measurements were acquired through use of a spear tip probe pH meter (Hannah Instruments, Van Nuys, CA) designed for meat samples. Measurements were taken 24 hours postmortem.

Water Holding Capacity

The water holding capacity (WHC) was determined as described by Samuel et al. (2008) using a centrifugation method. Measurements were done in triplicate.

Statistical Analyses

A multivariate analysis was used to examine the effect of several production and processing parameters on the paleness and the pH of broiler meat at 24 hr postmortem. A one-way design analysis in addition to t- tests comparisons were utilized to compare mean differences in L* between several growers. Pearson's correlations were conducted to examine the relationship between certain parameters and L*. All data was analyzed by use of the SAS software package (SAS Institute, Inc., Version 9.0, Cary, N.C.).

RESULTS

In previous research, the L* measured in turkeys ranged from 41.1 to 53.6, while measurements in broilers ranged from 43.1 to as high as 58.9 (Table 3.1). In this study the birds with a higher growth rate had a mean L* of 66.3 (dorsal) and 60.37 (ventral) and were significantly higher ($p<0.0001$) than mean L* of 62.7 (dorsal) and 58.4 (ventral) for those with a slower growth rate. However, there was no significant difference between the mean pH values of the two groups (Table 3.2). A histogram of the ventral surface measurements showed significant differences in the occurrence of pale birds (Figures 3.1 and 3.2). L* greater than 60 occurred 57% of the time from the birds selected for greater yield while only 26% of L* were greater than 60 in the slower growing birds (Figures 3.1 & 3.2). Previous research (Table 3.1) showed that L* for normal poultry breast meat were on average significantly less than 60 despite a greater variation in their measurements. Our present findings showed that the mean L* was consistent with values considered to be classified as pale in previous research indicating that selection for growth rate has increased the occurrence of paler birds.

Mean L* varied significantly ($P < 0.0001$) between different growers involved in the study (Table 3.3). Among the two commercial processors, there were a total of ten growers. Processors 1 and 2 had six and four growers, respectively. ANOVA analysis showed significant ($P < 0.0001$) mean L* differences within individual processors and between the two processors (Tables 3.4, 3.5 and 3.6). For processor 1, fifteen comparisons were made and eight were significantly different within the mean L* and seven showed no significant differences ($P \leq 0.05$) between mean L*. For processor 2, six comparisons were made. Four of the six grower comparisons exhibited significant differences in mean L*. However, when the comparisons involved both processors, there was a greater incidence of significant differences at the 0.05 probability level. Only three out of twenty four comparisons were not significantly different (t-tests results not shown). The average bird weight and mean L* for the individual growers are reported in Table 3.7.

Although a higher occurrence of paler birds was confirmed, the corresponding ultimate pH values did not show extremely high correlations with the lightness. Pearson's correlations for the relationship between pH and L* were -0.51 and -0.27 for the faster growing birds and slower growing birds, respectively (Figures 3.3 and 3.4). However, some samples with higher L* were preselected to determine the effect of subjective preselection on the correlation which increased to -0.77 (Figure 3.5).

The Pearson correlation coefficient for the relationship between water holding capacity and L* was -0.35. The Pearson correlation coefficient was 0.42 when examining the relationship between water holding capacity and pH (Figures 3.6 & 3.7). While previous research showed higher correlations of L* with pH and water holding capacity, their sample selection involved

subjective preselection prior to objective analysis. The present study is not based upon subjective preselection although a comparison was done for validation.

Also, Pearson correlations were examined between L*, a*, and b and age, weight, and growers (Table 3.8). Results showed that there was a higher correlation between production factors (age, weight, and grower) and a* than with L*. This was found to be true of b* as well when looking at the ventral measurements.

DISCUSSION

Meat color is the primary criterion by which consumers evaluate meat quality and acceptability (Conforth, 1991). Aberle (2001) states that the most important contributors to meat color are the pigments that absorb certain wavelengths of light and reflect others in addition to the structure and texture of the muscles which also influences the reflection and absorption of light. The two major proteins found in meat pigments are hemoglobin, the pigment of blood, and myoglobin, the pigment of muscles. In well-bled muscle tissue, myoglobin content varies between 80 to 90 percent of the total pigment depending upon the species, age, sex, muscle, and physical activity of the animal. Pigment content accounts for much of the variability in meat color (Aberle, 2001). Other factors found to contribute to the color of breast meat are early aging times during processing and during storage (Petracci and Fletcher, 2002); meat thickness (Sandusky and Heath, 1996; Bianchi and Fletcher, 2002); and the position on the breast where the color measurement is taken (Goshaw et al., 2000).

Moran (1977) defined animal growth as the sum of the growths of the component parts of the carcass (meat, bone, and skin). Chambers (1990) states that these parts not only differ in their rates of growth as age advances, but they are dependent upon levels of nutrition as well.

Growth rate can be indicated by the body weight at a specific age (Chambers, 1990). Our research results showed significant mean differences in L* with the faster growing birds having higher L* for both dorsal and ventral measurements with no significant differences in pH. In addition to significant differences between the fast and slow growing birds, significant differences in mean L* were also present between growers within the same processor as well as those not from the same processor. Fox and Bohren (1954) demonstrated that faster growing chickens were more efficient than the slower growing ones. The energy costs of production in both net energy and heat increment change as the chicken grows due to the changing composition of the rapid body weight gain (Chambers, 1990).

The present study shows a significant increase in L* and is consistent with literature suggesting that selection for growth has resulted in a paler meat. Our data were also consistent with a study discussed in Fletcher (1999) where research conducted in the Netherlands failed to show any significant relationship between broiler breast meat color variation and muscle pH. Although we found a higher occurrence of paler birds, the corresponding ultimate pH did not show high correlations with the lightness. Pearson's correlations were -0.51 and -0.27 for the large and small birds, respectively. Le Bihan-Duval et al. (1999) found a similar correlation value of -0.59 in his study on the effect of selection for increased carcass quality and estimates of genetic parameters. The rate of pH decline varies between genetic lines within broilers and between individual birds. The variation in pH can range typically from 6.2 to 6.6 at 15 minutes postmortem (Gardzielewska et al., 1995). Due to a variety of environmental factors, pH at 20 min postmortem can vary from 6.2 to 6.8 in breast muscle from 10-wk-old turkey hens. The variation is even greater between male and female broilers (Dransfield and Sosnicki, 1999). The present study did not differentiate between sexes. While the previous studies showed higher

correlations between pH and L*, their sample selection for data shown involved subjective preselection prior to objective analysis. The present study did not utilize prior subjective preselection but used random data. Also, Le Bihan-Duval et al. (2001) stated that their results suggest that selection for growth and muscle development would not alter the pH of the meat but should slowly modify its color by decreasing a* and b*. In this study,a* and b* showed negative correlations between age, bird weight, and grower in addition to no significant mean differences in pH's for the two groups. Boulianne and King (1995) found that pale breast fillets had significantly greater lightness, less redness, greater yellowness, less total pigments, less myoglobin, less iron, but higher pH. The same author reported that darker breast fillets had significantly greater total pigment, myoglobin, iron, pH, and redness and significantly lower lightness and yellowness (Boulianne and King, 1998). They concluded that the paleness in the breast meat was a result of leakage of the heme pigments during chilling and storage in ice slush.

The relationship between raw breast meat color and functional meat properties in poultry meat has been studied by many researchers (Boulianne and King, 1995; Van Laack et al., 2000; Qiao et al., 2001; Qiao et al., 2002; Cavani et al., 2002; Petracci et al., 2004; Barbut et al., 2005). As a result, L* is often used as a measurement of meat quality as it has been shown to be an indicator of water holding capacity. However, our Pearson correlation coefficient between water holding capacity and L* was determined to be -0.35 and 0.42 when examining the relationship between water holding capacity and pH, respectively. While previous research showed higher correlations of L* with pH and water holding capacity, their sample selection involved subjective preselection prior to objective analysis as opposed to ours which used a random sample. The results of our study support the findings in Le Bihan-Duval et al. (2001) that suggest that selection does not necessarily have a negative impact on meat quality.

CONCLUSION

L* for poultry have increased dramatically with more intense selections for growth. Significant mean differences in L* are seen within the same processor as well as between different processors. The faster growing birds exhibited much higher incidences of pale birds than the slower growing birds. Correlations between water holding capacity and L* as well as correlations between pH and L* were weak. These results support the findings in Le Bihan-Duval et al. (2001) that suggests that selection does not necessarily have a negative impact on meat quality but may be due to a difference in the amount of total pigment, myoglobin and iron content.

REFERENCES

Aberle, E. D., J.C., Forrest, D.E., Gerrard, and E.W., Mills. 2001. Principles of Meat Science. Dubuque, Iowa, Kendall/Hunt Publishing Company.

Alvarado, C. Z. and A.R. Sams. 2003. Injection Marination Strategies for Remediation of Pale, Exudative Broiler Breast Meat. Poultry Science **82**: 1332-1336.

Baeza, E., H. De Carville, M.R. Salichon, G. Marche and B. Leclercq. 1997. Effect of selection, over three or four generations, on meat yield and fatness in Muscovy ducks. British Poultry Science **38**: 359-365.

Barbut, S. 1996. Estimates and detection of the PSE problem in young turkey breast meat. Canadian Journal of Animal Science **76**: 455-457.

Barbut, S. 1997. Occurrence of pale soft exudative meat in mature turkey hens. British Poultry Science **38**: 74-77.

Berri, C. 2000. Variability of sensory and processing qualities of poultry meat. World's Poultry Science Journal **56**: 209-224.

Berri, C., N. Wacrenier, N. Millet, and E. Le Bihan-Duval. 2001. Effect of selection for improved body composition on muscle and meat characteristics of broilers from experimental and commercial lines. Poultry Science **80**(7): 833-838.

Bianchi, M. and D. L. Fletcher 2002. Effects of broiler breast meat thickness and background on color measurements. Poultry Science **81**(11): 1766-1769.

Boulianne, M. and A.J. Knight. 1995. Biochemical and color characteristics of skinless boneless pale chicken meat. Poultry Science **74**: 1693-1695.

Boulianne, M. and A.J. Knight. 1998. Meat colour and biochemical characteristics of

unacceptable dark-coloured broiler chicken carcasses. Journal of Food Science **63:**759-762.

Chambers, J. R. 1990. Quantitative genetics and selection. Genetics of growth and meat production in chickens. In Poultry Breeding and Genetics. R. D. Crawford. ed. Elsevier, Amsterdam, The Netherlands.

Conforth, D. 1991. Methods for identification and prevention of pink color in cooked meat. Proc. Reciprocal Meat Conference, Chicago, IL.

Dransfield, E. and A.A. Sosnicki. 1999. Relationship between muscle growth and poultry meat quality. Poultry Science **78**: 743-746.

Fletcher, D. L. 1999. Broiler breast meat color variation, pH, and texture. Poultry Science **78**(9): 1323-1327.

Fox, T. W. and B.B. Bohren. 1954. An analysis of feed efficiency among breeds of chickens and its relationship to rate of growth. Poultry Science **33**: 549-561.

Gardzielewska, J., J. Kortz, and M. Jakubowska. 1995. Postmortem kinetics of muscle pH fall in relation to strain crosses of chicken broilers. XII European Symposium on the Quality of Poultry Meat, Zaragoza, Spain.

Goshaw, D. R., L.P. Marquette, T.J. Buttles, and B.S. Walters. 2000. Broiler breast meat color evaluation. XXI World's Poultry Congress, Montreal, Canada, WPSA.

Le Bihan-Duval, E., C. Berri, E. Baeza, N. Millet, and C. Beaumont. 2001. Estimation of the genetic parameters of meat characteristics and of their genetic correlations with growth and body composition in an experimental broiler line. Poultry Science **80**(7): 839-843.

Le Bihan-Duval, E., S. Mignon-Grasteau, N. Millet, and C. Beaumont. 1998. Genetic analysis of a selection experiment on increased body weight and breast muscle weight as well as on limited abdominal fat weight. British Poultry Science **39**(3): 346-353.

Le Bihan-Duval, E., N. Millet, and H. Remignon. 1999. Broiler meat quality: effect of selection for increased carcass quality and estimates of genetic parameters. Poultry Science **78**(6): 822-826.

McCurdy, R. D., S. Barbut, and M. Quinton. 1996. Seasonal effect on pale soft exudative (PSE) occurrence in young turkey breast meat. Food Research International **29**(3-4): 363-366.

Moran, E. T., Jr. 1977. Growth and meat yield in poultry in Growth and Poultry Meat Production. K. N. Boorman, and B.J. Wilson. Edinburgh, Brit. Poultry Science Ltd.

Owens, C. M., E. M. Hirschler, S.R. McKee, R. Martinez-Dawson, and A.R. Sams. 2000. The characterization and incidence of pale, soft, exudative turkey meat in a commercial plant. Poultry Science **79**(4): 553-558.

Petracci, M. and D. L. Fletcher 2002. Broiler skin and meat color changes during storage. Poultry Science **81**(10): 1589-1597.

Pietrzak, M., M.L Greaser, and A.A. Sosnicki. 1997. Effect of rapid rigor mortis processes on protein functionality in pectoralis major muscle of domestic turkeys. Journal of Animal Science **75**: 2106-2116.

Piles, M., A. Blasco, and M. Pla. 2000. The effect of selection for growth rate on carcass composition and meat characteristics of rabbits. Meat Science **54**(4): 347-355.

Ramirez, J. A., M. A. Oliver, M. Pla, L. Guerrero, B. Arino, A. Blasco, M. Pascual, and M. Gil. 2004. Effect of selection for growth rate on biochemical, quality and texture characteristics of meat from rabbits. Meat Science **67**(4):617-624.

Remignon, H. and E. Le Bihan-Duval. 2003. Meat quality problems associated with selection for increased production. Pages**:** 53-66.In Poultry Genetics, Breeding and Biotechnology. W. M. Muir and S.E Aggrey, eds.

Samuel, D., D. Pringle, L. Billard, and L. Wicker. 2008 The relationship between production and processing factors on color changes in broiler breast meat and the implications on quality. Proceedings of XXIII World's Poultry Congress, World Poultry Science Journal.

Sandusky, C. L. and J.L. Heath. 1996. Effect of background color, sample thickness and illuminant on the measurement of broiler meat color. Poultry Science **75**: 1437-1442.

Sante, V., G. Bielicki, M. Renerre, and A. Lacourt. 1991. *Post mortem* evolution in the *pectoralis superficialis* muscle from two turkey breeds: relationship between pH and colour changes. 37th International Congress of Meat and Technology Kulmbach, Germany.

