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Dietary Creatine Induces PSE-like Broiler Meat

Amy Paulette Chandler
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To the Graduate Council:

I am submitting herewith a thesis written by Amy Paulette Chandler entitled "Dietary Creatine Induces PSE-like Broiler Meat." I have examined the final electronic 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 Animal Science.

Kelly R. Robbins, Major Professor

We have read this thesis and recommend its acceptance:

Michael O. Smith, Judith M. Grizzle

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Dietary Creatine Induces PSE-like Meat in Broilers

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

Amy Paulette Chandler
August 2008

Dedication

This thesis is dedicated to my fiancé Aaron and to my family for their continued support and patience through this process.

Acknowledgments

I would like to thank Dr. Kelly Robbins for acting as my major professor and providing me with the encouragement and humor necessary to survive the graduate program. I also extend my gratitude to Dr. Michael Smith and Dr. Judy Grizzle for serving as members of my graduate committee. I would like to give a special thanks to Eddie Jarboe and Linda Miller for their help in the lab and to Roger, Steven, and the rest of the JARTU staff for their help in the management and processing of the birds. Last but certainly not least; I would like to thank the graduate students for their continual help and support.

Abstract

Consumer demand for chicken breast meat has led poultry breeders to emphasize selection for high breast muscle yield, resulting in contemporary broilers that grow rapidly with heavy muscling. Genetic selection for fast growth and high yield has increased the incidence of PSE-like meat that occurs when low muscle glycogen levels decrease quickly resulting in lactic acid build-up, rapid pH decline, and early rigor mortis onset. Preliminary research in our laboratory suggests that dietary inclusion of creatine in broiler diets may reduce the incidence of Pale, Soft, and Exudative (PSE) like meat. We hypothesized that creatine supplementation in broiler diets increases muscle creatine concentration, which may slow glycogen breakdown, decrease the rate of pH decline, and delay the onset of rigor mortis. To test this hypothesis we fed fast-growth mixed sex broilers creatine-supplemented and non-supplemented (control) corn-soybean diets. Three nutritionally complete diets were formulated for each phase of boiler growth: starter, grower, and finisher. The control diet contained 0% creatine; the other two experimental diets each contained 0.05% creatine provided as either the monohydrate (CMH) or monohydrochloride (CMHC) form. Five birds from each pen were used to determine total body composition; the remaining ten birds in each pen provided samples from the breast muscle that were used to determine pH (<30 min, and 4, 7, and 24 hours), expressible moisture, drip loss, and CIE color values for L*, a*, and b*. Breast muscle from birds fed creatine-supplemented diets underwent a more rapid rate

of pH decline than birds fed the control diet ($P < .05$). The degree of lightness (L^*) and degree of redness (a^*) was higher in the muscle from birds fed creatine-supplemented diets compared to birds fed the control diet, but only the L^* value from birds fed the CMH supplemented diet and a^* value from birds fed the CMHC diet were found to be significantly higher than values from muscle of control birds ($P < .05$). The addition of creatine monohydrate in the diet resulted in markedly higher drip loss percentage compared to the birds fed a control diet ($P < .05$). Overall, supplementing creatine in the broiler diet adversely affected meat quality characteristics.

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Introduction

Over the last several decades, consumer demand for expedience and quantity of poultry products has shifted the goal of producers towards growing birds at a rapid rate with increasingly larger muscles (Anthony, 1998). In 1990 the poultry industry produced approximately twenty-three billion pounds of meat from broilers; by the year 2015 the United States is expected to produce an estimated forty billion pounds of meat from broilers (USDA, 2008). The impending increase in the amount of broiler production will result in an even greater need to grow heavily muscled birds quickly. Intense selection pressure has made the goal of rapidly growing, heavily muscled birds attainable but the success of reaching this goal is over shadowed by the increasing number of products affected by pale, soft, and exudative (PSE) meat.

