

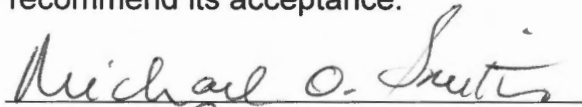
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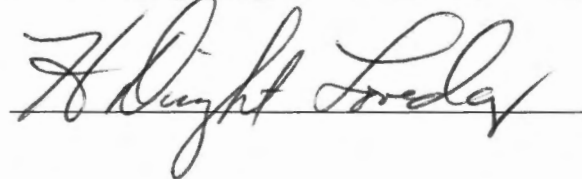
I am submitting herewith a thesis written by Cheng-Hwei Liu entitled "Characteristics and causes of pale, soft, exudative (PSE) broiler breast muscle." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science & Technology.



Riëtte van Laack, Major Professor

We have read this thesis and
recommend its acceptance:





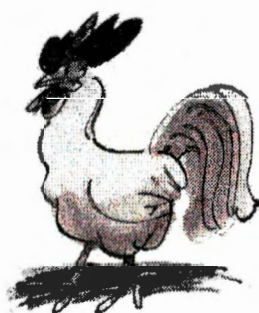
Accepted for the Council:



Associate Vice Chancellor and
Dean of The Graduate School

**CHARACTERISTICS AND CAUSES OF
PALE, SOFT, EXUDATIVE (PSE)
BROILER BREAST MUSCLE**

**A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville**



**Cheng-Hwei Liu
December 1998**

Ag-VetMed

Thesis

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, L58

DEDICATION

This thesis is dedicated to my uncle

Mr. Chang-Pei Liu, who was deceased while I was in the U.S.A.,

and who has given me unceasing and invaluable encouragement throughout my life.

His helpful spirit and good humor are much missed.

ACKNOWLEDGMENTS

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ABSTRACT

The problem of Pale, Soft, Exudative (PSE) chicken has been recognized only recently. Although PSE in chicken seems linked to stress, it is unknown if this condition is comparable to PSE pork which occurs in stress susceptible pigs. In Experiment 1 of the study, 20 normal and 20 pale color chicken breasts were evaluated. Compared with normal meat, the pale (PSE) chicken had a significantly ($P < 0.05$) lower pH (5.9 vs. 5.7), higher L^* -value (55.1 vs. 60.0), higher drip loss (0.87 vs. 1.33%), lower water-holding capacity (WHC, 44 vs. 31%), and lower cooking yield (106 vs. 96%). Differences in sarcomere length (1.77 vs. 1.72 μm) were small and insignificant. Protein solubility in PSE samples was slightly, but significantly, lower than in normal samples. Correlations between pH and L^* -value ($R = -0.76$), pH and WHC ($R = -0.65$), and sarcoplasmic protein and L^* -value ($R = -0.71$) were significant. Correlations between protein solubility and WHC were not significant.

Experiment 2, 3, 4, 5, utilizing a total of 195 commercial male broilers, were conducted to evaluate if a rapid change in pre-slaughter environmental temperature results in PSE quality. In Experiment 2, heat-stressed broilers were kept in a temperature-controlled chamber, in which temperature increased from 23.9 to 35°C within 3 h. In Experiment 3, 4 and 5, the temperature was increased to 36.1°C instead of 35°C. In Experiment 4 and 5, additional stress was imposed by chasing chicken on the floor for 10 min before catching. The heat-stressed birds were caught and kept in the crate for an additional hour before slaughter in order to simulate a commercial

environment. For each experiment the control group was kept at 23.9°C. Breast muscles were excised at 8 h post-mortem and stored at 0-2°C. At 24 h post-mortem, the quality characteristics pH, color (L*), and water-holding capacity, were assessed. In Experiment 2, heat stress did not affect any of the meat quality characteristics. In Experiment 3, heat stress resulted in significantly ($P<0.05$) higher L*-values (59.8 vs. 58.3), lower water-holding capacity (WHC, 36 vs. 52 %), and lower cooking yield (CY, 99 vs. 111%). In Experiment 4 and 5, the sudden change in temperature and extra stress (chasing) did not affect quality. A low ultimate pH seems to be the main factor for the PSE phenomenon in chicken. Results of this study indicate that under conditions of these experiments, temperature is not the main cause of PSE. Other factors, such as genetics, may interact or interfere with the development of PSE broiler breast muscle under (temperature) stress conditions.

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INTRODUCTION

Meat quality is defined by consumer-desirable characteristics; 'good' quality meat needs to look, smell and taste good. Color, juiciness and palatability are criteria used in the evaluation of meat quality and acceptability. Producing acceptable meat quality is a great challenge for the meat industry.

Pale, soft, exudative (PSE) quality is a well-known aberration in pork. PSE pork has a paler color than normal (reddish pink) meat, and the ultimate pH of PSE pork is often lower than that of normal meat (Wisner-Pedersen, 1959; Briskey and Wisner-Pedersen, 1961; Warner, 1994). The occurrence of PSE quality has been related to stress and genetics in pork. After animals have been slaughtered, the circulatory system discontinues the blood supply, and thus ceases the transfer of essential nutrients to the muscle, and the removal of waste products from cells (Greaser, 1986). As a result, muscle metabolism becomes anaerobic, and glycogen will be converted to lactic acid. The lactic acid is not transported out of muscle cells but accumulates in the muscle, leading to a pH decline. If the pH is low at a time that body temperature is high, denaturation of sarcoplasmic and myofibrillar proteins expressed in a loss of solubility is the result (Wisner-Pedersen 1959; Sayre and Briskey, 1963; Penny, 1967; Honikel et al., 1986; Offer, 1991). Consequently, the resulting meat will have a poor water-holding capacity, expressed as high drip losses.

Water-holding capacity (WHC) is an economically important characteristic for the meat industry. Among others, WHC impacts the weight of the marketable products,

cooking yield of further processed products, uptake of marinades, and emulsification capacity.

In pork, the occurrence of PSE has been recognized and widely discussed for past decades. In poultry, information on PSE muscle is limited. Barbut (1993) and Woelfel et al. (1998) reported that poultry breast muscle can exhibit PSE-like characteristics similar to those seen in pork. Limited data available on PSE in broilers suggest that pale breast muscle has twice as much purge as the darker muscle, and that cooking yields of PSE-like broiler product may be 8% lower than those of non-PSE product (Bacon, 1996). In addition, it seems that the pale birds are more difficult to debone, resulting in significantly lower deboning yields (Bacon, 1996).

The frequency of PSE in broilers seems to be related to the environmental temperature, and varies from as low as 2% on cool days, to more than 20% on warm days (Bacon, 1996). Barbut (1996) found that the incidence of 'PSE-like' turkey also varies with season. Although accurate figures of dollar losses associated with PSE in broilers, are not available, these can be estimated to be 16 to 160 million dollars ($355,725,000 \times 8\% \text{ PSE meat} \times \$ 3.00 \text{ boneless breast} \times 2\% \text{ to } 20\%$) per year.

There is limited research on PSE in broilers, and PSE chicken has not been characterized. At present, it is not known if PSE chicken is similar to PSE pork. The possible genetic basis of this phenomenon has not yet been explained (Anthony, 1998). Before it will be possible to solve the PSE problem in broilers, the causes of occurrence of PSE must be established. More knowledge about the phenomenon PSE in chicken is needed. Thus, the objectives of this research were:

1. To characterize PSE in broiler breast muscle

Factors that influence WHC (i.e. exudation), such as protein denaturation, sarcomere length and pH (Offer and Knight, 1988), were investigated. Proteins were analyzed by SDS-PAGE, to detect sarcoplasmic protein denaturation, specifically phosphorylase. Results on color (L^* , a^* , and b^*) and drip loss of normal and pale samples were used to establish cut-off values for PSE.

2. To investigate the causes and mechanisms of PSE in broiler breast muscle

The second objective was to determine if sudden temperature change causes PSE in chicken. Knowledge about the cause of PSE is needed before procedures to prevent PSE can be developed.

CHAPTER I

LITERATURE REVIEW

Introduction

The major quality attributes of meat are color, water-holding capacity (WHC), and palatability. Pale, soft, exudative (PSE) meat is well-known as a meat quality defect due to its unattractive color and reduced water-binding capacity. In the United States, estimated annual losses related to PSE broiler breast meat approximate 16 to 160 million dollars. 'PSE-like' chickens have not been studied extensively, but it seems that occurrence of PSE broiler meat is associated with season, and environmental temperature, particularly sudden changes in temperature.

Poultry meat products are increasingly used for production of value-added products, such as home replacement meals and ready-to-eat products. Poor water-holding capacity in PSE-like broiler would be a severe drawback for these types of products. Therefore, research into the characteristics and causes of PSE broilers is essential.