Woelfel, R. L., C. M. Owens, E.M. Hirschler, R. Martinez-Dawson, and A.R. Sams. 2002. The characterization and incidence of pale, soft, and exudative broiler meat in a commercial processing plant. Poultry Science **81**(4): 579-584.

Woelfel, R. L. and A. R. Sams 2001. Marination performance of pale broiler breast meat. Poultry Science **80**(10): 1519-1522.

TABLE 3.1. L* of poultry meat reported from previous research

Researcher	Poultry Type	L*	Instrument
Barbut 1993	turkeys	41.1-53.6	Colormet surface spectro-colorimeter
Barbut 1996	turkeys	48.4	Minolta CR-200
McCurdy et al. 1996	turkeys	46.84-47.75	Minolta CR-200
Barbut 1997	turkeys	48.2	Minolta CR-200
Pietrzak et al. 1997	turkeys	44.4 N	
Fletcher et al. 1998	broilers	51.13 P	Minolta CR-100
Fletcher 1999	broilers	43.1D, 45.6N, & 48.8P	Minolta CR-100
Le Bihan-Duval et al. 1999	broilers	50.7	Miniscan spectrocolorimeter Hunterlab
Wilkins et al. 2000	broilers	55.2	Minolta Chromameter
Berri et al. 2001	broilers	48.1-54.1	Miniscan spectrocolorimeter Hunterlab
Qiao et al. 2001	broilers	45.68D, 51.32N, & 55.95P	Minolta CR-300
Woelfel & Sams 2001	broilers	52.26	Minolta CR-200
Petracci et al. 2004	broilers	52.38	Minolta CR-300
Barbut et al. 2005	broilers	49.71-57.70	Minolta CR-200
Bianchi et al. 2005	broilers	50.9 N & 58.9 P	Minolta CR-300
Cavitt et al. 2005	broilers	47.06 F & 48.37 M	Minolta CR-300

D= Dark, N=Normal, P=Pale, F=Female, M=Male

TABLE 3.2. Mean L* and pH of broilers selected for different growth rates

Avg Broiler Weight	Growth Rate	Dorsal L*	Ventral L*	pH
3.36 kg[1]	59 g/day	66.3[a]	60.4[a]	5.8[a]
1.93 kg[2]	46 g/day	62.7[b]	58.5[b]	5.8[a]

[1]n=397

[2]n=294

Means followed by same letter in the same column do not differ significantly (α=0.0001)

TABLE 3.3. One-Way Analysis ANOVA for difference between mean L* of ten individual broiler growers from two commercial processors

Source	DF	Sum of Squares	Mean Square	F Value
Model	9	1057.83	117.55	19.02
Error	682	4215.03	6.18	
Total	691	5272.86		

$P < 0.0001$

TABLE 3.4. One-Way Analysis ANOVA for difference between mean L* of broiler growers within Processor 1

Source	DF	Sum of Squares	Mean Square	F Value
Model	5	226.94	45.39	9.70
Error	289	1351.75	4.68	
Total	294	1578.69		

$P < 0.0001$

TABLE 3.5. One-Way Analysis ANOVA for difference between mean L* of broiler growers within Processor 2

Source	DF	Sum of Squares	Mean Square	F Value
Model	3	209.44	69.81	9.58
Error	393	2863.28	7.29	
Total	396	3072.72		

$P < 0.0001$

TABLE 3.6. One-Way Analysis ANOVA for difference in mean L* between the two commercial broiler processing plants

Source	DF	Sum of Squares	Mean Square	F Value
Model	1	621.45	621.45	92.19
Error	690	4651.41	6.74	
Total	691	5272.86		

$P < 0.0001$

TABLE 3.7. Mean L*, a*, b*, and pH values for dorsal and ventral breast surfaces of broilers per grower

				Dorsal			Ventral			
Grower	N	Age (days)	avg BW(kg)	L*	a*	b*	L*	a*	b*	pH
1	24	42	1.84	62.5	13.0	10.7	57.6	14.5	11.6	5.9
2	24	42	2.08	64.6	10.9	10.9	58.7	14.1	12.4	5.9
3	22	42	1.88	60.7	12.8	9.6	57.3	13.7	11.4	5.9
4	80	40	1.91	62.9	12.2	10.5	58.7	14.0	12.8	5.8
5	82	43	1.80	63.2	13.1	11.3	59.5	14.7	13.0	5.8
6	63	41	2.05	62.0	12.0	11.5	57.4	14.1	14.1	5.7
7	99	59	3.42	66.0	9.7	10.2	59.6	12.9	11.4	5.8
8	100	57	3.45	67.5	10.1	10.1	61.5	12.9	10.9	5.8
9	100	56	3.15	65.6	10.3	10.7	59.9	13.2	11.4	5.9
10	98	56	3.43	66.2	9.9	10.3	60.5	12.1	11.4	5.8

Processor 1: Growers 1-6
Processor 2: Growers 7-10

TABLE 3.8. Pearson's correlation coefficients between production factors and L* a*, b* of broilers selected for different growth rates

	Dorsal			Ventral		
	L*	a*	b*	L*	a*	b*
Weight	0.56	-0.67	-0.15	0.34	-0.50	-0.39
Grower	0.55	-0.65	-0.15	0.34	-0.48	-0.41
Age	0.55	-0.64	-0.15	0.34	-0.46	-0.40

N=691
(P< 0.0001)

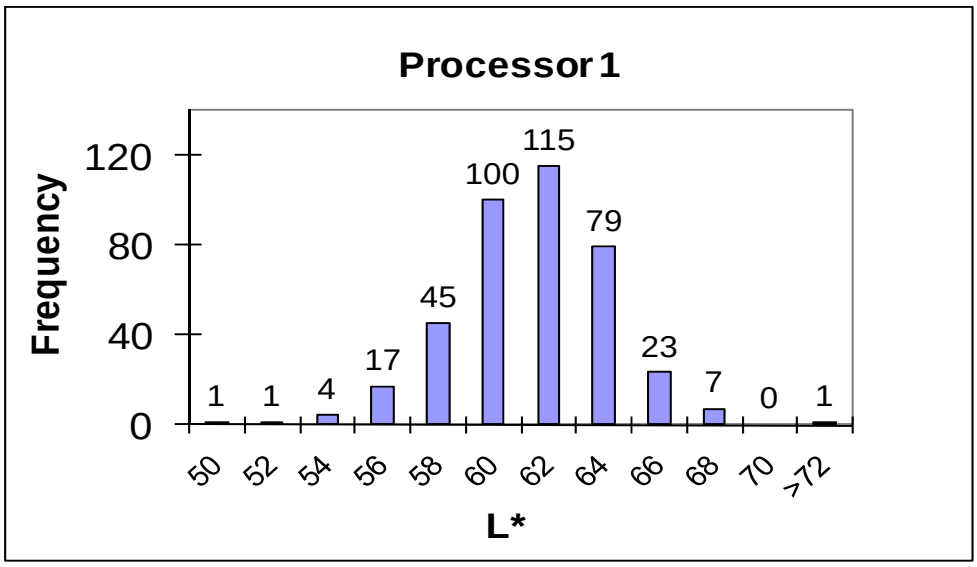

Fig. 3.1 L* of broilers averaging 3.36 kg at 57 days (Growth rate = 59 g/day). N=393

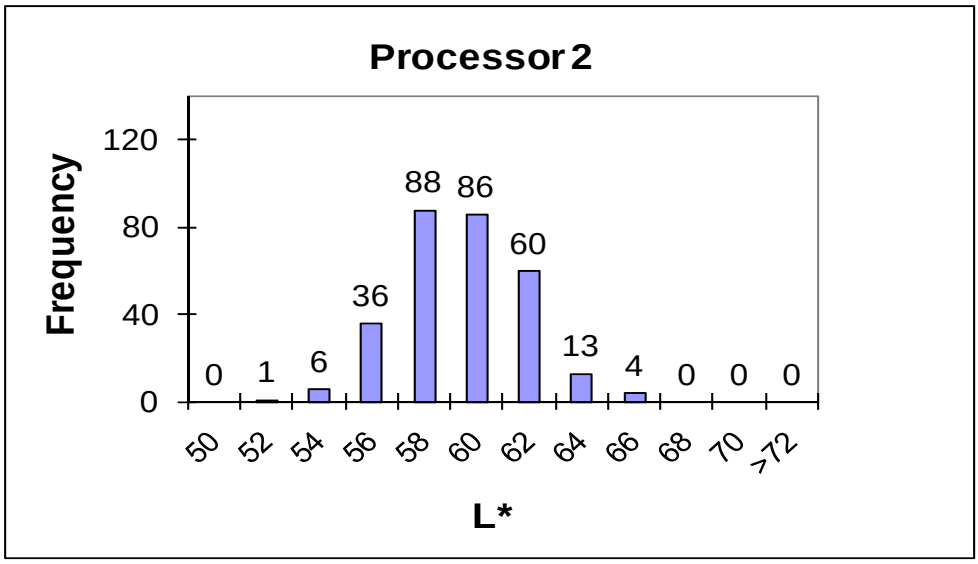

Fig.3. 2 L* of broilers averaging 1.93 kg at 42 days (Growth rate = 46 g/day). N=294

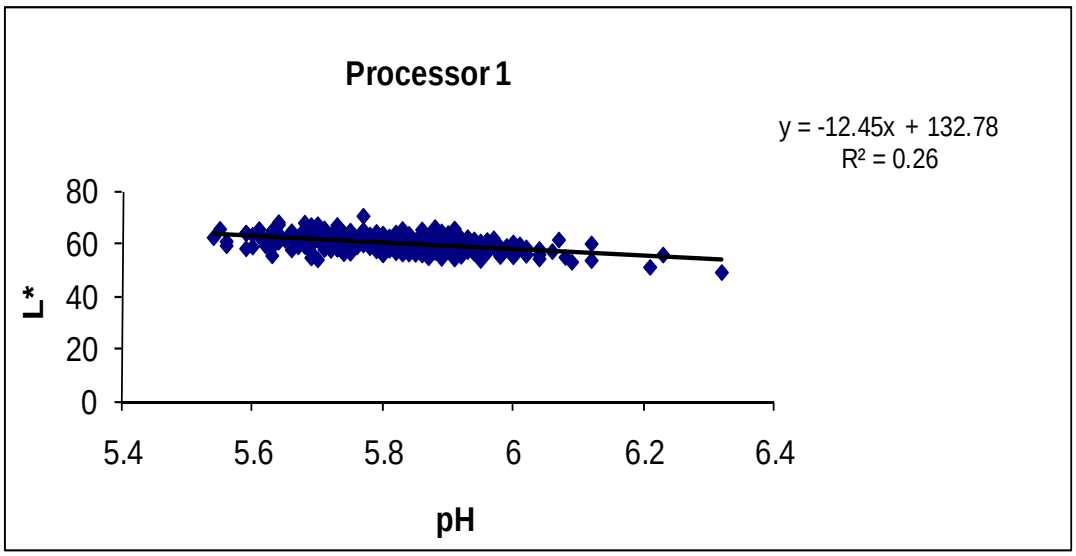

Fig.3.3 Relationship between L* and pH in broilers averaging 3.36 kg at slaughter age of approximately 57 days; Pearson's correlation coefficient = -0.51.

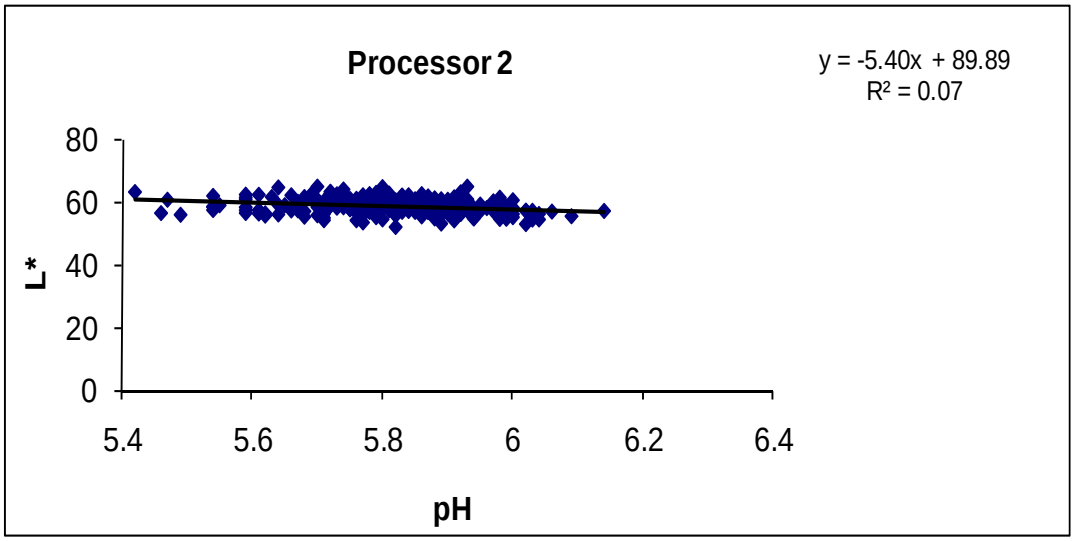

Fig. 3.4 Relationship between L* and pH in broilers averaging 1.93 kg at slaughter age of approximately 42 days; Pearson's correlation coefficient = -0.27.