PSE is a condition that results in meat that exhibits exceptionally light color with poor texture as a result of low water holding capacity (Van Laack et al., 2000). Biochemical changes that occur in the muscle post-mortem are a consequence of anaerobic glycolysis and have the potential to create conditions that will result in inferior quality meat. More specifically, the rate at which pH falls and lactic acid accumulates in the muscle determines the quality of meat that develops. Rapid rates of pH decline in the post-mortem muscle have been implicated in the development of PSE (Bate-Smith and Bendall, 1949; Berri et al., 2005; Goldspink G, 1964).

Although PSE was initially a concern in the swine industry; the increase of PSE affected poultry meat is becoming an exceptionally troublesome concern in the poultry industry because consumer desirability of poultry meat is an essential component necessary for the industry's success. When consumers are confronted with choosing between various poultry products, quite often buying decisions rest on the consumers' preference of color and texture, both important quality attributes of meat. PSE negatively affects the quality attributes of poultry meat that concern most consumers. In addition to consumer preference, PSE decreases the usability of poultry meat for further processed parts, a market that currently accounts for an estimated 50% of the total product market (Council, 2002). The rate of post-mortem pH decline in the muscle is affected by a variety of events that occur long before the product reaches the grocery shelves. Events that are likely to result in rapid rates of post-mortem pH decline include: stress incurred during transportation, excessive struggle during slaughter, and the rate of post-mortem temperature decline. The development of PSE is further compounded by the genetic make-up of the bird (Chiang et al., 2004; Sayre et al., 1963b).

The poultry industry has tried a variety of methods to eliminate the development of meat classified as pale, soft, and exudative. Attempts have been made to investigate alternative methods of slaughter, minimize struggle during slaughter, and adjust chilling temperatures. Currently, the methods designed to

combat pale, soft, and exudative meat have proved unable to eliminate or substantially decrease the occurrence of PSE (Barbut, 1998).

Alternatively, increasing the energy status of the muscle at the time of slaughter has the potential to effectively decrease the incidence of PSE. Human studies indicate that the supplementation of creatine in the diet is likely to increase the availability of energy to the muscle (Hultman et al., 1996; Snow et al., 1998); if the same occurs in broilers creatine supplementation could prevent the development of pale, soft, and exudative meat. Consequently, the objectives of this research were to:

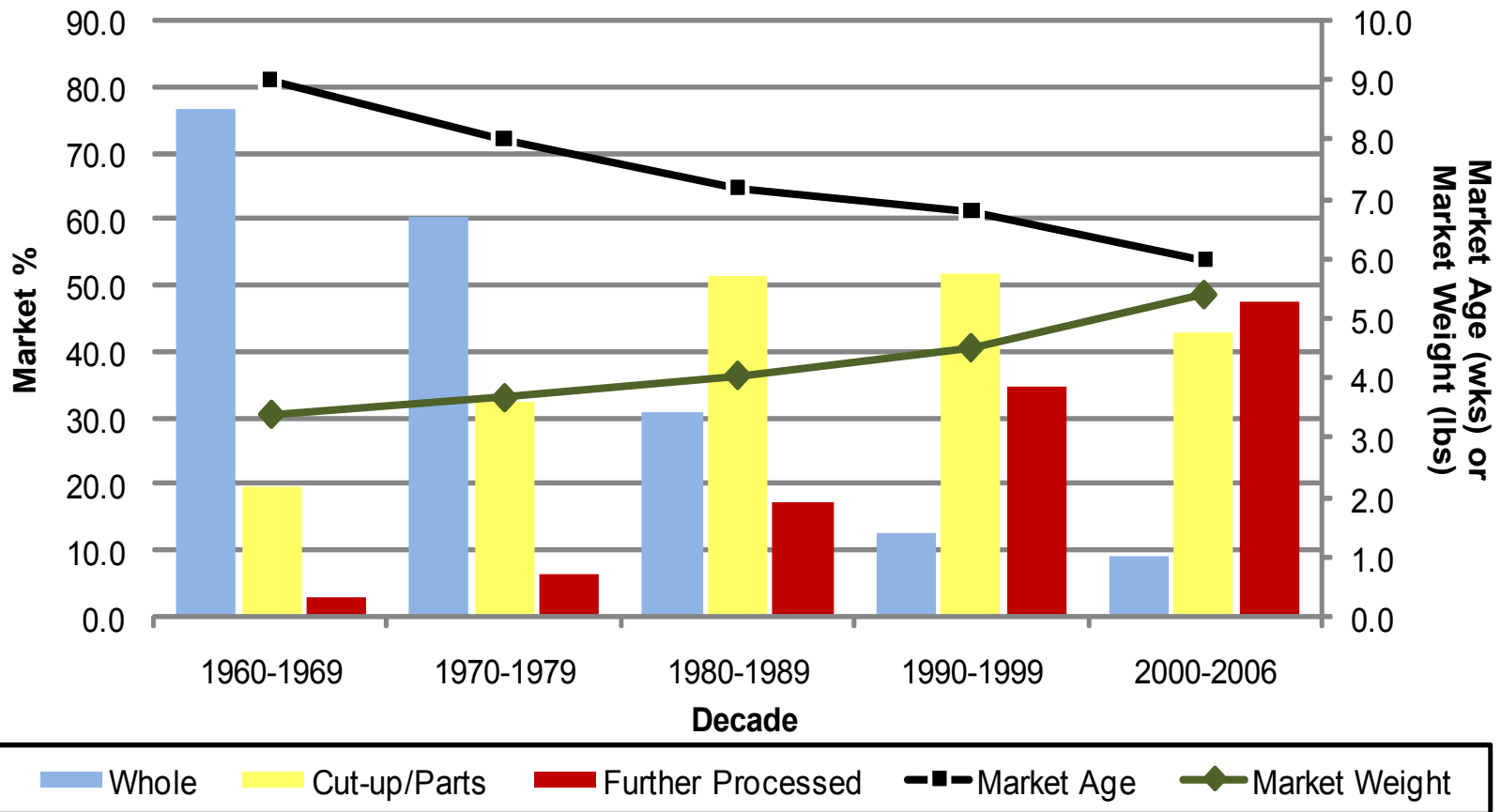
1. Determine the effects of creatine monohydrochloride and creatine monohydrate on the growth performance of broilers.
2. Determine creatine effects on breast muscle quality characteristics.

Literature Review

Introduction

Consumer demand for poultry has increased steadily over the last 50 years. During the period 1998 to 2007, broiler consumption per capita underwent an approximate 16-pound increase from an estimated 72 pounds to over 88 pounds (USDA, 2008). Producers have compensated by growing broilers that rapidly develop heavy muscling. Presently, the market age for the majority of broilers is six weeks; a 70% decrease from 1925 when market age was 20 weeks (Anthony, 1998). Over the same period of time, broiler market weight was increased from 2.5 pounds in 1925 to an estimated 5.25 pounds in 2005 (Council, 2002). The goal of producers to reduce broiler market age while at the same time increase broiler weight was driven, in part, by the change in consumer demand from whole birds to cut-up and further processed parts (Figure 1).

Although goals for rapid growth and heavy muscling have been accomplished, meat quality has suffered. The predominant quality concern in the broiler industry is a condition called pale, soft, and exudative (PSE). PSE-like broiler meat is described as having extremely pale color and a low water holding capacity (Van Laack et al., 2000). Initially, PSE developed as a problem in the swine industry. In the 1960's, meat science research was primarily focused on genetic selection for fast lean growing hogs, a highly heritable trait (Miller, 2002). This led to decreased pork quality because the incidence of PSE



*Data obtained from the National Chicken Council

Figure 1: Broiler Market Statistics

in meat shared a relationship with genetic selection for fast, lean growing animals. Estimates of the broiler industry indicate that the occurrence of PSE in typical slaughter facilities reach upwards of 30% (Barbut, 1998). Meat affected by PSE is known to have undesirable characteristics that include pale color and low water holding capacity. In the broiler industry, meat with a high ability to hold water is particularly important, as there is a relationship between water holding capacity and the suitability of meat for further processing.