Poultry Production

Poultry meat is one of the most popular animal protein sources. Economical production, a quick reproduction cycle, good feed efficiency, and continuous source of food (i.e. eggs) make poultry production a steadily growing industry (NASS, 1997). The main sources of poultry meat are chicken and turkey. Chicken is relative smaller in size, and less expensive than turkey. Moreover, turkey is still considered a festive

item for the holidays in the United States. Hence, chicken is the main poultry meat for human consumption.

In the modern hatchery, the use of an incubator operation for hatching is the most common practice. The incubation period takes approximately 21 days for chicken. Brooding is the time period in which supplemental heat is supplied to broilers to help them maintain body temperature and grow at an optimal level. From the time they are received from the hatchery, chicks are kept in the brooding house until they are 8-10 days old. The growing period (without supplemental heat) follows the brooding period. Commercial broiler growers commonly use brooding houses for brooding and growing. As chickens grow to desired market weight in the range from 4.5-6 lbs (ca. 42 to 49 production days), they are caught and transported to the processing plants.

Muscle Composition, Type, and Structure

Muscle composition and type

Muscle contains 75-80 % of water, 15-20 % of protein, 1-13 % of fat, and traces of carbohydrates and minerals (Pearson and Young, 1989). Muscle performs the function of locomotion and heat production in living animals. There are three types of muscles: skeletal, smooth, and cardiac muscle. Skeletal muscle, attached to the skeleton, comprises most of muscle tissue of the body. In avian species, skeletal muscle is distributed along with bone to meet the need of flight.

Muscles fibers differ from each other in appearance such as color: red and

white. Red muscle contains more mitochondria and more of the pigment myoglobin, whereas white muscle has few mitochondria and a low myoglobin content, e.g. chicken thigh (red) vs. breast (white) (Chandra-Bose et al., 1964).

Structure of muscle

Muscle tissue is enclosed by a thick sheath of connective tissue, the epimysium, and is split into several bundles of fibers by a coarse network of perimysial connective tissue. The network is further subdivided by thinner sheets of perimysial connective tissue. The features of muscle are shown in Figure 1. Muscle fibers run almost parallel to each other. Within the bundle, individual muscle fibers are separated by the endomysial connective tissue. The fibers are primarily composed of myofibrils, accounting for approximately 80% of the muscle cell. The myofibrils consist of three groups of proteins: contractile proteins (myosin and actin), regulatory proteins (tropomyosin and troponin), and cytoskeletal proteins (titin and desmin).

Chicken pectoralis muscle

The pectoralis muscle, commonly known as breast muscle, represents 25-30% of a broiler carcass (Winter and Clements, 1957; Smith, 1993). The function of the pectoralis muscle is to depress the wings, and the wings are then lifted by muscles on the back. The pectoralis has homogenic white fibers (George and Berger, 1966), and contains very little myoglobin (Chandra-Bose et al., 1964). Therefore, chicken

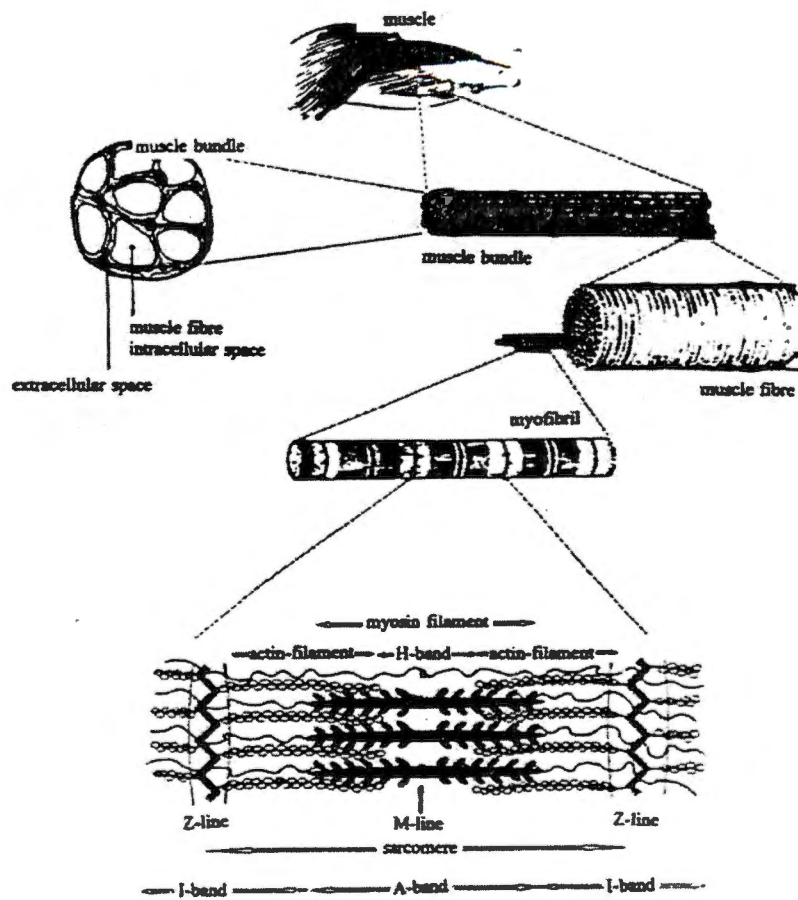


Figure 1. Structure of muscle fibers

Source: M.J.A. den Hertog-Meischke, R.J.L.M. van Laack and F.J.M. Smuldrs. Veterinary Quarter 1997. 19:175-181.

breast muscle is often referred to as an example of a white muscle. Muscle is not only classified by color, but also categorized in terms of muscle ATPase activity. White muscle has a fast contraction speed, whereas red muscle has a slow contraction speed. Ashmore and Doerr (1971) observed that white muscle has a higher ATPase activity and goes into rigor very quickly, and that dark muscle has lower ATPase activity, and it takes longer to form actomyosin.

Pre-slaughter Handling and Processing

Chickens grow to the desired market weights of 4.5-6.0 lbs, corresponding to a production time of 42-49 days. Subsequently, they are caught and transported to a slaughter processing plant. In the United States, the average duration of transportation is 1 h. The live birds are brought to the waiting area of the slaughter house for unloading. The waiting areas are usually under cover. In summer time, additional cooling systems, such as fans, are provided to protect the birds from heat stress. The various steps of processing poultry are illustrated in Figure 2. The birds are shackled and stunned by electrical stunning, which is predominately practiced for automated killing, followed by exsanguination for 3-5 min. Subsequently, the birds are scalded (ca. 50-60°C) and plucked. At the high scalding temperature, the epidermis of a bird is opened and as a result plucking is easier. The defeathered bird is eviscerated. At approximately 7-10 min post-mortem, chilling starts. USDA requires chilling to 4°C

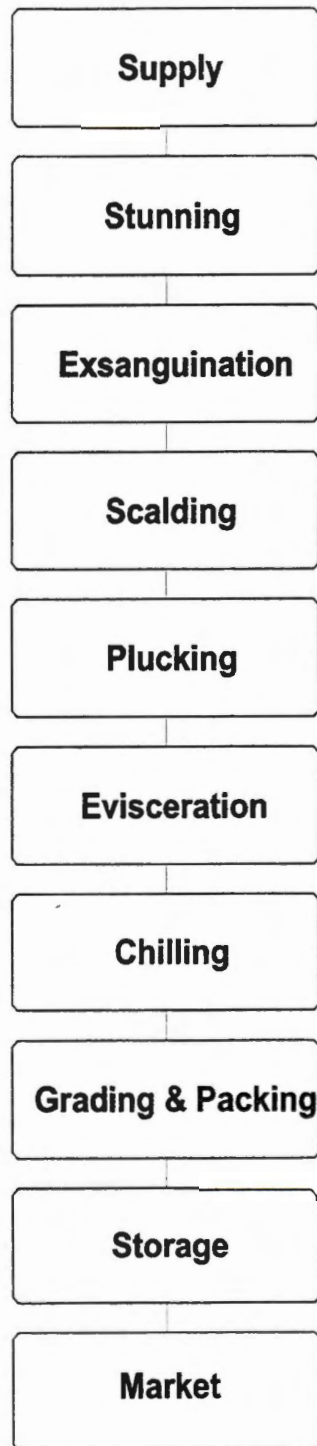


Figure 2. Poultry processing flow diagram

within 4 h post-mortem for birds weighing 4-8 lbs, and 8 h for birds more than 8 lbs (USDA, 1972). Chilling can be achieved in various ways. In the United States, chilling in water is the common procedure. The exact temperature profile varies from plant to plant. After chilling, grading is performed according to USDA Poultry-Grading Manual (USDA, 1989). The carcasses are cut and sorted as whole birds, cut-up parts, or further-processing products, and packed for the 'markets' demand. The products are stored as frozen or fresh products, and finally distributed to wholesale/retail stores.