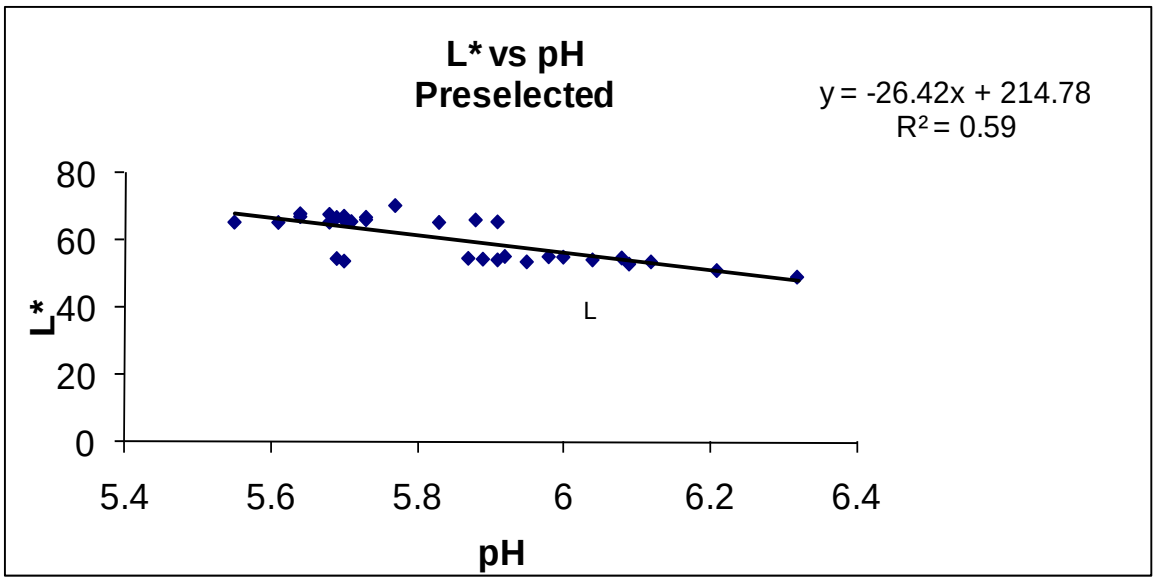

Figure 3.5. Relationship between L* and pH of broilers preselected on a subjective basis. Pearson's correlation coefficient = -0.77. N=28

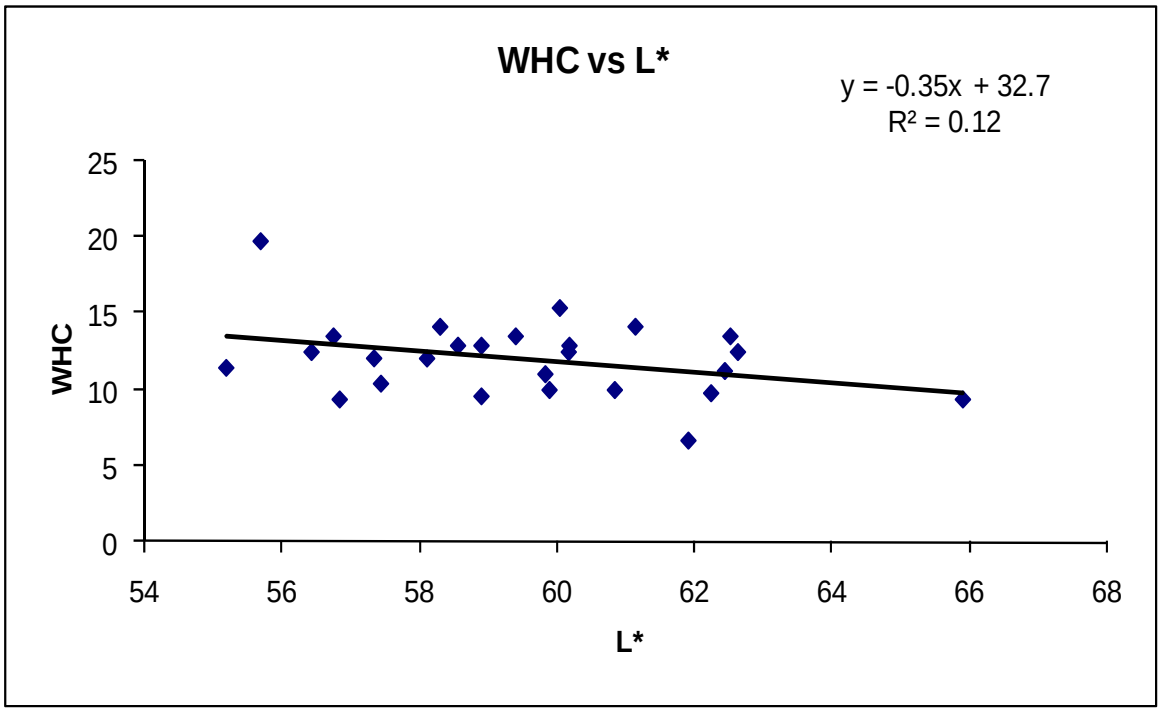

Figure 3.6. Relationship between water holding capacity and L* of broilers randomly selected. Pearson's correlation coefficient = -0.35. N=26

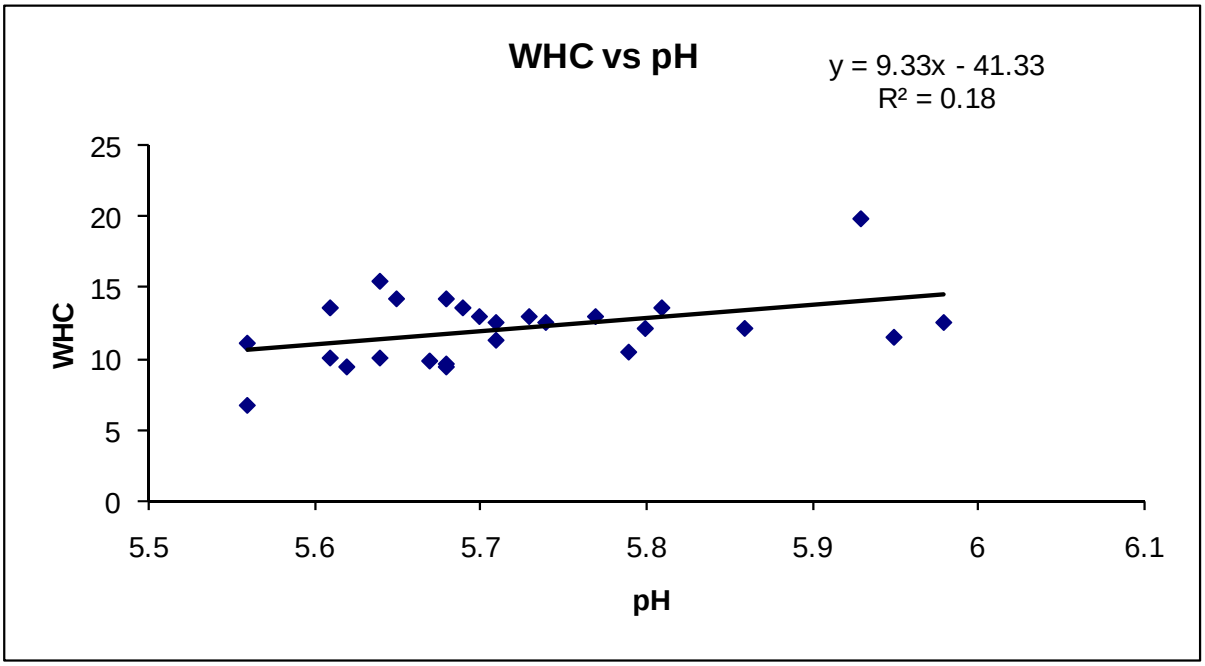

Figure 3.7. Relationship between water holding capacity and pH of broilers randomly selected. Pearson's correlation coefficient = 0.42. N=26

CHAPTER 4

EVALUATION OF PROTEIN DIFFERENCES BETWEEN PALE AND NORMAL BROILER BREAST MEAT THROUGH ONE DIMENSIONAL ELECTROPHORESIS

Samuel,D. and L.Wicker. To be submitted to *Poultry Science*

ABSTRACT

Samples from normal and pale broiler breast fillets were analyzed using SDS-PAGE, water holding capacity and protein solubility studies. Results showed that pale (L* = 66.0) broiler breast meat exhibited lower water holding capacity, protein solubility, and pH. The average protein concentration (2.25 mg/ml) of water soluble proteins in pale breast muscle was less than half the concentration (5.15 mg/ml) found in normal meat. The average water holding capacity for pale samples was 14.5 % compared to 18.3% for normal samples. The presence and intensity of protein bands on SDS-PAGE were similar in water soluble and salt soluble extracts from pale and normal muscle. A peptide that migrated to the molecular weight of water soluble phosphorylase was consistently present in myofibrillar fractions of both normal and pale broiler breast fillets. Thus, a paler color in poultry breast meat may influence protein solubilities and water holding capacity but cannot be traced back to specific protein differences.

INTRODUCTION

Anthony (1998) states that the success of the poultry industry has been fueled by the ability to economically produce acceptable products. High selection intensities, shorter generation intervals and reduced environmental influences have contributed to this success. However, the resulting reduced slaughter age, increased body weight at younger ages, and improved muscle yields and feed conversion have resulted in a reduction of meat quality associated with muscle texture and flavor (Anthony, 1998). Research has shown that the poultry industry is facing similar problems with meat quality that resemble the pale, soft and exudative (PSE) condition in pigs (Barbut, 1997; Owens et al., 2000; Woelfel et al., 2002). In contrast to pigs, the genetic basis of meat quality alterations in poultry is not known (von Lengerken, 2002).

Pale, soft, and exudative breast meat is associated with a rapid postmortem pH decline at high carcass temperatures resulting in protein denaturation. The reduced water-holding capacity (WHC) causes excessive yield losses (Alvarado, 2003) as studies have shown that PSE in poultry has been reported to occur as high as 50% in commercial plants (Barbut, 1996; Barbut, 1997; McCurdy et al., 1996; Owens et al., 2000; Woelfel et al., 2002; Woelfel and Sams, 2001).

However, a selection for rapid growth in poultry, both turkey and broilers, has resulted in abnormally large muscle fibers with less developed connective tissue (Swatland, 1989). It has been hypothesized that a certain population of commercial turkeys may have an altered sarcoplasmic reticulum Ca^{++} channel protein leading to the development of PSE meat (Wang et al., 1999). These alterations strongly influence the biochemical changes occurring in the muscle during rigor mortis, which is directly responsible for PSE (Wang, 1999).

Because proteomics can be used to translate genomic information such that it is useful in biological studies, a proteomic analysis of PSE- like meat in poultry meat may provide additional

information towards understanding the characterization of PSE meat to the poultry industry. Proteomic research in the poultry field has covered some important posttranslational modifications including myosin degradation and identification of specific oxidatively modified proteins in chicken meat (Ikeuchi, 2001; Kamiyama, 2001; Stagsted, 2004). Pietrzak, et al., (1997) used proteomics to determine that the development of PSE turkey breast muscle was due to irreversible myosin insolubility resulting from low pH and high-temperature conditions. Currently, the genetic basis for PSE in chicken has not been determined (Barbut et al., 2008). Mullen et al., (2006) stated that proteins are frequently the functional molecules and are most likely to reflect differences in gene expression. Thus, the objective of this research is to utilize one-dimensional electrophoresis to confirm obvious differences in protein expressions between PSE and normal broiler breast meat. PSE and normal breast meat were classified based upon L*, pH, and water holding capacity (WHC).

MATERIALS AND METHODS

Sample Collection

Boneless, skinless, broiler breast meat was obtained from a local commercial deboning plant. Samples were taken from birds selected for improved breast yield and an average weight of over 7 lbs. Samples were subjectively selected according to lightness before measurements were taken. The samples were put on ice and taken back to the university laboratory for water holding capacity analysis. The right breast portion was used to determine L* a* b* while the left breast portion was frozen until used to determine the corresponding WHC and protein solubilities of the breast sample. Birds with a pH < 5.6 and an L* greater than 66 were classified

as pale birds. There were a total of 33 pale and 49 normal samples classified. Excessive fat and connective tissue were avoided to minimize sampling errors.

Color and pH Measurements

L* was measured at the medial point of the dorsal and ventral surfaces of the pectoralis major using a Minolta Chroma Meter CR-310, Osaka, Japan. Reported values are the average of three measurement per sample. The pH measurements were acquired through use of a spear tip probe pH meter (Hannah Instruments, Van Nuys, CA) designed for meat samples. All measurements were taken 24 hours postmortem as described by Samuel et al., (2008).

Water Holding Capacity (WHC)

The water holding capacity (WHC) was determined as described by Samuel et al., 2008 using a centrifugation method. Measurements were done in triplicate.

Protein Extraction and Quantification

Extraction procedures were similar to those used by Barbut et al. (2005). A phosphate buffer was used in addition to NaCl. Sarcoplasmic proteins were extracted from 2 gm of meat sample using 20 ml of 0.025M sodium phosphate buffer (Buffer A, pH=7.2). The samples were homogenized with a PRO300A Proscientific homogenizer (Sparks Technologies, Buford, GA) on ice with low setting for 1 min. The samples were centrifuged in a Sorvall RC-5B refrigerated super-speed centrifuge (Du Pont Instruments, Wilmington, DE) at 7000 g for 15 min at 4°C. The supernatants were collected and labeled as sarcoplasmic or water soluble proteins. The pellets were then mixed with 0.025 M of sodium phosphate buffer, pH=7.2 containing 0.6 M of NaCl (Buffer B) and homogenized for 1 min. To ensure solubilization of the salt soluble proteins, the

samples were stirred for 4 hours on ice. The samples were centrifuged at 7000 g for 15 min at 4°C and the supernatants were collected as myofibrillar or salt soluble proteins. The concentration of proteins in the supernatants was determined in triplicate using the Better Bradford Assay (Coomassie Plus-The Better Bradford Assay Kit, PIERCE, IL) and measured using a micro-plate reader (Bio-Rad model 550 micro-plate reader, Hercules, CA) at 595nm. The standard protein used was bovine gamma globulin.

SDS-PAGE

SDS-PAGE was performed using a Mini-PROTEAN II electrophoresis cell unit (BioRad Laboratories, Hercules, California) and a PhastSystem Unit (GE Healthcare, USA) according to manufacturer's instructions. An aliquot of 15 µg per sample in sample buffer was applied to a 4-20% gradient gel (BioRad Laboratories, Hercules, California) for the Mini-PROTEAN II and an aliquot of 3 µg was applied to a 4-15% gradient gel (GE Healthcare, USA) for the PhastSystem. A broad range of molecular weight standards from Biorad (Catalog # 161-0317, Control 310002095) were also applied to the gel for a reference. Two pale and two normal samples were each ran three times on each system using coomassie staining.

Densitometry

Gels were scanned using the Biorad Model GS-700 Imaging Densitometer. Analysis and molecular weights were determined using the Molecular Analyst/PC Software (Version 1.5).