Muscle Anatomy and Physiology

Anatomy

Skeletal muscle is comprised of muscle fibers that run the length of the muscle bound together by connective tissue. Sarcolemma, a membrane layer, encloses each fiber. Fibers enclose numerous cylinder shaped myofibrils containing thick and thin filaments. A myofibril's working portion is located from Z line to Z line and called the sarcomere. Z lines are situated on the myofibril perpendicular to the longitudinal alternating light and dark lines, respectively termed I bands and A bands. The lighter I band corresponds to thin filaments while the darker A band corresponds to mostly thick and some thin filaments. Thick and thin filaments give the muscle its striated appearance. Thin filaments are comprised of two twisted protein strands containing spherical actin molecules while thick filaments contain the protein myosin. At the A band, thin filaments slightly overlap with thick filaments. Thick and thin filaments are the cornerstones of the sliding filament contraction model. This model describes

muscle contraction as the shortening of the muscle as a result of thin filaments sliding across thick filaments thus bringing I bands closer together. Sliding occurs when myosin projections of the thick filament form a cross-bridge with the actin molecules of the thin filament, actively moving thin filaments across thick filaments (Hanson and Huxley, 1953; Huxley, 1953a, b; Huxley and Hanson, 1954).

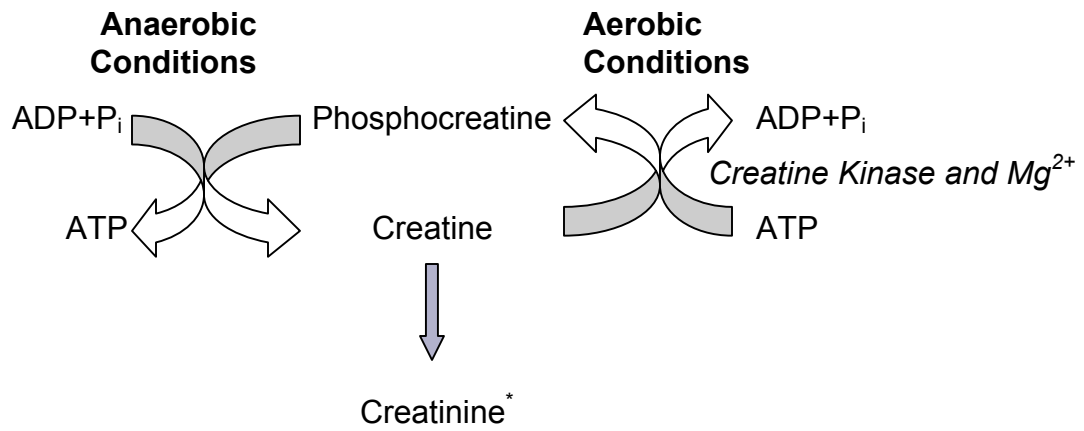
Physiology

Signals received by motor neurons located on the muscle depolarize the sarcolemma. The depolarization of the sarcolemma results in the release of calcium from the sarcoplasmic reticulum, located within the fiber. Calcium allows for the myosin projection to form the cross-bridge necessary to move the filaments (Davies, 1963). The active movement of filaments occurs as a result of adenosine triphosphate (ATP) availability. The myosin head breaks down ATP forming adenosine diphosphate (ADP) and a phosphate group (P_i); the resulting energy is stored in the myosin head. Calcium is released resulting in the binding of the myosin head to the actin molecule. The myosin head uses the stored energy to slide the thin filament across itself resulting in the muscle shortening. The stored energy used by the myosin head needs to be replaced. The myosin head breaks from the cross-bridge with actin to bind with ATP. The binding of ATP by the myosin head is the action that breaks the actin-myosin cross-bridge allowing the muscle to relax. ATP is regenerated in the muscle from the donation of a phosphate group from creatine phosphate (CP) in a creatine kinase (CK)

reaction (Figure 2). Creatine, the product of the CK reaction, is either removed from the muscle to be excreted as creatinine by the kidneys or will be recycled in the muscle by accepting P_i from the myosin head breakdown of ATP reforming CP.