Poultry Grades and Quality

The poultry grading system is a classification based on sorting poultry and poultry products by visual evaluation. The current USDA poultry grading system for quality is based on physical appearances, such as conformation, fleshing (the bulk of the meat in drumsticks, thighs, and breast), processing defects (discoloration, dehydration), fat covering, and deformities (USDA, 1989). Excess fat covering, or processing defects would lead to downgrading of the products, and influence the use and economic value of poultry. The causes for defective products are mostly either mechanical forces (catching, loading, transport, unloading, and shackling) or physical factors (a high scalding temperature and improper packaging handling). Quality is not only physical appearance, protein properties of meat itself are also involved. The desirable quality includes a good visual appearance, eating pleasure (taste and texture). The muscle properties refer to the factors: pH, water, protein, fat of muscle. The interaction among these four major components influences the palatability. For instance, the higher pH

value, the better water binding by myofibrillar proteins, and therefore, more juicy meat.

Ante-Mortem Factors Affecting Quality

As a live animal receives stressors (e.g. cold, heat, fatigues, etc.), the animal produces somatic changes, and deviates from its normal state. The changes include biochemical reactions such as enlargement of the adrenal cortex, and discharge of its hormones (Selye, 1950), and will influence meat quality characteristics (Hedrick, 1965). An inherent factor, such as genetic stress susceptibility, and the extrinsic stressors, such as sudden change in environmental temperature, long transportation distance, loading, unloading, and stunning, affect quality.

Genetics

There is little documentation on the effect of genetics on the incidence of PSE poultry quality. In pork, PSE is associated with the porcine stress syndrome (PSS) related to the presence of the stress gene. The presence of the stress gene influences Ca^{+2} release into the muscle, thus, influencing rate of contraction and glycolysis. In poultry, a similar gene has not yet been found (Sosnicki, 1996).

The incidence of PSE seems to be increased in fast-growing animals. In pigs, scientists have used halothane gas to identify stress susceptible pigs. Pigs that develop stiffness within a certain time period after inhalation of halothane are called halothane positive (Eikelenboom et al., 1978). These pigs tend to be more sensitive to stress and prone to development of PSE quality meat. In a study by Owens et al. (1998) halothane gas was used to detect positive halothane carriers in turkey. The

results indicated that the strain with the larger breast muscle would be more susceptible to develop PSE meat. Anthony (1998) suggested that a long term study on breed for PSE poultry is necessary. At present, it is not clear if genetics are involved to in occurrence of PSE muscle in poultry.

Environmental temperature

A significant interaction between environmental temperature and the production performance of broilers is well known; when live birds are exposed to increased ambient temperatures above a limit of the comfort zone, the result is a reduced growth rate and lowered feed efficiency (Prince et al., 1961; Adams et al., 1962; Teeter et al., 1985). The heat distress occurs when the ambient temperature or the ambient temperature-relative humidity combination exceeds the chickens' comfort level; temperature above 23.9°C (75°F), and relative humidity over 50% (North, 1984). When the heat produced by the bird is greater than that dissipated, birds lose their ability to rapidly and efficiently dissipate heat, and this results in rising body temperature. Sayre et al. (1963) reported that when environmental temperature was elevated immediately preslaughter, pork muscle temperature increased, and post-mortem glycolysis was accelerated. The incidence of the PSE in pork was highest in stress susceptible pigs subjected to heat stress prior to slaughter (Judge et al., 1966; Forrest et al., 1968).

In poultry, Wood and Richards (1975) reported heat stress (38°C) for 3 hours prior to slaughter caused a faster rate of post-mortem glycolysis, a lower ATP level in

the muscle, a lower final pH, and tougher broiler breast meat. Northcutt et al. (1994) found that chickens that were subjected to heat (40–41°C for 1 h) and preconditioned (3 consecutive days to 35–36°C for 3 h) exhibited PSE meat characteristics.

Stunning

Stunning is a way to make an animal unconscious before exsanguination. The purpose of stunning is to minimize the risk of distress, and to immobilize the bird to allow neck cutting to be performed easily. Stunning can be conducted by electricity or gas. Some research indicated that electric stunning results in broken bones and an increased incidence of leg bruising (Wilson and Brunson, 1968; Ma and Addis, 1973). In chickens, the pH decline in breast muscle was slower, and the meat was more tender in birds treated with electrical stunning compared to non-stunned birds (Lee et al., 1976). Also, Van Hoof (1979) found that the quality of turkey breast in terms of shear force, color, and water-holding capacity was lower in turkeys that were not stunned before slaughter.

In gas stunning, carbon dioxide (CO₂) is the most commonly used gas. It is used as the only gas or as part of a gas mixture. Because carbon dioxide easily crosses the blood-brain barrier, it decreases the pH of the cerebral spinal fluid and makes the living creature rapidly unconscious (Sams, 1997). Birds that were stunned with 40% carbon dioxide for 60 sec had a lower incidence of blood splash, and fewer broken bones (wishbone, collarbone) than birds stunned with electricity (Hirschler and Sams, 1993). Raj et al. (1992) observed that a gas mixture of CO₂ and argon reduces

the incidence of carcass damage compared with electrical stunning at 120 mA for 4 sec. This mixture gas (argon and carbon dioxide) also resulted in acceleration of rigor mortis and more tender meat at 2 h post-mortem than the electrical stunning (120 mA for 4 sec) (Raj, 1994).

Post-Mortem Factors Affecting Quality

Muscles terminate all their living functions and convert to meat in a gradual process. The chemical and physical changes take place over a period of several hours or even days. The quality characteristics-tenderness, palatability, and color may be influenced by changes during conversion of muscle to meat. For instance, pigs contain high fast glycolytic muscle fiber types; therefore, muscle has a fast rate of post-mortem glycolysis. The myofibrillar proteins are severely damaged resulting in functionality of the proteins.

Scalding and defeathering

Poultry differs from most animal species used for meat production because there are feathers attached to skin, and the carcasses or cuts are sold with or without the skin. The appearance of whole and/or portions of carcass is profoundly important to consumers' acceptance.

The use of hot water is the most common practice for opening the epidermis for the next step, defeathering. The scalding stage can have a strong effect on carcass appearance (raw vs. cooked). The efficiency of removal of the birds' feathers depends

on the scalding temperature and time. At higher temperatures (i.e. 57.2-60°C), feathers removal is greatly facilitated. Pearce and Lavers (1949) studied the influence of scalding time and temperature in chickens and found that the force required to pull feathers ranged from 15 ounce with 30 seconds at 51.7°C, to 5 ounce with 15 seconds at 57.8°C. Dickens and Shackelford (1988) studied scalding time 1, 1.5, 2 and 2.5 min, and temperature 52 and 56 °C. They concluded that a temperature of 56°C for 1 min resulted in the lowest feather-pulling forces, whereas at a temperature of 60°C the pin feathers were more easily removed by the mechanical pickers (Pool et al., 1954).

Chilling

Product shelf-life is of major importance in the distribution and consumption of poultry meat. The chilling process is an important (required) step in processing, and applied to reduce the rate of microbial growth (USDA, 1972).

In the live bird, the body temperature is approximately 41°C. Chilling can be accomplished by two different methods, water and air chilling. In the United States, water chilling, consisting of a combination of slush ice and water, is the most commonly used method. For the water chilling method, carcasses are moved through chilled water by mechanical rakes or rotating paddles. Counter-current chillers generally utilize augers for circulation pumps. The amount of ice needed to cool poultry from 38°C to 0°C is about 0.38 kg of ice per kg of bird. However, in most processing plants,

the actual use varies from 0.4 to 1.0 kg (A.S.H.R.A.E., 1971). Most immersion chillers reduce breast muscle temperature to less than 4°C in 30-50 min (Stadelman, 1961).

Biochemical Changes after Animal Death

Glycolysis

A live animal uses adenosine triphosphate (ATP) as the primary source of energy in cells for growing and maintaining the energy itself. Under aerobic conditions, ATP is produced via oxidative phosphorylation, and glucose is broken down to carbon dioxide and water. When an animal is killed, the blood supply ceases. Consequently, oxygen supply to the muscle is disrupted, and the energy production via mitochondria (oxidative phosphorylation) stops. As a result, pyruvate (produced from glucose) production cannot be converted into carbon dioxide and water. Rather, lactic acid is formed (Briskey and Wismer-Pedersen, 1961). The loss of ATP during post-mortem anaerobic glycolysis is the event causing the actin and myosin to remain locked, and yield the stiff muscle, known as onset of rigor. Beatly et al. (1963) reported that the rate of glycolysis is faster in white than in red muscles. Ma and Addis (1973) observed that turkey breast muscle has a fast rate of post-mortem processes.

pH

The pH value of muscle in living animals is 'neutral' (pH=7.0). After slaughter, the pH in muscle declines as a result of the accumulation of lactic acid. The measurement of pH is an indicator of the rate and the extent of post-mortem glycolysis. Watery meat is the result of rapid post-mortem glycolysis, causing pH to be lower than 6.0 within 45

minutes post-mortem (Bendall and Swatland, 1988). In poultry, the ultimate pH is reached relatively rapidly (Landes et al., 1971; Ma and Addis, 1973; Lee et al., 1976). In chicken breast muscles, some investigators observed a slight and temporary pH-rise very early post-mortem (Dodge and Peters, 1960; Landes et al., 1971). Landes et al. (1971) reported that pH of turkey decreased rapidly (pH of 6.6-6.0) in the first 15 min post-mortem, and then increased slightly (pH of 6.0-6.25) from 15 to 30 min. After 30 min post-mortem, the pH dropped very little through the first 6 h post-mortem (pH=5.95) to a final pH=5.75-5.8. In a study by Ma and Addis (1973), the pH value of turkey breast was 6.6, 6.4, 6.3 at 0, 15, 30 min, respectively. The rigor development among different locations in the broiler breast muscle is different. The anterior locations of the pectoralis major reached the onset of rigor faster than posterior locations (1 h vs. 2 h post-mortem) (Papa and Fletcher, 1988).