RESULTS AND DISCUSSION

The pale breast fillets had lower protein concentration and lower water holding capacity than the normal and dark breast fillets. Protein solubility samples (Table 4.1) were taken as a

subset from a larger sample size (Table 4.2). As shown in Table 4.1, the pale breast fillets had an average L* of 66.0. The average water holding capacity was 13.8. The average protein concentrations for the water soluble proteins and salt soluble proteins were 2.2 mg/ml and 7.01 mg/ml, respectively. The normal breast fillets had an L* lower than the pale breast fillets. These also exhibited higher water holding capacities as well as higher protein concentrations than pale samples. The average L* for normal breast fillets was 57.9 and the water holding capacity was 24.9%. Protein concentrations for water soluble and salt soluble proteins were 5.1 mg/ml and 7.7 mg/ml, respectively. Offer and Knight (1988) found that WHC of meat was highly dependent upon pH and protein denaturation which can be measured by protein solubility. Van Laack et al. (2000) found very small but significant differences in total protein solubility between pale and normal breast fillets. However, they found no correlation between the total protein solubility and water holding capacity. Table 4.2 shows data from the larger sampling plan used to correlate L8, pH, and WHC. The data is consistent with the findings of Van Laack et al. (2000). The average L* for all pale breast fillets was 63.2 with a pH of 5.5 yielding an average water holding capacity of 14.5 percent. Normal breast fillets had an oveall mean L* of 59.1, pH of 5.7, and water holding capacity of 18.3 percent. ` Van Laack et al. (2000) found that low ultimate pH was the main determinant in water holding capacity of PSE chicken breast muscle.

Also the data in Table 4.1 shows that the protein concentration of the water soluble proteins in the normal breast fillets was more than twice the concentration found in the pale breast fillets. The comparison of protein concentrations within the salt soluble proteins was not as profound as that in the water soluble proteins. Van Laack et al. (2000) reported that the protein solubility of sarcoplasmic or water soluble proteins in pale breast fillets was significantly lower than that in normal breast fillets. Bendall and Wismer-Pedersen (1962) showed that

sarcoplasmic proteins were denatured in meat with a low WHC, an observation which was confirmed by several other researchers (Lopez-Bote et al., 1989; Van Laack et al., 1994; and Warner et al., 1997). It has been suggested that the denatured sarcoplasmic proteins, mostly phosphorylase, precipitate onto the myofibrils covering the charged groups responsible for water binding (Bendall and Wismer-Pedersen, 1962). Van Laack et al. (2000) also reported a significant correlation between sarcoplasmic protein solubility and water holding capacity. However, Offer and Knight (1988) found that the myofibrillar proteins had greater influence on water holding capacity than the sarcoplasmic proteins. Several researchers reported that in PSE pork, the solubility of both sarcoplasmic and myofibrillar proteins correlated with water holding capacity (Bendall and Wismer-Pedersen, 1962; Bendall and Swatland, 1988; Warner et al., 1996). In addition, Warner et al. (1996) showed that protein solubilities were similar in reddish, soft, exudative and normal pork (RSE) despite lower water holding capacity of the RSE pork.

SDS-PAGE was utilized to determine if there were any pronounced differences in proteins between pale and normal breast meat. Estimated molecular weights of water and salt soluble proteins were identified by the molecular analyst software used with densitometry. There were a total of nine protein bands found in the water soluble protein extracts (Figure 4.1). Their molecular weights ranged from approximately 97,000 to 20,000 daltons. The highest molecular weight identified migrated approximately the same distance as phosphorylase found in the molecular weight standards. This protein precipitated onto the myofibrils in PSE turkey meat (Pietrzak, 1997) and in PSE broiler meat (Barbut et al., 2005). In our study, the protein band associated with phosphorylase was present in water soluble protein extracts of both normal and pale breast meat. Notably, there were no missing protein bands in water soluble protein extracts of normal and pale samples (Figures 4.1 and 4.2). Although the same peaks were consistently

identified, peak intensities were different among pale and normal samples but with great variation within replications (data not shown).

In salt soluble protein extracts, an estimated fourteen protein bands were observed. The salt soluble proteins showed no distinct differences between the protein bands. In contrast to a report by Barbut et al. (2005), myosin was present in all samples. The difference may be attributed to the difference in extraction buffers. For this study, a phosphate containing buffer was used whereas only NaCl was used by Barbut et al., (2005). Pietrzak et al. (1997) found that more myosin was solubilized when a combination of 2% NaCl and 0.15% Na-tripolyphosphate was used compared to when 2% NaCl was used alone as an extraction solvent.

Within the salt soluble protein extracts, a protein band that migrates between 98,000 and 101,000 daltons, putatively identified as phosphorylase, was observed in pale and normal breast. Van Laack et al. (2000) also found phosphorylase in the myofibrillar fraction in both normal and pale broiler samples, which could indicate that protein denaturation is similar in both normal and pale broiler meat. Wilson and Van Laack (1999) studied PSE and normal meat in pork and reported the presence of phosphorylase bands in the myofibril fractions of PSE meat but not in the normal meat. In the present study, phosphorylase was found to be present in the myofibril fractions of both normal and pale samples. This was consistent with characteristics exhibited by reddish soft exudative (RSE) pork (Warner et al., 1997). Warner et al. (1997) also reported that RSE pork exhibited lower water holding capacity compared to normal pork. The similarity between RSE pork and findings in the present study may provide an answer to changes in meat quality, such as lower water capacity resulting from birds selected for growth (Piles et al., 2000; Ramirez et al., 2004). While the missing protein bands seen in pork samples may relate to the

genetic differences responsible for PSE pork meat, it may also explain why no specific genes have been identified with PSE broiler meat.

CONCLUSIONS

Pale broiler breast meat exhibited lower water holding capacity, protein solubility and lower pH. Protein concentrations of sarcoplasmic proteins were almost half the concentration found in normal and dark meat. While salt soluble proteins are associated with functional properties of muscle foods, lower concentrations of water soluble proteins extracted from pale muscle was observed in conjunction with lower WHC in this study. SDS-PAGE showed no distinct protein bands absent within the water soluble or salt soluble proteins of either normal or pale samples. The use of a combined phosphate and NaCl extraction buffer may have resulted in the solubility of more myosin. Putative water soluble phosphorylase was consistently present in myofibrillar fractions of both normal and pale broiler breast meat. This suggests that changes in water soluble proteins such asloss of protein solubility or denaturation is involved in lower WHC. This data is consistent with what was seen in RSE pork reported by Warner et al., (1997) and may provide an answer to changes in meat quality such as lower water capacity resulting from birds selected for growth. Thus, PSE in poultry meat may influence water holding capacity but may not be able to be traced back to specific proteins and genes.

REFERENCES

Alvarado, C. Z. and A.R. Sams. 2003. Injection marination strategies for remediation of pale, exudative broiler breast meat. Poultry Science 82:1332-1336.

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

Barbut, S. 1996. Estimates and detection of the PSE problem in young turkey breast meat. Canadian Journal of Animal Science 76:455-457.

Barbut, S. 1997. Occurrence of pale soft exudative meat in mature turkey hens. British Poultry Science 38:74-77.

Ikeuchi, Y., K., Kamiyama, A., Suzuki, T., Hirose, K., Kim, T., Hayashi, and T., Ito. 2001. Monitoring myosin degradation during conditioning in chicken meat using an immunological method. Journal of Food Science 66:1119-1125.

Kamiyama, K., Y., Ikeuchi, A., Suzuki, T., Hirose, K., Kim, T., Hayashi, and T., Ito, 2001. An immunological assessment of myosin degradation in pressurized chicken muscle. Journal of Food Science 66:1126-1129.

McCurdy, R. D., S. Barbut, and M. Quinton. 1996. Seasonal effect on pale soft exudative (PSE) occurrence in young turkey breast meat. Food Research International 29:363-366.

Mullen, A. M., P. C. Stapleton, D. Corcoran, R. M. Hamill, and A. White. 2006. Understanding meat quality through the application of genomic and proteomic approaches. Meat Science 74:3-16.

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

Pietrzak, M., M.L. Greaser, and A.A. Sosnicki. 1997. Effect of rapid rigor mortis processes on protein functionality in pectoralis major muscle of domestic turkeys. Journal of Animal Science 75:2106-2116.

Samuel, D., D. Pringle, L. Billard, and L. Wicker. 2008 The relationship between production and processing factors on color changes in broiler breast meat and the implications on quality. Proceedings of XXIII World's Poultry Congress, World Poultry Science Journal.

Stagsted, J., E. Bendixen,and H. Andersen. 2004. Identification of specific oxidatively modified proteins in chicken muscles using a combined immunologic and proteomic approach. Journal of Agricultural and Food Chemistry 52:3967-3974.

Swatland, H. J. 1989. Physiology of muscle growth. . Pages 167-182 in Recent Advances in Turkey Science. C. a. G. Nixey, T.C. ed. Buttersworths & Co., Borough Green, United Kingdom.

von Lengerken, G., S. Maak, and M. Wicke. 2002. Muscle metabolism and meat quality of pigs and poultry. Veterinarija IR Zootechnika 20:82-85.

Wang, L. J.,T.M. Byrem, J. Zarosley, A.M. Booren, and G.M. Strasburg. 1999. Skeletal muscle calcium channel ryanodine binding activity in genetically unimproved and commercial turkey populations. Poultry Science 78:792-797.

Warner, R. D., R. G. Kauffman, and M. L. Greaser. 1997. Muscle protein changes post mortem in relation to pork quality traits. Meat Science 45:339-352.

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

Woelfel, R. L., and A. R. Sams. 2001. Marination performance of pale broiler breast meat.

Poultry Science 80:1519-1522.

Table 4.1. Protein solubilities of pale and normal broiler breast fillets

Sample	L*	pH	WHC (%)	WSP (mg/ml)	SSP (mg/ml)
Pale	66.0(0.8)	5.2(0.3)	13.8(2.4)	2.2(0.8)	7.0(1.2)
Normal	57.9(2.1)	5.7(0.2)	24.9(0.4)	5.1(0.4)	7.7(0.7)

WHC= water holding capacity, WSP= water soluble protein, SSP= salt soluble protein
Average values reported with standard deviation in parenthesis.

Table 4.2. Means for L*, pH, and water holding capacity from pale and normal broiler breast fillets

	L*(STDEV)	pH (STDEV)	WHC (STDEV)
Pale	63.2 (1.89)	5.5 (0.13)	14.5 (0.023)
Normal	59.1 (1.71)	5.7 (0.10)	18.3 (0.038)

[a]N=33
[b]N=49

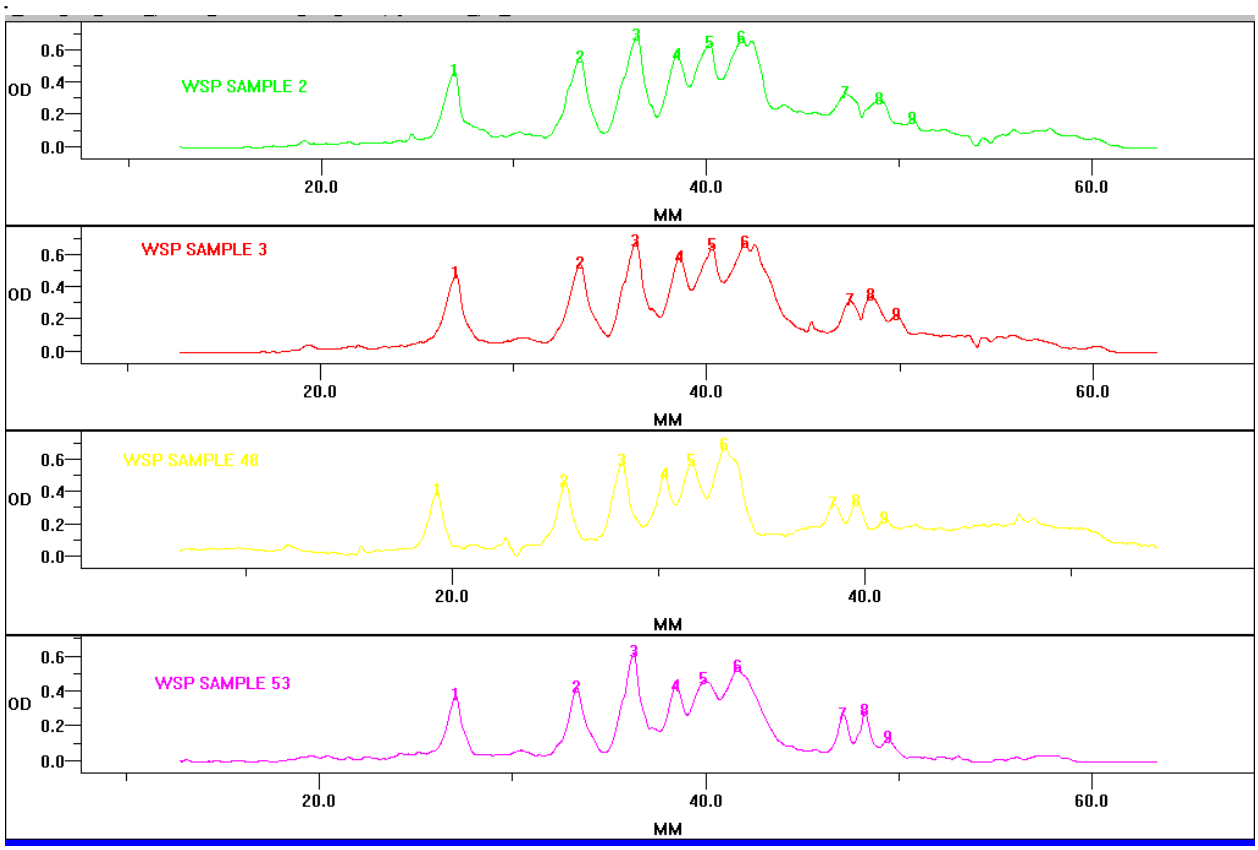

Figure 4.1. Peak identifications of water soluble proteins from normal and pale broiler breast fillets.. Samples 2 and 48 are from normal muscle and samples 3 and 53 are from pale muscle.