The rate of pH decline is markedly affected by temperature (Honikel et al., 1981). Marsh (1954) studied the influence of temperature on the rate of pH decline in beef. In the range from 7 to 37°C, rate of pH decline increased with increasing temperature. According to Cassens and Newbold (1967), the rate of pH decline in beef increases slightly at temperature less than 10°C.

The pH decline is dependent on muscle and species. Beef contains mostly slow-oxidative, red muscle, and beef muscle reaches onset of rigor mortis in 20-24 h (Lawrie, 1985). Pork has an abundance of fast-glycolyzing, white muscles, and rigor develops in 1/4 to 3 h. The chicken breast muscle is a homogenous white muscle and

is relatively small. Therefore, the rigor mortis in broiler breast muscle may set in faster than in beef (20-24 h) and in pork (0.25-3 h) (Lawrie, 1985; Judge et al., 1989).

Color

Meat color is the most important quality characteristic of meat because it influences the purchasing decision by the consumers. Meat color is determined by the amount of muscle pigment (myoglobin, hemoglobin, and cytochrome), and the chemical state of the pigment.

Myoglobin is the main pigment in muscle. The myoglobin molecule consists of a protein, the globin, and a heme group comprising the flat protoporphyrin IX ring with a central iron atom. Myoglobin is found in high concentrations in red muscle fibers (James et al., 1983; Cassens, 1970), and in low concentrations in white muscle. Muscle myoglobin content is also affected by species (Ashmore and Doerr, 1971). The concentration of myoglobin is 1-3 mg/g for pigs, 4-8 mg/g for lamb, 4-10 mg/g for beef (Cross et al., 1986), and 0.1-2 mg/g for poultry (Boulianne and King, 1995).

The chemical state of the heme pigments determines muscle color. The typical color of the interior of a piece of fresh beef is dark purple. This purple color is characteristic of deoxymyoglobin in its reduced state, and is the predominant form present in meat. When fresh meat surfaces are exposed to air, myoglobin reacts with oxygen to form bright-red oxymyoglobin (MbO_2). This process is known as “bloom” of fresh meats. The oxygen is continually associating and dissociating from the heme complex. The reduced pigment is subject to oxidation by oxygen or other oxidants.

Oxidation results in a brownish color of meat, known as metmyoglobin. The presence of metmyoglobin results in an undesirable discoloration.

PSE meat has a paler color because a fast glycolysis (pH decline) and a high carcass temperature resulting in denaturation of proteins. The denaturation and associated shrinkage of myofibrils results in increased light scatter and thus, paler color (Offer and Cousins, 1992).

In a study by Ma et al. (1971), birds were divided into 3 different groups (slow >125 min, intermediate 50-125 min, fast <50 min) based on the rate of rigor mortis completion on turkey. They found meat color was similar to that of PSE pork in the fast glycolysis group. Barbut (1993, 1996, 1997, 1998) has used color to select PSE turkey.

Water-holding capacity

Water-holding capacity (WHC) is important for both consumers and processors. From the consumers' point of view, WHC influences many quality traits such as nutritional value, appearance, and palatability (Kauffman et al., 1994). For the processing industry, WHC greatly impacts the economic value of weight for fresh meat products and the yield in processed products (the more water remains in meat, the heavier weight of the meat, the better).

WHC can be defined as the ability of meat to hold its own or added water (Hamm, 1960). Meat contains 75-80 percent water. Water is physically bound within in the muscle in three different forms: bound, immobile, and free (Pearson and Young,

1989). The bound and immobile water comprise only 5 % of the total water in the muscle (Hamm, 1986). The bulk of water in muscle is free. Free water can migrate throughout the muscle tissue and, therefore, is of interest in WHC.

After carcass or muscle are dissected, a red fluid exudes (drip) from the muscle surfaces. The mechanism of fluid loss is explained by Offer and Trinick (1983), who hypothesize that the losses of water from muscle are related to shrink of the myofibrils and myofilament lattice. The shrunken myofibrils or myofilament lattice cause the water to be expelled from the cell into the extracellular space. The myofibrils shrink as a result of denaturation of proteins in the muscle increased by a rapid pH decline and high carcass temperature. With decreasing pH, the charges on the filaments, and the negative electrostatic repulsion between the filaments is reduced. The occurrence of PSE meat with high drip loss is due to denaturation of proteins (Bendall and Wismer-Pedersen, 1962; Wismer-Pedersen, 1971; Hamm, 1986; Offer and Knight, 1988).

Proteins

Proteins are essential constituents of the animal body. The quantity of protein in the muscle is approximately 20%. The major proteins are classified as myofibrillar, sarcoplasmic, and connective tissue. Myofibrillar proteins are responsible for muscle contraction. Of all the muscle proteins, two third form the contractile elements of the myofibrills. The second major group of protein, the sarcoplasmic proteins, include the muscle pigment (myoglobin) and the enzymes for the glycolytic function. The connective tissue proteins constitute the third major class. Connective tissue makes

up the extracellular matrix, which supports and integrates the muscle bundles. Muscle proteins are important in the post-mortem changes involved in the conversion of muscle to meat. In addition, they are a major source of high quality proteins in the human diet.

Myofibrillar proteins

Myofibrillar proteins are responsible for contraction in the living animal. The two major contraction proteins are thick filaments-myosin, and thin filaments-actin. In terms of meat quality, the significant functions of the myofibrillar proteins are water binding and tenderness. Hamm (1986) concluded that 70% of the water of fresh meat is 'held' by the myofibrillar proteins. Most of the tissue water is located within the filament (Offer and Trinick 1983). Offer and Trinick (1983) suggested that water in the muscle tissue is held by capillary forces. With decreasing pH, the filaments spaces are reduced due to the changes in the structure of myosin and changes in the negative electrostatic repulsion between the filaments (Hamm, 1986). Irving et al. (1990) studied the effect of pH on myofilament spacing in pork; they reported that filament spacing increased from 40–46 nm in the pH range of 5.2 and 6.4. Beyond pH=6.4, the filament spacing did not change.

PSE meat, caused by a combination of high body temperature and low pH, results in denaturation (and shrinkage) of the heads of myosin. According to Offer and Knight (1988), myosin denaturation is the main factor responsible for the low WHC of PSE meat. There is good evidence for this hypothesis. Myosin denaturation is related

to the rate of glycolysis (Penny, 1967). Furthermore, myosin solubility and ATP-ase activity, both measures of denaturation, are reduced in PSE vs. normal pork (Lopez-Bote et al., 1989; Warner et al., 1997). Offer (1991) developed a model to predict and explain the effect of pH decline and temperature on the WHC of meat. Based on this model, the faster the rate of glycolysis, the greater the degree of protein denaturation and the lower the WHC. Only if glycolysis is very fast, the rapid rigor onset will limit myosin denaturation somewhat (Offer, 1991).

Sarcoplasmic protein

Sarcoplasmic proteins are in the sarcoplasm, the fluid bathing the muscle cells or fibers, and are soluble in water or in low ionic strength salt solutions. Approximately 30-35% of the total muscle proteins are sarcoplasmic proteins. Sarcoplasmic proteins contribute only 3% to total WHC of muscle (Wismer-Pedersen, 1971). Sarcoplasmic proteins have an important role in determining meat quality during the post-mortem period and meat processing. Many soluble enzymes are involved in anaerobic metabolism, and muscle pigments proteins are involved in color changes. Bendall and Wismer-Pedersen (1962) first reported that sarcoplasmic proteins are denatured in pork, resulting in a low water-holding capacity. They concluded that denatured sarcoplasmic protein are partly responsible for the watery condition of PSE meat. Fisher et al. (1979) reported that solubility and activity of the enzyme phosphorylase was decreased in PSE meat. Monin and Laborde (1985) suggested that spaces between the filaments decrease because of electrostatic repulsion and causes water

appearing. More recent studies (Warner, 1994; Wilson, 1997) indicate that a reduction in solubility of sarcoplasmic proteins has a negative impact on WHC.