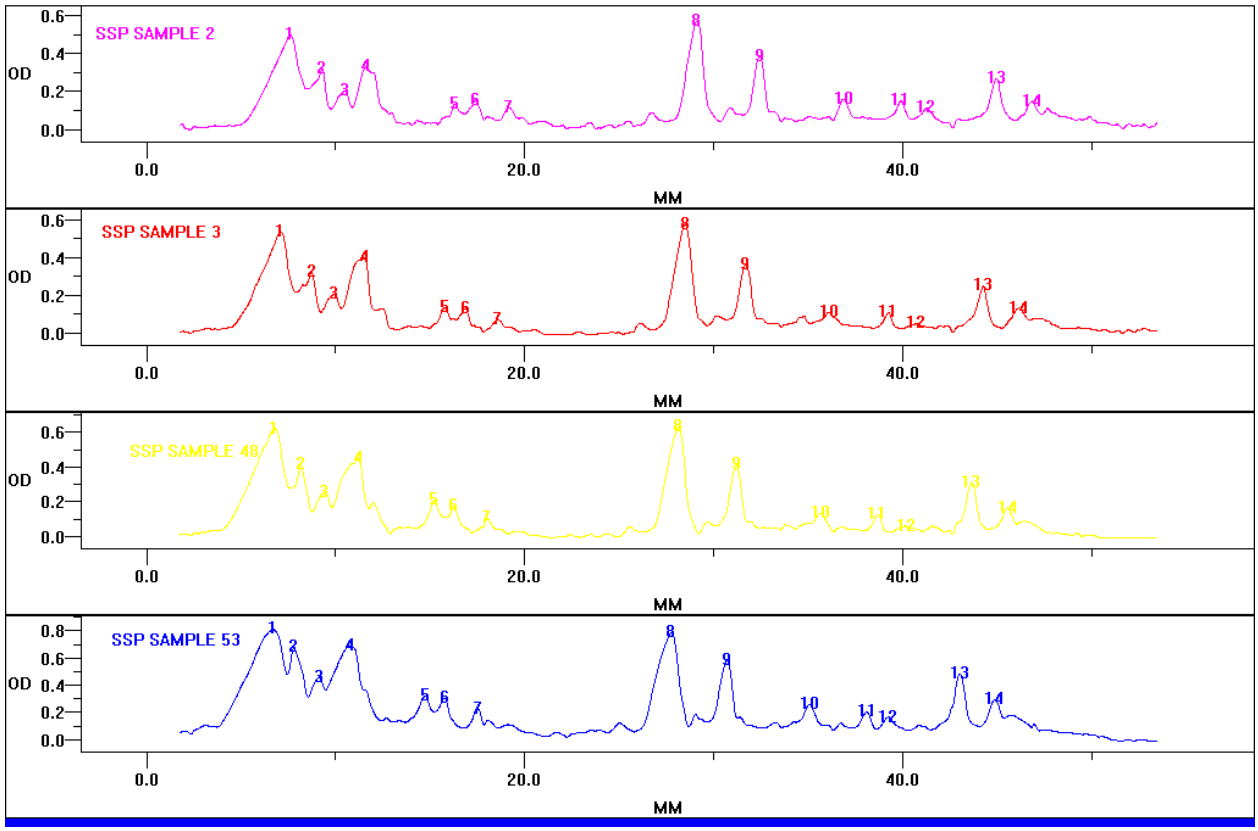

Figure 4.2. Peak identifications of salt soluble proteins from normal and pale broiler breast fillets. Samples 2 and 48 are from normal muscle and samples 3 and 53 are from pale muscle.

CHAPTER 5

VISIBLE/NEAR-INFRARED SPECTROSCOPY TO PREDICT PALE BROILER BREAST MEAT BY MEASURING WATER HOLDING CAPACITY

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ABSTRACT

In this study, Visible/Near-infrared spectroscopy (Vis/NIRS) was examined as a tool for rapidly determining water holding capacity (WHC) in broiler breast meat. Both partial least squares (PLS) and principal component analysis (PCA) models were developed to relate Vis/NIRS spectra of 85 broiler breast meat to WHC, pH, and L*. Partial least squares modeling resulted in coefficients of determinants (R^2) of 0.72, 0.67, and 0.62 for WHC, pH, and L*, respectively. The mean spectra from the PLS loading plots showed that absorption peaks, in the range of 415 nm to 609 nm (heme pigments) and 1140 nm,~1385 nm, and ~1880 nm (fat, water, and protein bands), had the greatest influence on the predictive models. PCA and discriminant analysis showed distinct differences between samples with pH values above and below 5.6. The results suggest that Vis/NIRS predicts water holding capacity in broiler breast meat.

INTRODUCTION

The meat industry has increased interest in rapid screening techniques used to predict quality characteristics (Geesink et al., 2003). Spectroscopy has become a more desirable method for analyzing qualitative characteristics in food due to a decrease in instrument prices, an improvement in equipment design, and an improvement in data-analysis methodology such as chemometrics (Brondum et al., 2000a). Brondom et al. (2000a) lists the main advantages of using spectroscopic measurements as rapid data acquisition with minimum sample preparation, the possibilities for simultaneous determination of several quality parameters, and the ability to replace expensive and slower reference techniques. Monin (1998) considered near infrared spectroscopy (NIRS) to be one of the most promising of these techniques for large-scale meat quality evaluation.

Several applications of NIRS as a qualitative and quantitative measurement tool have been applied to the meat industry. Research involving NIRS and meat include fat and moisture contents in emulsions of meat products (Ben-Gera and Norris, 1968; Iwamoto et al., 1981; Kruggel et al., 1981; Lanza, 1983); fat, moisture, and protein contents (Renden et al., 1986; Valdes and Summers, 1986; Isaksson, et al., 1995; Kadim et al., 2005; Berzaghi et al., 2005); fat depths and softness (Swatland, 1995); and meat texture and tenderness (Lyon et al., 2001; Park et al., 2001; Liu et al., 2003; Muellenet et al., 2004). NIRS has also been utilized to evaluate pigment content in meat (Mitsumoto et al., 1991; Chen and Massie, 1993; Chen et al., 1994; Chen et al., 1996a; Chen et al., 1996b). Huang et al. (2008) concluded that NIRS analysis is capable of rapid assessment of fat, water, and protein composition. In addition, Cozzolino and Murray (2004) used NIRS to correctly classify 80% of meat samples according to the muscle species (beef, lamb, pork, or chicken). Muellenet et al. (2004) suggested that near infrared

reflectance could be used to predict poultry meat texture and to classify muscles according to tenderness levels. Park et al. (1998) used NIRS to predict Warner-Bratzler shear force values of beef longissimus steaks.

More recent applications of NIRS involved the determination of water binding characteristics, water holding capacity and drip loss in fresh pork. NIRS analysis at 24 h post mortem was used to predict drip loss in pork 24 h after slaughter (Forest et al., 2000). NIRS was used to predict intra-muscular fat and drip loss, (Geesink et al., 2003; Hoving-Bolink et al., 2005) with varying predictive accuracy. Their results indicated that NIRS does enable the classification of pork longissimus muscles with a superior or inferior water-holding capacity.

Aberle et al. (2001) describes water holding capacity as the ability to retain naturally occurring or added water during application of external forces such as cutting, heating, grinding, or pressing. Muscle tissue consists of approximately 75% water, 20% protein, 5% fat, 1% carbohydrate, and 1.5% vitamins and minerals (Huff-Lonergan and Lonergan, 2006). Belitz (2004) states that approximately 5% of the water found in muscle is bound by hydrophilic groups on the proteins and the other 95% is held by capillary forces between the thick and thin filaments. Some of the water in muscle is also found in free form and will be expelled during even the mildest form of applications. The ability of meat to retain water is influenced by several factors. Those factors include production of lactic acid and loss of adenosine triphosphate (ATP), which influences rate and extent of pH decline, protein oxidation, and changes in cell structure associated with proteolytic enzyme activity (Aberle et al., 2001; Huff-Lonergan and Lonergan, 2005). Many of the quality attributes of meat such as color, texture, and juiciness are influenced by water-holding capacity (Aberle et al., 2001).

One of the major contributors to decreased WHC in the poultry industry has been attributed to pale, soft, and exudative (PSE) broiler breast meat. PSE breast meat is caused by a rapid postmortem pH decline while carcass temperatures are still warm. The resulting protein denaturation leads to a pale color and a decrease in WHC, causing excessive yield losses (Alvarado and Sams, 2003). PSE incidence in broilers and turkeys has been reported to occur as high as 50% in commercial plants (Barbut, 1996; Barbut, 1997; McCurdy et al., 1996; Owens et al., 2000; Woelfel et al., 2002; Woelfel and Sams, 2001).

In the poultry industry good water holding capacity is essential to maintain high yields, to avoid purge, to ensure juicy muscle and to maintain quality. The objective of this study was to evaluate the role of L*, pH on WHC of broiler breast muscle and to use Vis/NIRS to predict WHC.

MATERIALS AND METHODS

Sample Collection

Boneless, skinless, broiler breast meat was obtained from a local commercial processing plant. Samples were subjectively pre-selected based upon visible lightness or darkness and pre-classified into pale and normal categories. The samples were put on ice and taken back to the university laboratory for water holding capacity analysis and Russell Research Center for Vis/NIRS measurement. The left breast portion was used to determine the WHC of the breast sample while the right breast portion was used to determine L*, a*, b*, and Vis/NIR spectra. The left breast fillets were individually packed in Ziploc bags on day 1 and frozen at -30° C. Broiler breasts were classified into pale or normal based on pH values less than or greater pH 5.6, respectively. For broiler breasts classified as pale, the mean WHC and L* were 14.2% and

66.7%, respectively. These values were used to construct the mean Vis-NIR spectra for WHC and L* in pale and normal broiler breast samples. Excessive fat and connective tissue were avoided to minimize sampling errors.

Color and pH Measurements

L* was determined on the dorsal surface of the pectoralis major using a Minolta Chroma Meter (CR-310, Osaka, Japan). The pH measurements were acquired through use of a spear tip Hannah pH meter (Hannah Instruments, Van Nuys, CA) designed for meat samples. All color and pH measurements were taken 24 hours postmortem.

Water Holding Capacity

The WHC was determined as described by Barbut (1993) with some minor modifications. Frozen samples were thawed overnight at 4°C. All skin and visible fat were removed from the breast meat. Approximately 75 to 100g of the medial portion of the breast meat was chopped for approximately 60 seconds in a small chopper to mince meat. A 10 g aliquot of the chopped muscle was mixed with 16 ml of 0.6 M NaCl and then incubated for 30 min at 4°C. Afterwards samples were centrifuged at 7000 g at 4ºC for 15 minutes and the excess fluid was decanted. The WHC was defined as the portion of fluid retained by the sample. Measurements were done in triplicate assays.

Visible/Near-infrared Spectral Analysis

Samples for Vis/NIRS analysis were taken from the anterior portion of broiler breast meat and cores with a 38 mm diameter and 10 mm depth were collected for Vis/NIRS in the

quartz optical cell as described earlier (Liu et al., 2004; Park et al., 1998). The raw core samples from broiler breast meat were scanned using a scanning monochromator (XDS Instrument, Foss NIRSystems, Inc.Laurel, Md) and analyzed with Vision Spectral Analysis Software for Windows (Foss NIRSystems Vision). Reflectance measurements were recorded over the 400 to 2500 nm wavelength range at 2 nm intervals and 32 scans. A sample size of 85 cored breast samples were utilized (Park et al., 2001). For mean spectra, delineation between pale and normal was based on the mean value of WHC or L* for pale muscle.

Chemometric Analysis

The spectra of 85 breast fillets were divided into a calibration subset and a validation subset. Partial least squares (PLS), multiple linear regression (MLR), and principal component regression (PCR) analyses were performed to develop calibration models.

The PLS procedure was applied directly to the log(1/R) where R=reflectance spectra in the wavelength region of 400 to 2500 nm. Cross-validation was performed during model development. A mean-centering data processing algorithm was applied to the calibration model. On completion of calibration, the model was applied to the validation data set. Model performance was reported as the coefficient of determination (R^2), root mean square error of calibration (RMSEC), and root mean square error of cross-validation (RMSECV).

For spectral pretreatment of the MLR procedure, mean smoothing was conducted with three-point averaging (Hruschka, 1987) followed by log(1/R) transformation and computation of second derivatives. The MLR procedure was performed in a stepwise manner to yield the highest coefficient of determination (R^2).

PCR based discriminant analysis was obtained for classification of pale and normal breast. PCA was used to decompress spectra and produce a reduced representation of the data based upon maximum variations between the spectra. PCA score plots were generated from this data.

RESULTS AND DISCUSSION

The data in Table 5.1 shows the range, mean, and standard deviation for WHC, pH, and L* of the samples analyzed by Vis/NIRS in evaluating its use as a rapid method for detecting pale birds indicative of low water holding capacity. L* ranged from 59.6 to 71.7 with a mean of 65.6 and a standard deviation of 2.33. The pH values ranged from 5.0 to 6.2 with an average pH of 5.6 and a standard deviation of 0.16. Corresponding water holding capacities were as low as 9.4% and as high as 26.9%, with a mean of 16.7% and a standard deviation of 3.8 (Table 5.1). The data in Figure 5.1 shows the mean Vis/NIRS spectrum and the total Vis/NIRS spectra for these 85 samples. The absorbance bands of the spectra show distinct differences between samples with the greatest difference seen between wavelengths ~1400- 2400 nm. The data in Figures 5.2 through 5.4 show differences in mean spectra of high and low values of pH, WHC, and L*, respectively. The mean VIS/NIRS spectra for breast muscle samples with pH values above and below 5.6 (Figure 5.2) show differences in the spectra at approximate absorbance peaks between 400 and 800 nm and between 1400 through 2500 nm. The absorbance of high pH samples was higher than absorbance of low pH samples around these wavelength bands. Mean spectra comparing high and low WHC and L* show similar differences observed in the mean pH spectra with the exception that differences are not as great at wavelengths greater than 1900 nm for L* and greater than 2000 nm for WHC values (Figures 5.3 and 5.4). The differences between

spectra at wavelengths 400 nm to ~850 nm and ~1500 nm and 2000 are the greatest in the mean spectra for L* above 68 and below 66 (Figure 5.4). The samples with higher L* showed lower absorbance at those wavelength bands. The broiler breasts were first sorted into pale or normal by pH less or greater than 5.6. No definitive congruity between segregation of broiler breasts by pH and L* and WHC was observed. Therefore, mean spectra were collected one unit away from the mean for L* and WHC. While broiler breasts that were classified as normal had one or two of 42 readings that were out or range for WHC and L*, respectively, broiler breasts that were classified as pale had nine and ten of 43 readings that were out of range for WHC and L*, respectively. These results highlight the difficulty of identifying pale or PSE muscle in poultry based on L* or pH value. Nevertheless, Vis/NIRS provides clues to potential biochemical differences between pale and normal muscle.

Isengard (1995) states that water gives signals at 1460 and 1910 nm wavelengths that are usually stronger than those of other components. However these signals from water can hide signals from other components in the spectra, such as proteins, which are usually found around 1485 nm and above approximately 2055 nm (Brøndum, et al., 2000b). From spectra obtained from intact chicken muscles, Cozzolino et al. (1996) identified bands at 422 nm and 552 nm related to myoglobin and at 1936 nm related to water. Absorption bands at 430 nm and 574 nm were associated with hemoglobin and oxyhemoglobin, respectively (Mitsumoto et al., 1991). Cozzolino et al., (2004) showed absorption bands at 540 nm and 580 nm that were associated with both myoglobin and oxymyoglobin while Cozzolino and Murray (2002) showed an absorption band at 762 nm related to the oxidation of myoglobin or deoxymyoglobin. Results from the present study show that the mean spectra of breast muscle with pH values below 5.6 fall

slightly below the mean spectra for samples with a pH above 5.6 at wavelengths less than 800 and greater than 1200 (Figure 5.2).