Conclusions

The demand of chicken breast meat has increased in recent years (USDA, 1997). Meanwhile, the incidence of chicken with pale, exudative condition also increased (Vimini, 1996). PSE quality is undesirable to both retail meat buyers and processing industry. Much of the research on PSE concerns (stress-susceptible) pigs. There is little information on PSE in poultry, especially in chicken. This study emphasizes the importance of awareness the quality defect in chicken meat.

The purpose of review of literature was to present fundamental knowledge on PSE poultry muscle and WHC. This information will be used to address characteristics of PSE chicken, and the possible causes of this quality problem in chicken. The ultimate objective is to prevent occurrence of PSE chicken.

CHAPTER II

MATERIALS AND METHODS

Breast Muscle Samples

In Experiment 1, the samples were provided by Tyson Food, Inc. (Springdale, AR), and collected at a plant in Arkansas. Butterfly breasts were removed from the bird at approximately 6-8 h post-mortem. Selection of the samples was based on visual evaluation of color by Tyson meat researchers. Breast muscle was packed in the plastic bags, and stored and shipped overnight in an ice-packed Styrofoam cooler. Twenty normal and 20 pale samples were analyzed.

Birds

In **Experiments 2, 3, 4, and 5**, birds were obtained from a breeder flock at average weight 2.5 kg, provided by Burnett Poultry Inc. (Norris, TN). In each experiment, 60 chickens were used, except for **Experiment 4**, in which 30 chickens were used. The chickens were slaughtered on 2 separate days, each with 30 chickens. In **Experiment 5**, all 60 chickens were killed in one day.

Broilers arrived at the Poultry Research Farm at the University of Tennessee, a week prior to slaughter. Water and broiler feed were provided. For all chickens, feed was withdrawn 12 h prior to processing. Water was available up to 1 h before slaughter. The experiments were conducted by using two treatments, control and heat

stress. Each treatment had 2 replications of 15 birds per replication. Chickens were randomly assigned to each treatment.

Treatments

In **Experiment 2**, the control group was kept at room temperature (23.9°C) before slaughter. The to be stressed broilers were kept at 23.9°C, until one day before slaughter. At that time, the stressed chickens were moved to a temperature- controlled chamber. Four h before slaughter, the temperature in the chamber was increased from 23.9°C to 35°C within 3 h. Subsequently, the birds were caught, crated, and kept at 35°C for one extra hour. In **Experiment 3**, the temperature for the stress group was increased to 36.1°C instead of 35°C. In **Experiment 4 and 5**, the temperature of the stress group was the same as in **Experiment 3**. Moreover, the stress group was exposed to extra stress by chasing the birds on the floor for 10 min prior to catching and crating. This procedure was used to simulate catching procedures in an industry setting. The procedure for each experiment is summarized in Table 1.

Slaughter Processing

Chickens were stunned individually with a hand-held electrical knife (Model G, 6432, Voltage 120, 1 amp, Cervin Automated System Inc. Minneapolis,MN) and killed by

Table 1. The source of chicken, sample number and description of each experiment

	Source of Chicken	Sample No.	Purpose
Experiment 1	Tyson Foods	40	To characterize Pale or PSE chicken breast
Experiment 2	Burnett Poultry Inc.	60	To induce Pale or PSE breast by rapidly increasing temperature pre-slaughter Temperature change from 23.9 to 35°C within 3 h and followed by an extra hour at final temperature for the heat stress group. The control group stayed at 23.9°C all the time before slaughter.
Experiment 3	Burnett Poultry Inc.	60	The temperature for the heat stress group was increased from 23.9 to 36.1°C within 3 h; followed by an extra hour at the final temperature, for the heat-stress group. The control group stayed at 23.9°C all the time before slaughter.
Experiment 4	Burnett Poultry Inc.	30	The temperature and time setting of the stressed group were the same as for Experiment 3 ; stress was increased by chasing chickens on the floor for 10 min, followed by an extra hour at the final temperature.
Experiment 5	Burnett Poultry Inc.	60	The same procedures as Experiment 4

cutting the right carotid artery and jugular vein. After 4 min bleeding, broilers were immersed in water of 60°C for 50 sec. Subsequently, the feathers were removed by placing the broilers in a rotary drum picker (Ashley Machine Inc. Greensburg, IN) for 30 sec. Carcasses were manually eviscerated. Chilling was accomplished in an open stainless steel water tank at 15.5°C for 20 min, followed by slush ice for 75 min. The chilled carcasses were covered with ice and placed in a cooler at 0-2°C, until deboning, at 8 h post-mortem. The deboned breasts were packaged and kept overnight at 0-2°C. At 24 h post-mortem, the breasts were sampled for meat quality.

Quality Measurements

pH Measurement

Several pH determination methods were used.

(1) Direct probe method

The pH of each muscle was determined using an Orion pH meter (Model 250 A, Orion, Boston, MA). A spear glass probe was inserted into the center 0.5-1 cm below the pectoralis major muscle of one side of the breast sample.

(2) Slurry method

The pH of the ground meat was measured, using the same pH meter as in (1), by blending 1 g of sample in 10 mL deionized water as described by Stewart et al. (1984).

(3) Iodoacetate method

This procedure was used in **Experiment 4**. Iodoacetate was used to stop the glycolysis. A solution 5 mM of iodoacetate in 0.15 M KCl, adjusted to pH 7.0, was used. One g of pulverized samples was added to 10 mL of iodoacetate solution, and homogenized with Polytron® (Model PT 10/35, Brinkmann Instruments, Inc., Westbury, KY), for 15 sec. A Sentron pH meter (Model 2001, Federal Way, WA) was used to measure the pH of each sample.

The samples were randomly taken from the anterior, middle and posterior breast, at 10 and 30 min post-mortem. The sample was cut into small pieces, bagged in a WHIRL-PAK®, frozen with liquid nitrogen, and then temporarily stored on dry ice. Later, the samples were stored at -70°C. Before analysis of pH (and R-value), the frozen sample was pulverized with a hammer (crush sample by wrapping with thick towel to prevent a broken bag).

Color Measurement

Measurements were taken on the thickest part of the ventral surface, in the middle of the cranial third of pectoralis major muscle as depicted in Figure 3. A MINOLTA, surface spectrophotometer, (model CM-508d, SCI setting, D65 and 2° viewing angle, standardization using a pink reference tile, Minolta, Resume, NJ), was used. International Commission on Illumination color values L^* , a^* , and b^* , (L^* =lightness, a^* =redness and b^* =yellowness) were determined. Each sample was measured in triplicate and then averaged.

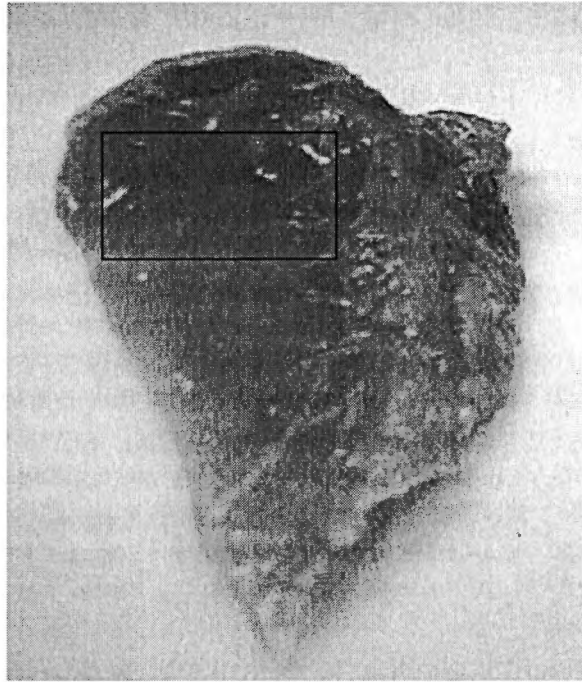


Figure 3. Location of color measurement

Drip Loss Measurement

Drip was measured in 1-L polymethylpentene jars, by suspending breast meat from a steel wire hook attached to the lid of the jar. A cored sample (Figure 4) of 4 cm diameter (one core per bird) was collected, weighed, and suspended. Samples were stored at $2\pm 2^{\circ}\text{C}$ for 48 h. After 48 h, samples were reweighed, and drip loss was calculated.

Sarcomere Length Measurement

The sample was cut (Figure 5) at 5 different locations across the center of breast. Fixation and measurement by laser diffraction method were as described by Koolmees et al. (1986).

Protein Solubility Measurement

Protein solubility was used as a measure of protein denaturation in the muscle. Total protein, myosin protein, and sarcoplasmic protein solubility were evaluated.

Sarcoplasmic proteins

The homogenates were stored overnight at $2\pm 2^{\circ}\text{C}$. The following day, homogenates were filtered through filter paper Whatman No. 1. The protein concentration of filtrate was measured, using the Biuret procedure (Gornall et al., 1949), and bovine serum albumin (BSA) as standard.