The data in Figure 5.5 displays the PLS model and loading plot that corresponds with water holding capacity of the samples analyzed by Vis/NIRS. The coefficient of determination in cross validation (R^2), the root mean square error of calibration (RMSEC), and root mean square error of cross-validation (RMSECV) were 0.719, 0.019, and 0.028, respectively.

The PLS loading plots in Figure 5.5 show that the wavelengths of ~400 nm to 600 nm that were previously associated with heme pigments (Mitsumoto et al., 1991; Cozzolino et al., 1996, Cozzolino and Murray, 2002; and Cozzolino et al., 2004) and the wavelengths at ~1450 nm to 1950 nm that were previously associated with water (Isengard, 1995) had the greatest influence in developing the calibration models. The PLS models and loading plots for the pH values and L* (Figures 5.6 and 5.7) of broiler breast samples resulted in lower prediction values than the PLS model for water holding capacity. The coefficients of determination were 0.674 and 0.617, respectively. The resulting RMSEC values for pH and L* were 0.07 and 1.27, respectively, while the respective RMSECV values were 0.10 and 1.54. PLS models for water holding capacity and pH both utilized two latent variables. Latent variables are the common orthogonal structures in which PLS project the spectral data after centering it and are used to describe the maximum covariance between the spectral information and the references respectively (Brøndum et al., 2000b). The PLS loading plots based on pH of the breast fillets were similar to the WHC PLS loading plots in that the wavelengths relating to respiratory pigments and water had the greatest influence in developing the calibration models. While PLS loading plots were extremely similar, the PLS loading plots for L* of the samples showed influence by fewer wavelengths associated with the heme pigments and additional wavelengths

between 1100 nm and 1900 nm, most likely associated with water and other proximate compositions.

When analyzing spectroscopy data, many of the data points are reduced to a few factors, or scores, because they are extremely co-linear. These scores are combined with the latent variables to form the principal components (Brondum, et al., 2000). The PCA score plots for pH of the breast samples are shown in Figure 5.8. A distinct difference between the samples at pH values lower or higher than 5.6 can be seen. The discriminate analysis shown in Figure 5.9 also shows differences seen between the low and high pH based upon four principal components. The resulting coefficient of determination was 0.93 along with a RMSEC of 0.12. The cross-validation model showed a root mean standard error of prediction (RMSEP) value of 0.20. These results indicate the importance of pH in determining the WHC of broiler meat and its influence in using Vis/NIRS as a predictor of WHC. Offer and Knight (1988) found that pH was one of the main determinants of water holding capacity. Diesbourg et al. (1988) showed that as the pH reaches ~5.2-5-5, the isoelectric point of myosin, the distance between thick filaments in pork muscle was drastically reduced. The decline in pH induces lateral shrinkage of the myofibril leading to expulsion of water from the myofibrillar into the extramyofibrillar spaces of the muscle cells (Bendall and Swatland, 1988). Huff-Lonergan and Lonergan (2005) states that it is thus likely that the gradual mobilization of water from the intramyofibrillar spaces to the extramyofibrillar spaces may be responsible some drip loss.

CONCLUSIONS

The results from the PLS model suggest that Vis/NIRS has the potential to predict water holding capacity in broiler breast meat. PLS also showed that pH was a better predictor of

WHC than the L*. Discriminant analysis showed distinct differences between low and high pH samples. The mean spectra for the low and high pH, WHC, and L* of the samples showed differences in wavelengths associated mainly with the heme pigments, myoglobin and hemoglobin, water and protein.

REFERENCES

Alvarado, C. Z. and A.R. Sams. 2003. Injection marination strategies for remediation of pale exudative broiler breast meat. Poultry Science 82:1332-1336.

Barbut, S. 1993. Colour measurements for evaluating the pale soft exudative (PSE) occurrence in turkey meat. Food Research International 26:39-43.

Barbut, S. 1996. Estimates and detection of the PSE problem in young turkey breast meat. Canadian Journal of Animal Science 76:455-457.

Barbut, S. 1997. Occurrence of pale soft exudative meat in mature turkey hens. British Poultry Science 38:74-77.

Ben-Gera, I. and K. H. Norris. 1968. Direct spectrophotometric determination of fat and moisture in meat products. Journal of Food Science 33:64.

Berzaghi, P., A. Zotte, L.M. Jansson, and I. Andrighetto. 2005. Near-infrared reflectance spectroscopy as a method to predict chemical composition of breast meat and discriminate between different n-3 feeding sources. Poultry Science 84:128-136.

Brøndum, J., D. V. Byrne, L. S. Bak, G. Bertelsen, and S. B. Engelsen. 2000a. Warmed-over flavour in porcine meat -- a combined spectroscopic, sensory and chemometric study. Meat Science 54:83-95.

Brøndum, J., L. Munck, P. Henckel, A. Karlsson, E. Tornberg, and S. B. Engelsen. 2000b. Prediction of water-holding capacity and composition of porcine meat by comparative spectroscopy. Meat Science 55:177-185.

Chen, Y. R., and D.R. Massie. 1993. Visible/near-infrared reflectance and interactance spectroscopy for detection of abnormal poultry carcasses. Trans. ASAE 36:863-869.

Chen, Y. R., B. Park, and R.W. Huffman. 1994. Instrument inspection of poultry

carcasses. in ASAE-Paper No. 94-6026St. Joseph, MI.

Chen, Y. R., R.W. Huffman, and B. Park. 1996a. Changes in the visible/near-infrared spectra of chicken carcasses in storage. J. Food Process Engineering 19:121-134.

Chen, Y. R., B. Park, M. Nguyen, and R.W. Huffman. 1996b. Instrumental system for on-line inspection of poultry carcasses. SPIE 2786:121-129.

Cozzolino, D., I. Murray, and R. Paterson. 1996. Visible and near infrared reflectance spectroscopy for the determination of moisture, fat and protein in chicken breast and thigh muscle. Journal of Near Infrared Spectroscopy 4:213-223.

Cozzolino, D. and I. Murray. 2002. Effect of sample presentation and animal muscle species on the analysis of meat by near infrared reflectance spectroscopy. Journal of Near Infrared Spectroscopy 10:37-44.

Cozzolino, D. and I. Murray. 2004. Identification of animal meat muscles by visible and near infrared reflectance spectroscopy. Lebensm.-Wiss. u.-Technol. 37:447-452.

Forrest, J. C., M. T. Morgan, C. Borggaard, A. J. Rasmussen, B. L. Jespersen, and J. R. Andersen. 2000. Development of technology for the early post mortem prediction of water holding capacity and drip loss in fresh pork. Meat Science 55:115-122.

Geesink, G. H., F. H. Schreutelkamp, R. Frankhuizen, H. W. Vedder, N. M. Faber, R. W. Kranen, and M. A. Gerritzen. 2003. Prediction of pork quality attributes from near infrared reflectance spectra. Meat Science 65:661-668.

Hoving-Bolink, A. H., H. W. Vedder, J. W. M. Merks, W. J. H. de Klein, H. G. M. Reimert, R. Frankhuizen, W. H. A. M. van den Broek, and e. E. Lambooij. 2005. Perspective of NIRS measurements early post mortem for prediction of pork quality. Meat Science 69:417-423.

Hruschka, W. R. 1987. Data analysis: Wavelength selection methods. in Near-Infrared Technology in the Agricultural and Food Industries. P. C. Williams and K. H. Norris eds. American Association of Cereal Chemists, Inc., St. Paul, Minnesota.

Huff-Lonergan, E., and S. M. Lonergan. 2005. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Science 71:194-204.

Isaksson, T., G. Togersen, A. Iversen, and K.I. Hildrum. 1995. Non-destructive determination of fat, moisture and protein in salmon fillets by use of near-infrared diffuse spectroscopy. J. Sci. Food Ag 69:95-100.

Isengard, H.-D. 1995. Rapid water determination in foodstuffs. Trends in Food Science and Technology 6:155-162.

Iwamoto, M., K.H. Norris, and S. Kimura. 1981. Rapid prediction of chemical compositions for wheat, soybean, pork, and fresh potatoes by near-infrared spectrophotometric analysis. J. Japanese Soc. Food Sci. and Technology 28:85-90.

Juncher, D., Ronn, B. Mortensen, E.T., Henckel, P., Karlsson, A., and Skibsted, L.H. 2001. Effect of pre-slaughter physiological conditions on the oxidative stability of colour and lipid during chill storage of pork. Meat Science 58:347-357.

Kadim, I. T., O. Mahgoub, W. Al-Marzooqi, and K. Annamalai. 2005. Prediction of crude protein, extractable fat, calcium and phosphorus contents of broiler chicken carcasses using near-infrared reflectance spectroscopy. Asian-Australian Journal of Animal Sciences 18:1036-1040.

Kruggel, W. G., R.A. Field, M.L. Riley, H.D. Radloff, and K.M. Horton. 1981. Near-

infrared reflectance determination of fat, protein, and moisture in fresh meat. J. Asso. Off. Anal. Chem. 64:692-696.

Lanza, E. 1983. Determination of moisture, protein, fat, and calories in raw pork and beef by near infrared spectroscopy. Journal of Food Science 48:471-474.

Liu, Y., B. G. Lyon, W. R. Windham, C. E. Realini, T. D. D. Pringle, and S. Duckett. 2003. Prediction of color, texture, and sensory characteristics of beef steaks by visible and near infrared reflectance spectroscopy. A feasibility study. Meat Science 65:1107-1115.

Liu, Y., Lyon, B.G., Windham, W.R., Lyon, C.E., Savage, E.M. 2004. Prediction of physical, color, and sensory characteristics of broiler breasts by visible/near infrared reflectance spectroscopy. Poultry Science 83:1467-1474.

Lyon, B. G., W.R. Windham, C.E. Lyon, and F.E. Barton. 2001. Sensory characteristics and near-infrared spectroscopy of broiler breast meat from various chill-storage regimes. Journal of Food Quality 24:435-452.

Martinaud, A., Y. Mercier, P. Marinova, C. Tassy, P. Gatellier, and M. Renerre. 1997. Comparison of oxidative processes on myofibrillar proteins from beef during maturation and by different model oxidation systems. Journal of Agricultural and Food Chemistry 45:2481-2487.

McCurdy, R. D., S. Barbut, and M. Quinton. 1996. Seasonal effect on pale soft exudative (PSE) occurrence in young turkey breast meat. Food Research International 29:363-366.

Mitsumoto, M., S. Maeda, and T. Mitsuhashi. 1991. Near-infrared spectroscopy determination of physical and chemical characteristics in beef cuts. Journal of Food Science 56:1493-1496.

Monin, G. 1998. Recent methods for predicting quality of whole meat. Meat Science 49:S231-S243.

Muellenet, J. F., E. Jonville, D. Grezes, C.M. Ownes. 2004. Prediction of the texture of cooked poultry pectoralis major muscles by near-infrared refelctance analysis of raw meat. Journal of Texture Studies 35:573-585.

Offer, G., and P. Knight. 1988. The Structural basis of water-holding in meat. Pages 173-243 in Developments in Meat Science. R. A. Lawrie ed. Elsevier Applied Science, London.

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

Park, B., Chen, Y.R., Hruschka, W.R., Shackelford, S.D., Koohmaraie, M. 1998. Near-Infrared reflectance analysis for predicting beef longissimus tenderness. Journal of Animal Science 76:2115-2120.

Park, B., Y.R. Chen, W.R. Hruschka, S.D. Shackelford, and M. Koohmaraie. 2001. Principal component regression of near-infrared reflectance spectra for beef tenderness prediction. Trans. ASAE 44:609-615.

Pietrzak, M., Greaser, M.L., and Sosnicki, A.A. 1997. Effect of rapid rigor mortis processes on protein functionality in pectoralis major muscle of domestic turkeys. Journal of Animal Science 75:2106-2116.

Remignon, H. and E. Le Bihan-Duval. 2003. Meat quality problems associated with selection for increased production. Pages 53-66 in Poultry Genetics, Breeding and Biotechnology. W. Muir and S.E. Aggrey, eds.

Renden, J. A., S.S. Oates, and R.B. Reed. 1986. Determination of body fat and moisture in dwarf hens with near-infrared reflectance spectroscopy. Poultry Science 65:1539-1541.

Swatland, H. J. 1995. On-line evaluation of meat. Technomic Publishing Company, Inc., Lancaster, PA.

Valdes, E. V. and J. D. Summers. 1986. Determination of crude protein and fat in carcass and breast muscle samples of poultry by near-infrared reflectance spectroscopy. Poultry Science 65:485-490.

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

Woelfel, R. L., and A. R. Sams. 2001. Marination performance of pale broiler breast meat. Poultry Science 80:1519-1522.

Table 5.1. Mean reference values of broiler breast meat samples (n=85) used for Vis/NIRS analysis.

	Range	Mean	SD
WHC (%)	9.4-26.9	16.7	3.8
pH	5.0-6.2	5.6	0.16
L* (d)	59.6-71.7	65.6	2.33

[a] WHC is water holding capacity. L* (d) is L* on dorsal side of muscle. Range, mean standard deviation (SD) is reported for 85 samples.

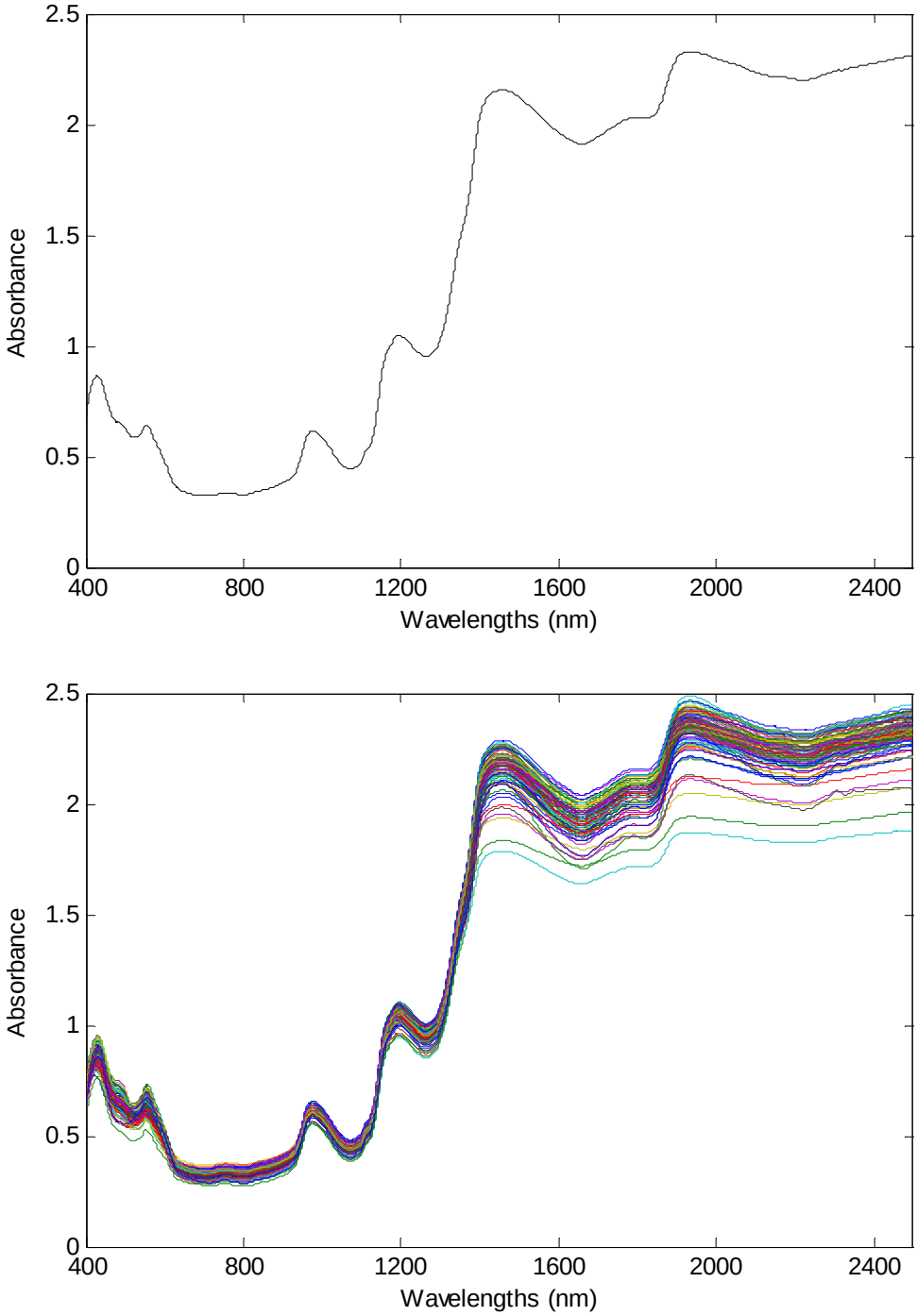


Figure 5.1. Vis/NIRS spectra of broiler breast meat samples (n=85). Mean spectrum (top) and total spectra (bottom).

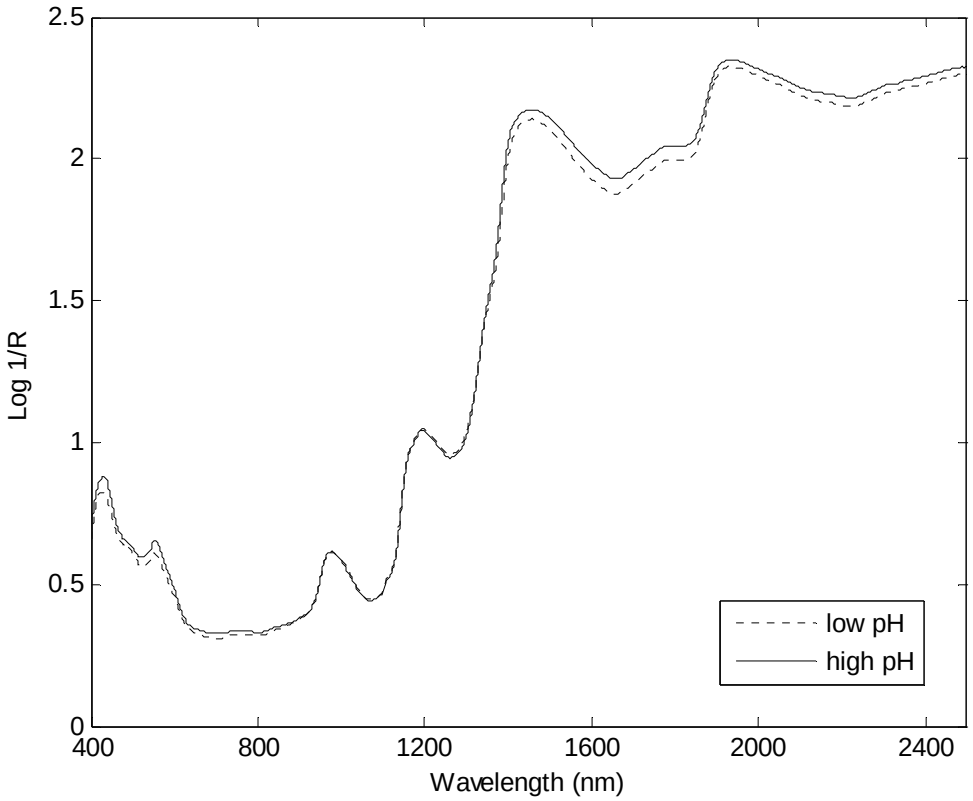


Figure 5.2. Mean spectra of low pH (<5.6) and high pH (>5.6) broiler breast samples. Mean value of pH 5.6 was used to segregate samples based on pH.

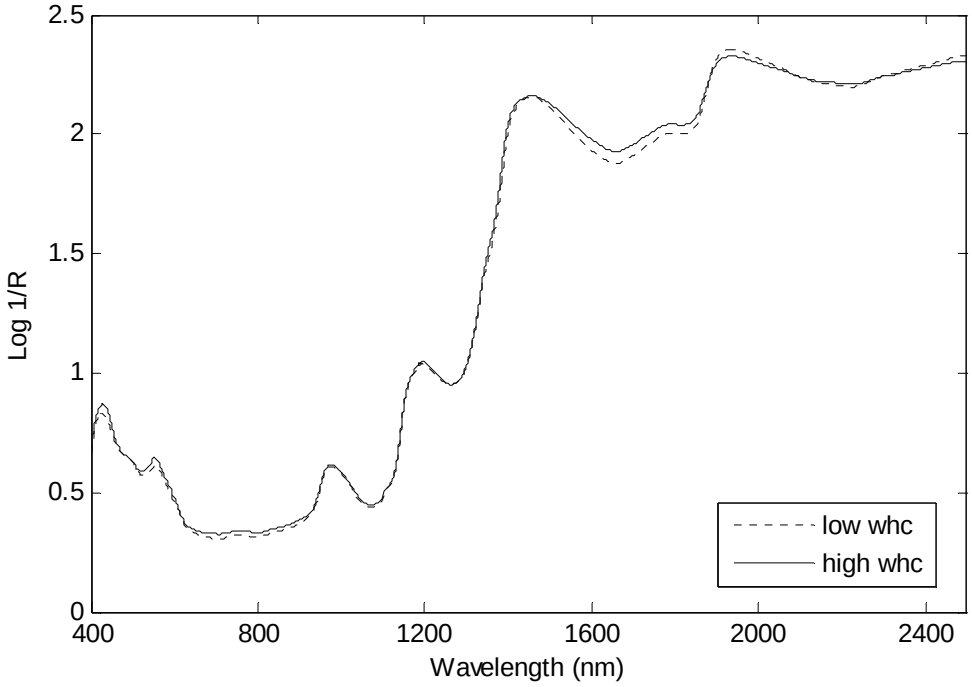


Figure 5.3. Mean spectra of low WHC (<13%) and high WHC (> 15%) broiler breast samples. Mean WHC (water holding capacity) value for pale samples was 14.2%. Cutoff values of 13% and 15% were used to further delineate spectra of pale and normal muscle. WHC values correspond with mean pH value of 5.6.

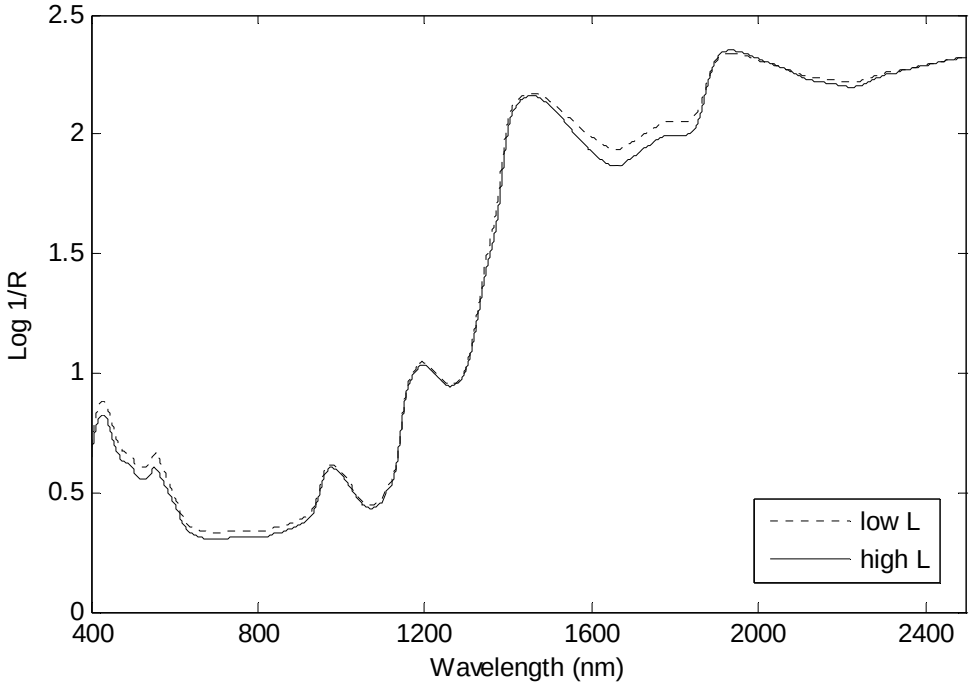
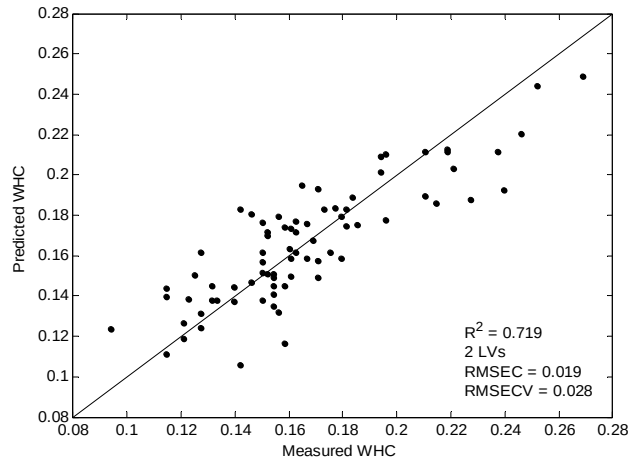


Figure 5.4. Mean spectra of low L* (<66) and high L* (>68) broiler breast samples. L* classified based on combination of pH and WHC values.



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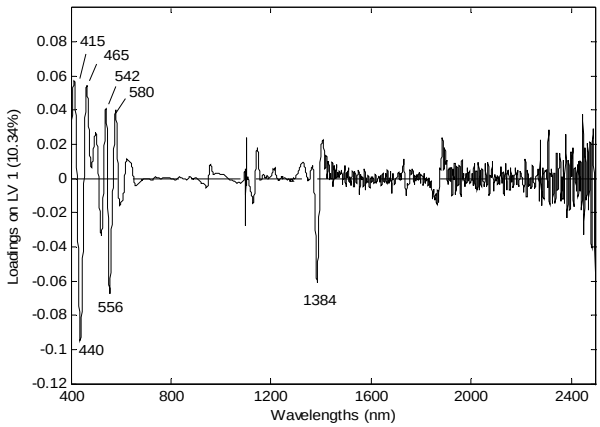

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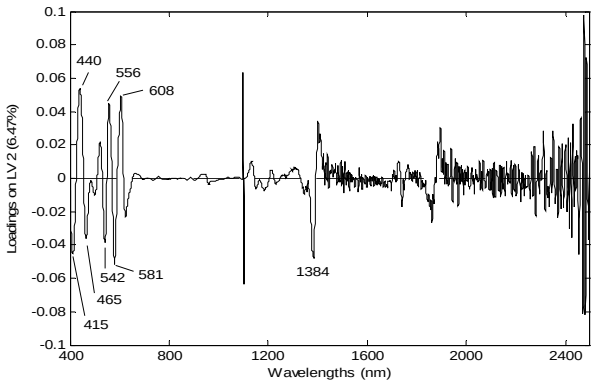
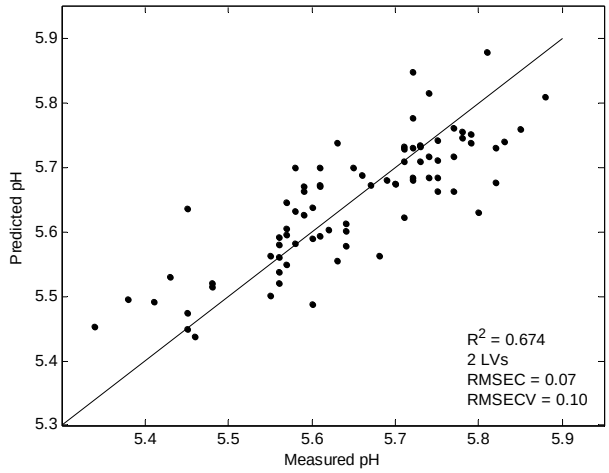


c

Figure 5.5. Water holding capacity prediction in broiler breast meat by near infrared spectroscopy using a partial least squares (PLS) model (a). The two bottom figures are PLS loading plots for WHC of samples using one (b) or two (c) latent variables. Identified peaks at the given wavelengths (415, 440, 465, 542, 556, 558, 580,581, 608, 609) have greatest influence on the prediction model.