Figure 4. Location of drip loss measurement



Figure 5. Location of sarcomere length measurement

Myosin

Twenty mL of ice-cold 0.5 M KCl, 0.1 M potassium phosphate, 1 mM MgCl₂, 10 mM tetrapyrophosphate, (pH 6.8) was added to 2 g ground breast meat sample, in a 50 mL plastic tube. After overnight extraction at 0–4°C, the homogenates were centrifuged (2000 xg for 15 min) and filtered (Whatman No. 1). Protein concentration was determined as for sarcoplasmic proteins.

Total protein

Twenty-five mL of ice-cold 0.05 M potassium phosphate buffer (pH 7.4) and 0.55 M KI was added to 1 g ground breast meat sample in a 50 mL plastic tube. The samples were homogenized, stored, filtered and protein concentration was determined as described for sarcoplasmic proteins.

Water-Holding Capacity (WHC) and Cooking Yield (CY)

Water retention (uptake) and cooking yield were determined. Cooking yield was measured as the release of fluid after heating. The 50 mL plastic tubes were coded, and weighed. Exactly 6.00 g of the ground breast meat was weighed into each tube. Ten mL 3.5% NaCl solution was added, and shaken vigorously for 15 sec. The tubes were incubated for 30 min at 25°C. After incubation, the suspensions were centrifuged for 15 min at 3000 xg. The supernatant was discarded, and the tubes were inverted for 10 min, to assure complete removal of any supernatant. The weight of the tube and pellet was assessed, and WHC was calculated:

$$\{[(\text{Pellet weight} + \text{tube}) - \text{tube weight} - 6.00 \text{ g ground meat}] / 6.00\} * 100\% = \text{WHC} (\%)$$

After weighing of the pellets, the tubes were recapped loosely, and incubated for 20 min at 80°C. After cooking, the juices were poured off, and the tubes were inverted to assure removal of the cooking juices. After cooling thoroughly, the samples were weighed and cooking yield was calculated:

$$\{[(\text{Pellet weight} + \text{tube}) - \text{tube weight}] / 6.00\} * 100\% = \text{CY (\%)}$$

SDS-PAGE

SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) on the myofibrillar fraction (all proteins insoluble in rigor buffer) was performed to detect the present of phosphorylase, a sarcoplasmic protein that becomes insoluble when denatured. Two g ground meat was homogenized in 20 mL cold rigor buffer (75mM KCl, 5mM KPO₄, 2mM EGTA, 2mM MgCl₂, pH 7.2). The Polytron® homogenizer was set medium speed and the sample was homogenized for 10-15 sec. The sample was kept on ice while being homogenized. After homogenizing, the solution was centrifuged at 1000 xg for 15 min. Following centrifugation, the supernatant was poured off; the pellet was resuspended in rigor buffer, and centrifuged again. This process was repeated 4 times. After the final centrifugation, the pellet was resuspended in tris buffer (0.0625 M, pH 6.8). The protein concentration was determined by using biuret (Gornall et al., 1949) and Bovine serum albumin (BSA) as a standard. Proteins were analyzed on 12% acrylamide gels (Fritz et al., 1989). After fixation (0.025% Coomassie Brilliant Blue R 250, 50% methanol, and 9.2% acetic acid) and staining, the density of the various protein bands was determined with a

laser densitometer (GelScan XL version 2, Pharmacia LKB, Biotechnology). To get a measure of the amount of phosphorylase present in the myofibrillar fraction, independent of amount of protein loaded, the density of phosphorylase vs. actin was calculated.

Pigment

Total pigment was used to determine the concentration of myoglobin, cytochrome, and hemoglobin in the muscle. Ground sample (2.5 g) was weighed in to a 50 mL plastic tube and 10 mL ice-cold acetone was added. The suspension was mixed with a glass rod, for approximate 15 sec. Then, 0.5 mL deionized H₂O and 0.25 mL 12N HCl were added. The sample was kept in the dark overnight at 2±2°C. The following day, the sample was filtered over Whatman filterpaper No. 42 into a screwcap glass tube. After 30 min in the dark, at room temperature, absorbance at 512, 640, and 700 nm was assessed. The concentration of pigment in meat was calculated.

$$E = \varepsilon \times c \times d$$

E = absorbance

ε = molar absorption coefficient (640 and 512 nm)

c = concentration (mol/l or mmol/ml)

d = light path (1 cm)

Total pigment in 12.6 mL: at 640 nm $\Rightarrow$ 686 x E mg/kg (ppm)

at 512 nm $\Rightarrow$ 342 x E mg/kg (ppm)

R-value

The R-values was used to monitor the development of rigor. During post-mortem, glycolysis, adenosine triphosphate (ATP) is dephosphorylated to adenosine diphosphate (ADP) and adenosine monophosphate (AMP). AMP is then deaminated to inosine monophosphate (IMP). All adenine nucleotides show an absorption maximum at 259-260 nm, whereas the maximum of IMP is 248.5-249 nm. Therefore, the ratio of absorbances at 250 and 260 nm can be used to determine a R-value (Honikel, 1977).

Preparation of the samples is described under the iodoacetate procedure for pH. Three g of pulverized meat was thoroughly homogenized with 10 mL 1 M perchloric acid with Polytron® for 30 sec. The homogenate was filtered (Whatman No. 1). Then, 0.1 mL of the filtrate was diluted with 4.9 mL 0.1 potassium phosphate buffer pH 7.0. The absorption at 250 and 260 nm was measured with deionized water as reference, and the R-value was calculated.

Statistical Analysis

Data from all experiments were analyzed as complete random design using the SAS PROC MIXED (SAS Institute, Inc., 1996) to evaluate the differences between quality classes. PDIFF was used as the mean separation technique.

CHAPTER III

RESULTS AND DISCUSSION

Experiment 1

The physical and biochemical characteristics of two classes, normal and pale samples, are presented in Table 2. 'Normal' and pale muscle were significantly ($p < 0.05$) different in pH, L*-value, WHC, cooking yield, and solubility of sarcoplasmic proteins, 'myosin', and total proteins. The data of pH, WHC, and color are consistent with observations by Barbut (1993; 1997), who suggested that poultry breast muscle can exhibit the same pale soft exudative (PSE) characteristics as seen in pork.

The pale samples had a pH value of 0.26 units lower, and a lightness (L*-value) approximately 4 units higher than the normal samples. This is consistent with earlier observations by Van Hoof (1979) and Barbut (1993;1996;1997;1998). Van Hoof (1979) reported that the pale color in poultry meat is associated with lower pH, and that turkey meat is susceptible to a PSE-like condition as described in pork. Barbut (1993;1996;1997;1998) also observed that the pale breast turkey has a significantly lower pH and higher cooking loss compared to dark muscles, and suggested that use of L*-value is a fast method to detect PSE product without damaging the muscle.

Water-holding capacity and cooking yield were significantly ($p < 0.05$) lower in pale muscle than in normal muscle. The fact that pH is related to WHC has been

Table 2. The quality characteristic of pale and normal broiler breasts muscle (Experiment 2)(least square means±standard errors)

	Normal n=20	Pale n=20
pH	5.96±0.04 ^a	5.70±0.04 ^b
L*-value	55.1±0.5 ^a	60.0±0.5 ^b
Drip Loss (%)	0.87±0.2 ^a	1.33±0.2 ^b
WHC ¹ 25°C (%)	44±2 ^a	32±2 ^b
Cooking Yield (%)	106±2 ^a	96±2 ^b
Sarcomere Length (µm)	1.77±0.4	1.72±0.4
Sarcoplasmic protein (mg/g)	50±1 ^a	44±1 ^b
Myosin (mg/g)	172±2 ^a	165±2 ^b
Total Protein (mg/g)	217±2 ^a	210±2 ^b
Phosphorylase:actin	0.15±0.02	0.14±0.02

^{ab} Within rows, means with unlike superscripts are significantly different (p<0.05)

¹ Water-holding capacity

demonstrated in previous work on turkey meat (Froning et al. 1978; Barbut 1993;), in chicken breast meat (Northcutt et al., 1994; Barbut, 1996) and in pork (Bendall and Swatland, 1988). Cooking loss is a very important characteristic for the poultry industry. For processed products, water retention ability is a main point for profit. In the present study, only 3.5% salt was added because this amount of salt is the most commonly applied in the industry. Barbut (1993) found a 9% difference in cooking loss between the darkest and palest sample. This is similar to the observation in the present study in which the difference in cooking yield between pale and normal samples was 12%.

Drip loss was measured 48 h after slaughter. The drip losses were also significantly different, but very small. Compared to pork sample (>6% in PSE pork) (Warner, 1994), the drip losses from broiler breast were quite low. The amount of water bound to myosin is minimum at its isoelectric point (pH 5.3). At this pH, there is minimum repulsion between the charged group on myofibrillar proteins, and thus the spacing between myofibrils is minimized, therefore, drip loss would be high. The ultimate pH of broiler breast muscle is away from isoelectric point, drip loss would be expected to be low.

The differences in protein solubility are small but significant ($p < 0.05$). Protein solubility values indicate that pale muscle had more protein denaturation; sarcoplasmic, myosin, and total protein solubility values were lower ($p < 0.05$) in pale samples than in normal samples (Table 2). However, it is not clear if these small differences would explain the differences in color and water-holding capacity. In

comparison with total protein solubility of PSE by Warner (1994), the total protein solubility of pale muscle in pork is 90 mg/g, the total protein solubility of pale breast muscle in broiler is relatively high.

The ultimate pH in chicken breast was found higher than in pork loin muscle. The data from the study for total proteins and sarcoplasmic proteins indicate that pale chicken has less protein denaturation than PSE pork (Warner, 1994). In PSE pork, myofibrillar proteins have a poor extractability, and sarcoplasmic proteins have low solubility; these two proteins are highly correlated with WHC (Bendall and Wismer-Pedersen, 1962; Warner, 1994). However, RSE (red, soft, exudative) muscle, another pork quality defect, has no myofibrillar proteins denaturation. The only difference between normal and RSE muscle is precipitation of phosphorylase (part of sarcoplasmic proteins). In turkey, Pietrzak et al. (1997) found that phosphorylase was precipitated in the myofibrillar fraction of PSE muscle. The sarcoplasmic proteins are denatured, precipitated and ended up in the myofibrillar fraction. The quantitative phosphorylase bands are measured by the laser densitometry. The ratio of phosphorylase to actin is determined because actin is resistant against denaturation and not degraded by proteolytic muscle enzymes. Pale samples did not have a significant higher amount of bound phosphorylase than the normal samples (Table 2).

Sarcomere length has been associated with water-holding capacity. Shorter sarcomeres results in lower WHC (Warner, 1994). Honikel et al. (1986) investigated the effect of shortening on drip loss. It was concluded that drip losses within the first

24 h post-mortem were hardly affected by shortening. In the present study, sarcomere length was not significantly different between the two classes.

The L*-value was significantly ($p < 0.01$) correlated with the pH of the meat (Table 3). A strong negative correlation between pH and L*-value ($R = -0.76$) indicated that as the pH value decreases, the L*-value increases (meat paler). The correlation between pH and WHC ($R = -0.71$) suggests that pH is an important determinant of WHC. The correlation between L*-value and WHC and protein solubility are low (Table 3). Thus, results suggest that paleness in broiler is due to a low ultimate pH rather than to denaturation of proteins (resulting from a rapid post-mortem glycolysis).

Low ultimate pH is partially related to the presence of substrates in the muscle of the live animal at the time of slaughter. After slaughtering, the substrates are available for the anaerobic metabolism to produce lactic acid, resulting in lowering pH (Crouse et al., 1984; Warriss et al., 1988). A higher ultimate pH may be achieved by withdrawing feed some time before slaughter to empty of the bird's digestive tracts before slaughter (Northcutt and Buhr, 1997).

According to the significant correlation between WHC and pH, cooking yield and pH, and pH and L*-value, the ultimate pH and L*-value can be used to identify normal and pale samples. Based upon the results of the present study, if the ultimate pH is less than 5.9, and the L*-value is greater than 58, the muscle can be considered PSE chicken.

Table 3. Pearson correlation coefficients (R) among muscle quality parameters

	pH	L*	DL ¹	WHC ²	CY ³	SP ⁴	MP ⁵	TP ⁶
pH	1.00	-0.76	N/S	0.71	0.64	0.45	N/S	N/S
L*		1.00	0.37	-0.75	-0.63	-0.71	-0.36	-0.31
DL			1.00	-0.33	-0.43	-0.41	-0.47	-0.35
WHC				1.00	0.68	0.66	N/S	N/S
CY					1.00	0.44	N/S	N/S

¹. Drip loss

². Water-Holding Capacity

³. Cooking Yield

⁴. Sarcoplasmic protein

⁵. Myosin protein

⁶. Total protein

N/S: not significant

Experiment 2

The second objective of this study was to attempt to determine the cause of PSE-like condition in broilers. According to information from the industry, the incidence of pale muscle is dependent on season, especially sudden changes in temperature. Therefore, in this study, ambient temperature was used in an attempt to induce PSE quality in broiler breast muscle. The temperature for heat stress chicken (35°C) was chosen because it induces considerable physiological stress without lethal consequences (Smith and Teeter, 1987).

As shown in Table 4, chicken exposed to ante-mortem heat stress was not significantly different from the control for any of the parameters studied. Based upon the previous experiment, PSE meat has a low ultimate pH and high L*-value. In Experiment 2, the stressed group had similar pH (5.66 vs. 5.70) and L*-value (56.1 vs. 57.4) as the control group. Consequently, WHC and CY were not affected. According to Experiment 1, if pH < 5.9, the meat is PSE quality. However, the L*-values for both treatments are below 58. The data indicate a wide range in pH (control 5.43-6.0 vs stressed 5.34-6.1) and L*-value (control 42.1-64.2 vs. stressed 45.3-62.3) for both treatments.

If lightness L*-value=58 is used as a cut-off point (based upon the data of Experiment 1), then the incidence of pale muscle is 23.3% (control) and 13.6% (stressed). These numbers were not significantly different. In Experiment 1, there were

Table 4. The quality characteristics of breast meat from heat-stressed (temperature increased from 23.9 to 35°C in 3 h, followed by 1 h at 35°C) and control (23.9°C constantly) broilers (Experiment 3)(least square means±standard errors)

	Control n=24	Stressed n=27
pH	5.72±0.03	5.66±0.03
L*-value	56.1±0.8	55.4±0.8
WHC ¹ 25°C (%)	61±3	57±3
Cooking Yield (%)	115±2	113±2
Pigment (mg/g)	0.172±0.008	0.166±0.008

¹ Water-holding capacity

three samples in the normal group with a L*-value in the range from 58.1-58.9. Therefore, it was decided to increase the cut-off for L*-value to 60. Using L*-value=60, there was still no significant difference between control and stressed (control 6.8 vs. stressed 3.4%). According to information provided by the industry, the incidence of PSE on 'cool' days is only two percent. It is unclear what caused the high incidence of PSE in the control group in this experiment.

Experiment 3

In the previous experiment, there were no significant differences between the heat-stressed and control group. Therefore, for the heat-stress treatment, the temperature was increased from 35 to 36.1 °C. This temperature was chosen because it has been reported to be high enough to stress birds but does not cross a dangerous zone at which birds may die from heat prostration (Smith, 1997). As can be seen in Table 4, the higher temperature resulted in significantly ($p < 0.05$) higher L*-values, lower WHC, and lower cooking yield in the heat stressed group than in the control group. The effect of stress, as noted in this study, is consistent with previous work (Froning et al., 1978; Barbut, 1993; Northcutt et al., 1994).

Using L*-value=58, the stress group has 40% PSE, whereas 24% of the control group was PSE (Table 5). By using this cut-off point, there were significant differences ($p < 0.05$) between the two classes. No significant difference was found by using L*-value>60.

Table 5. The quality characteristics of breast meat from heat-stressed (temperature increased from 23.9 to 36.1°C in 3 h, followed by 1h at 36.1°C) and control (23.9°C constantly) broilers (Experiment 3)(least square means±standard errors)

	Control n=29	Stressed n=29
pH	5.73±0.03 ^a	5.68±0.03 ^b
L*-value	58.3±0.5 ^a	59.8±0.5 ^b
a*-value	1.07±0.2	1.26±0.2
b*-value	12.2±0.3	12.99±0.3
WHC ¹ 25°C (%)	51±2 ^a	37±2 ^b
Cooking Yield (%)	111±2 ^a	99±2 ^b
Pigment (mg/g)	0.12±0.005	0.13±0.005
Total Protein (mg/g)	237±3 ^a	206±3 ^b
Incidence PSE (%)		
L*-value>58	24 ^a	40 ^b
L*-value>60	14	26

¹ Water-holding capacity

^{ab} Within rows, means with unlike superscripts are significantly different (p<0.05)

Pigment concentrations were not significantly different. a^* -value indicates very low red pigment concentrations in the muscle for both treatments. There is a slight significant negative correlation ($p < 0.05$, $R = -0.38$) between L^* -value and pigment. As the pigment concentration of the muscle decreases, the corresponding L^* -value of the muscle increases.

Low pH values are usually associated with lower meat quality such as lower WHC, cooking yield, and higher shear values (Bendall and Wismer-Pedersen, 196; Bendall and Swatland, 1988; Barbut, 1993; McKee and Sams, 1998). This was shown in this study where pH, WHC and cooking yield, were less desirable in breast meat obtained from the preslaughter heat-stressed condition. The low ultimate pH value in pale meat was consistent with the results of Experiment 1; a low pH was associated with low WHC, and a pale color which indicates low meat quality.