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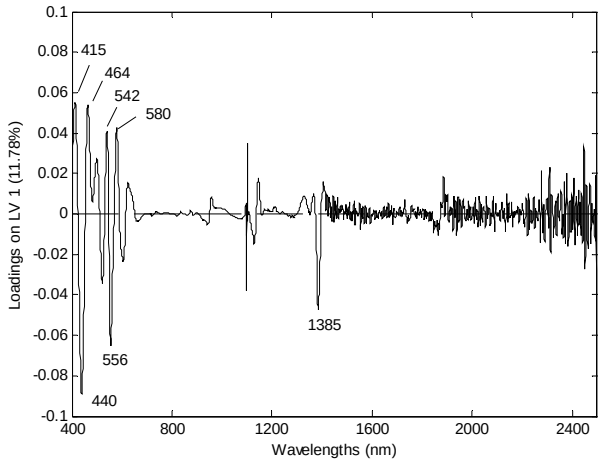

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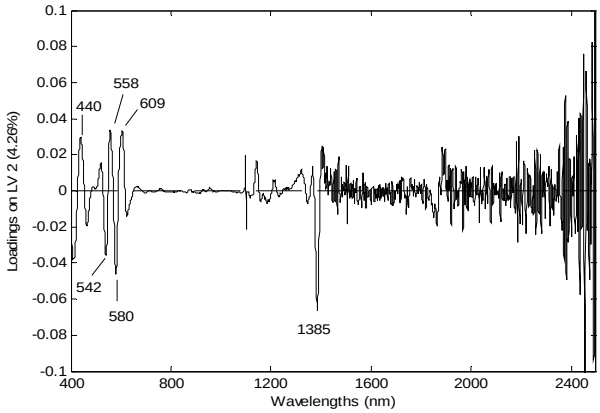

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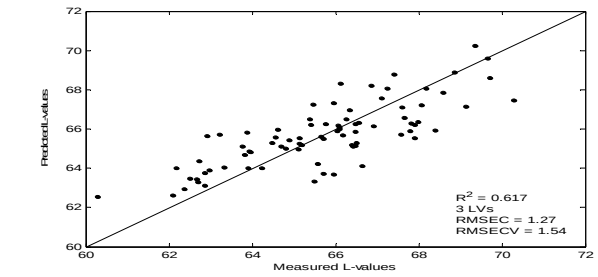
Figure 5.6. The pH prediction of broiler breast meat by near infrared spectroscopy using a partial least squares (PLS) model (a). The two bottom figures are PLS loading plots for pH of samples using one (b) or two (c) latent variables. Identified peaks at the given wavelengths (415, 440, 464, 542, 556, 558, 580, 609) have greatest influence on the prediction model.



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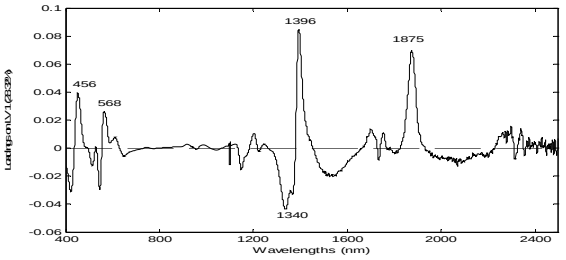
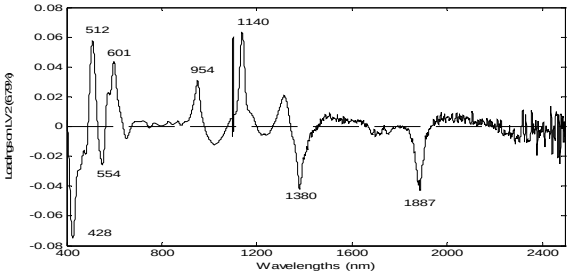
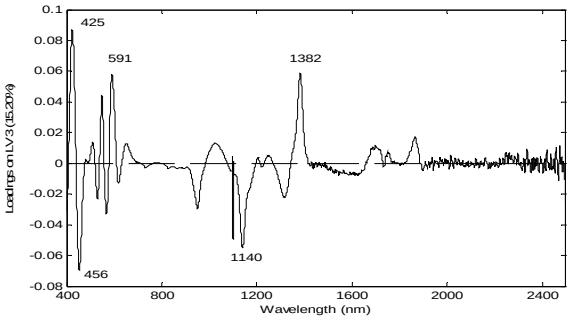


b



c



d

Figure 5.7. The L* prediction of broiler breast meat by near infrared spectroscopy using a partial least squares (PLS) model (a). The two bottom figures are PLS loading plots for L*of samples using one (b) or two (c) or three (d) latent variables. Identified peaks at the given wavelengths (425, 428, 456, 512, 554, 568, 591, 601, 954, 1140,1340, 1380, 1382, 1396, 1875, 1887) have greatest influence on the prediction model..

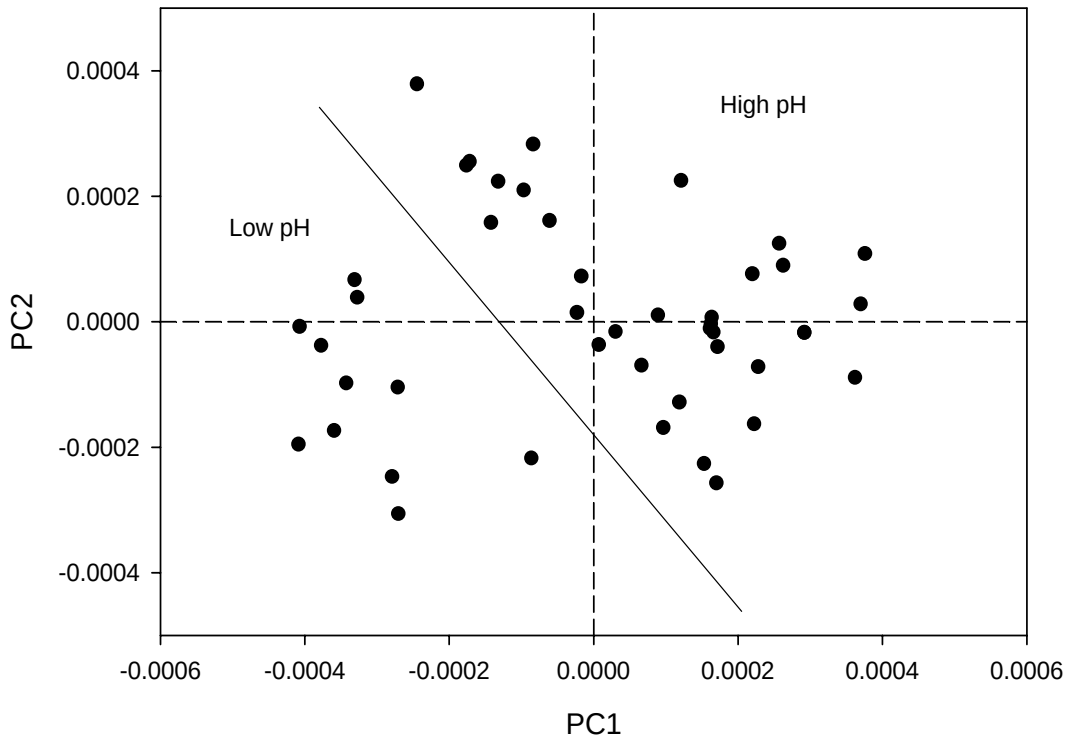


Figure 5.8. Principal component analysis score plots for low pH (<5.6) and high pH (>5.6) broiler breast samples.

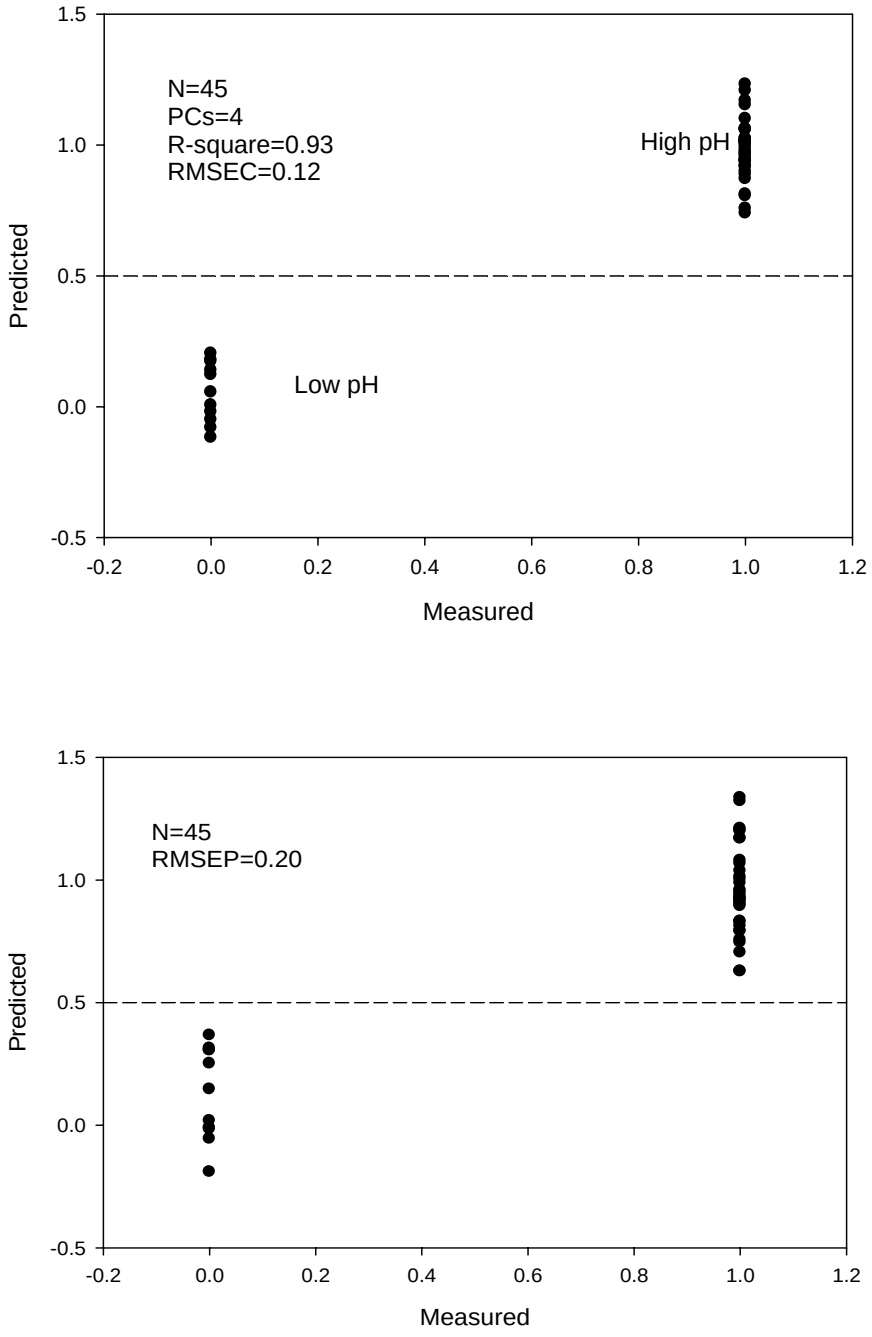


Figure 5.9. Discriminant analysis for low pH (<5.6) and high pH (>5.6) broiler breast samples. Calibration (top) and cross-validation (bottom). Predicted values are plotted against measured values. Low pH values were assigned a value of 0 and high pH's were assigned a value of 1.

CHAPTER 6

SUMMARY AND CONCLUSIONS

The existence of pale, soft and exudative meat and its causes has been established in pork while the incidence in broiler meat has not been fully explained. Currently, the poultry industry has not identified a reliable genetic marker responsible for PSE meat but continues to search for indicators to minimize production losses resulting from PSE meat. Therefore, several hypotheses should be taken into consideration for future research into the PSE condition within broilers (Barbut et al., 2008). Boulianne and King (1995) found that color characteristics (*L, a*, and *b*), pH, total pigment, myoglobin, and iron concentrations were all significantly different in pale meat when compared with normal chicken breast meat. Dransfield and Sosnicki (1999) suggested that higher growth rates may induce morphological abnormalities, larger fiber diameters and a higher proportion of glycolytic fibers, and a lower proteolytic potential in the muscles; and subsequently increase the occurrence of paler color, reduced water holding capacity, and poorer quality of further processed products. Additionally, preslaughter environmental conditions have shown to be major contributors to the increased incidence of PSE meat in poultry (Berri, et al., 2005; Debut, et al., 2005; McKee and Sams, 1997). Thus, there are several factors that influence meat quality and the purpose of this research was to investigate the incidence of a PSE-like meat in broilers and examine changes in proteins that may be responsible for the condition. In addition, a tool for rapid assessment of meat quality was evaluated in an effort to provide a short term solution to minimize yield losses resulting from this condition of a PSE-like meat.

The results showed that L* was heavily influenced by several factors such as grower, age, and bird weight. The research also showed an increase in L* compared to previous research, with a* having a greater correlation between the production and processing factors than the L*. Color measurements, pH, and water holding capacity studies showed that the heavier birds with a higher growth rate exhibited a higher occurrence of pale birds than the lighter birds with a slower growth rate. L* greater than 60 was observed in 57% of birds selected for greater yield and in 26% of slower growing birds. Analysis from SDS-PAGE, water holding capacity and protein solubility studies showed that pale broiler breast meat exhibited lower water holding capacity, protein solubility, and pH. The presence and intensity of protein bands on SDS-PAGE were similar in water soluble and salt soluble extracts from pale and normal muscle. A peptide that migrated to the molecular weight of phosphorylase was consistently present in myofibrillar fractions of both normal and pale broiler breast fillets. Vis/NIRS was examined as a tool for rapidly determining water holding capacity. Results suggested that NIRS had the potential to predict water holding capacity in broiler breast meat with a coefficient of determination (R^2) of 0.72.

REFERENCES

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

Berri, C., M. Debut, V. Sante-Lhoutellier, C. Arnould, B. Boutten, N. Sellier, E. Baeza, N. Jehl, Y. Jego, M. J. Duclos, and E. Le Bihan-Duval. 2005. Variations in chicken breast meat quality: implications of struggle and muscle glycogen content at death. British Poultry Science 46:572-579.

Boulianne, M. and A.J. Knight. 1995. Biochemical and color characteristics of skinless boneless pale chicken meat. Poultry Science 74:1693-1695.

Debut, M., C. Berri, C. Arnould, D. Guemene, V. Sante-Lhoutellier, N. Sellier, E. Baeza, N. Jehl, Y. Jego, C. Beaumont, and E. Le Bihan-Duval. 2005. Behavioural and physiological responses of three chicken breeds to pre-slaughter shackling and acute heat stress. British Poultry Science 46:527-535.

Dransfield, E., and A. A. Sosnicki. 1999. Relationship between muscle growth and poultry meat quality. Poultry Science 78:743-746.

McKee, S. R., and A. R. Sams. 1997. The effect of seasonal heat stress on rigor development and the incidence of pale, exudative turkey meat. Poult Science 76:1616-1620.