The incidence of pale muscle in the control group is quite high compared to reports from the industry, which is 2% in the cool days. Even if the occurrence of pale breast broiler meat is related to sudden increases in high environmental temperature, it is unknown what caused the high incidence of pale muscle in the control group.

Experiment 4

In Experiment 2 and 3, temperature was used as the only stress factor. In Experiment 4, to increase the level of stress and make the situation closer to the real life situation of catching broilers, an extra stress treatment was added by chasing the broilers before putting them in the crates.

Despite the increased level of stress, there were no significant differences between the control and heat-stresses group (Table 6). These results were unexpected since, compared to Experiment 3, an extra stress factor (chasing practice) had been added. A heat-stressed broiler would have displayed hormone secretion, i.e. epinephrine, resulting in increased body temperature and faster exhaustion of energy (ATP). R-value is used as an indicator of how much ATP is converted to inosine monophosphate (IMP). If ATP runs out, in the absence of oxygen, anaerobic metabolism takes place, resulting in a pH decline. The data indicate that there are no significant differences in pH at 6 min and 24 h post-mortem and R-value at 6 min, but there is a small significant difference in pH at 30 min post-mortem and in R-value at 30 and 24 h post-mortem of control vs. stressed chickens (Table 7).

According to our observations, pH value slightly increased between 6 and 30 min post-mortem. This finding is consistent with the results reported by Dodge and Peters (1960) and Landes et al. (1971). De Fremery (1966) hypothesized that the slightly increased pH value is due to alkaline creatine produced as a result of breakdown of creatine phosphate. This accumulates temporarily in the muscle, resulting in increasing pH. It is unclear why the control group has a lower pH at 6 and 30 min than the stressed group.

Table 6. Quality characteristics of breast meat from heat-stressed (temperature increased from 23.9 to 36.1°C in 3 h, followed by 1 h at 36.1°C) and control (23.9°C constantly) broilers. Extra stress was applied by chasing chicken on the floor for 10 min before one hour at 36.1°C (Experiment 4)(least square means±standard errors)

	Control	Stressed
	n=14	n=15
pH	5.73±0.04	5.71±0.04
L*-value	58.3±1.0	58.3±1.0
WHC ¹ 25°C (%)	21±2	20±2
Cooking Yield (%)	88±2	86±2

¹ Water-holding capacity

Table 7. The rate of pH and temperature post-mortem on chicken breast muscle (Experiment 4)(means±standard deviation, control n=14, stress n=15)

	6 min		30 min		24 h	
	<u>Control</u>	<u>Stress</u>	<u>Control</u>	<u>Stress</u>	<u>Control</u>	<u>Stress</u>
pH ¹	6.2±0.1	6.4±0.1	6.3±0.1 ^a	6.6±0.1 ^b	5.7±0.0	5.7±0.0
Temp ²	38.2±0.6	38.6±0.6	21.9±0.5	21.5±0.5	N/D ³	N/D
R -value ³	0.83±0.00	0.83±0.00	0.82±0.0 ^a	0.83±0.0 ^b	1.43±0.02 ^a	1.42±0.01 ^b

^{ab}Results at a specific sampling time bearing a different superscript are different (p<0.05)

¹ Iodoacetate method

² Temperature (°C)

³ The ratio of inosine to adenine nucleotides, calculated by the ratio of absorbance at 250 nm to absorbance at 260 nm

³ Not determined

According to the rate of glycolysis, the control birds received as much stress as the stressed birds. This result is not consistent with the report of Mckee and Sam (1998) who studied the rigor development at 15, 30 min, 1, 2, and 4 h in turkey at different temperatures; they found that the R-value was significantly higher ($R\text{-value} > 1$) at 15 min in 40°C vs. 25°C treatment. When kept at 40°C, rigor onset ($R > 1.0$) occurred the first 15 min post-mortem. In the present study, $R\text{-value} > 1$ occurred some time between 30 min and 24 h post-mortem.

In PSE porcine longissimus muscle a typical pH decline results in a pH 6.0 at 45 min post-mortem (Bendall, 1973; Bendall and Swatland 1988; Offer 1991). In Experiment 4, we found that pH dropped 6.3 at 30 min post-mortem. Broilers did not reach the onset of rigor at 30 min.

Experiment 5

The results of quality characteristics of Experiment 3 and 4 were not consistent. Less stress (Experiment 3, only temperature) seems to result in more PSE than more stress (Experiment 4, temperature +chasing exercise). It might be suggested that results of Experiment 4 were influenced by the sampling for pH and R-value. Therefore, we decided to repeat Experiment 4, but not sample for R-value and pH. Thus, all factors, except the additional chasing stress in Experiment 5, were the same as in Experiment 3. The results of Experiment 5 are included in Table 8. Again, as in Experiment 4, both treatment groups were comparable in meat quality characteristics.

Table 8. The quality characteristics of breast meat from heat-stressed (temperature increased from 23.9 to 36.1°C in 3 h, followed by 1 h at 36.1°C) and control (23.9°C constantly) broilers. Extra stress was applied by chasing chicken on the floor for 10 min before one hour at 36.1°C (Experiment 5)(least square means±standard errors)

	Control n=28	Stress n=28
pH	5.76±0.03	5.71±0.03
L*-value	56.6±0.6	57.9±0.6
WHC ¹ 25°C (%)	24.5±1.4	26.5±1.4
Cooking Yield (%)	92.9±1.4	96.0±1.4

¹ Water-holding capacity

According to Experiment 1, PSE quality muscle has an ultimate pH<5.9 and L*-value >58. In Experiment 5, both control and stressed muscle had an ultimate pH below 5.9. Yet, the L*-value was lower than 58, i.e. not pale. The results of Experiment 5 are consistent with those of Experiment 4. Thus, the question is whether the stress group does not get stressed enough, or if the control group suffered stress as well. The possible factors that may have influenced the results are processing procedures (stunning) and animal strains (genetics).

Stunning is primarily used to render the animal unconscious for easier handling during slaughter and to make the animal insensible to pain. Stunning also affects the tenderness of meat (Lee et al., 1979) because electrical stunning may delay post-mortem glycolysis. Papinaho and Fletcher (1995) studied stunning amperages from 0 to 200 mA and monitored the rigor development. They found that the higher the amperages, the higher the pH at 15 min post-mortem. However, there were no difference at 24 h post-mortem. Papinaho and Fletcher (1996) reported that stunning (50 and 125 mA) increased pH and decreased R-value until 6 h post-mortem compared to the control group (0 mA). In the stunning step in our experiments, the current was 1 A. There are no data on the influence of such high currents. Based upon the results by Papinaho and Fletcher (1995), a higher ultimate pH may be expected. Yet, we found a low pH in both the stressed and the control group. At present, there is no information on the effect of both stunning and heat stress on meat quality.

In pigs, PSE quality is known to be associated with the presence of a stress gene. It is not known if a similar genetic defect exists in chicken. The possible genetic influences on PSE have not yet been determined (Anthony, 1998).

CONCLUSIONS

Sudden changes in the environment induce a response of the animal's physiology in an effort to acclimate to the environment. Changes that occur immediately before slaughter affect the muscle's biochemistry post-mortem, and further determine the meat quality. Improving or preventing a defect quality (PSE) of broilers requires an understanding of PSE muscle in broiler, and the causes of the PSE quality muscle in broiler. The purpose of the research presented was to address these issues.

The results indicate that the PSE condition in chicken is different from that in pork. PSE chicken has less protein denaturation, a higher ultimate pH, and less drip loss than PSE pork. In broiler, paler muscle has a lower pH, WHC and cooking yield than normal color muscle. A low ultimate pH seems to be the main reason for the occurrence of PSE in chicken.

Sudden changes in ambient environment temperatures did not successfully induce PSE quality breast muscle in broiler. In pork, PSE is related to genetics (porcine stress syndrome). Genetic effects in broiler may potentially exist, and need to be identified. At present, there are few reports on poultry strain variation in response to stress. More research is needed including (i) test the genetic basis involved in the PSE condition in chicken (ii) intensive time observation of development of rigor mortis in broilers to have better understanding of the rate of glycolysis (iii) how much myosin protein denaturation will result in a low WHC (in pale muscle). The ability

to control the rate of glycolysis to prevent quick protein denaturation by chilling or stunning methods could reduce the amount of PSE muscle in broiler.

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VITA

Cheng-Hwei Liu, born June 16, 1970, in Taipei, Taiwan, received her elementary and secondary education in the Taipei public school system. After graduating from Ching-Mei Girls' High School in 1988, she attended Soochow University in Taipei. In September 1992, she transferred to Utah State University, Logan, Utah, U.S.A. In August 1994, she was an exchange student at Kansai Gaidai, Hirakada, Japan. A year later, in June 1996, she completed the requirements for a Bachelor of Science degree in Agricultural Systems Technology and Animal Science. She entered the graduate school of the University of Tennessee in January 1997, and received the Master of Science degree in Food Science and Technology in December 1998.

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