Chicken Pectoralis (Breast) Muscle and Post-Mortem Physiology

The square area of the chicken pectoralis muscle is composed of 98% white fibers (Kiessling, 1977). Being glycolytic by nature, white fibers rely on glycolysis to produce energy. One glucose entering the glycolytic cycle produces two ATP and two molecules of pyruvic acid. Aerobic conditions preferentially send the two pyruvic acid molecules through the Krebs citric acid cycle to produce additional ATP. Glycolysis and the Krebs cycle yield a net total of 38 ATP produced for every glucose molecule entering the cycle. After death, the availability of oxygen ceases and the muscle undergoes glycolysis anaerobically. Under anaerobic conditions, rather than move through the Krebs Cycle, pyruvic acid formed during glycolysis is reduced to lactic acid causing muscle pH to drop. Skeletal muscle relies on ATP as its main source of energy. In order to adequately supply the muscle with energy, ADP must be phosphorylated to ATP. Phosphorylation is catalyzed by CK, the phosphorous group (P_i) from phosphocreatine binds with ADP to form ATP. ADP is phosphorylated until the availability of P_i ceases (Scopes, 1973). The availability of P_i is a direct result of



*Creatine is removed from the muscle, converted to creatinine in the kidney, and excreted through the urine

Figure 2: Creatine Phosphorylation in the Muscle

the CK reaction. CK activity is dependent on three conditions: the availability of Mg^{2+} , skeletal muscle pH, and phosphocreatine/ATP concentrations (Kuby et al., 1954; Noda et al., 1954). In addition to the pH drop, without the replenishment of ATP (Batesmith and Bendall, 1947) and in the presence of calcium, as is the case after death, the muscle fails to relax and stays in a continued state of contraction termed rigor mortis. Muscle relaxes after a period of time as a result of protein denaturation.

Pale, soft, and exudative (PSE)

Genetics

PSE is a result of multiple factors: the genetic make-up of the individual, the environmental conditions the live animal is exposed to just prior to death, and the resulting biochemical changes that occur within the first twenty-four hours post-mortem. Genetic differences exist that predispose individuals to producing meat with PSE-like characteristics (Sayre et al., 1963b). Breed influences the amount of modifications in the glycogen structure that occur post-mortem. In swine, Sayre et al. (Sayre et al., 1963a) observed more pronounced glycogen alterations in chester whites compared to poland chinas and as a consequence, chester whites experienced a slower rate of anaerobic glycolysis while poland chinas experienced a faster rate of anaerobic glycolysis.

More recently, a genetic predisposition to producing PSE-like meat has been associated with malignant hyperthermia, a metabolic condition involving the increased release of Ca^{++} from the sarcoplasmic reticulum in conjunction with

ryanodine binding to the ryanodine receptor located on the sarcoplasmic reticulum in skeletal muscles. Ryanodine receptors are the channels through which calcium exits the sarcoplasmic reticulum and enters the cell's cytosol. Mutations found in the ryanodine receptor gene (RYR1) have been causally linked to the incidence of malignant hyperthermia (Mickelson et al., 1988), a condition that results in tetany of the skeletal muscle. A second causative gene responsible for an individual's susceptibility to malignant hyperthermia induced by stress is the halothane gene (HAL) (MacLennan et al., 1990). The allele (n) is responsible for stress-susceptibility with both nn and Nn genotypes considered at a high risk to produce PSE affected meat but homozygous nn genotypes (carriers) are twice as likely as both Nn (carrier) and NN (normal) genotype swine to produce meat classified as PSE (Guardia et al., 2004). Limited research has been performed investigating the genetic factors that contribute to PSE-like meat in poultry. Chiang et al. (2004) indicate the homozygous α RYR1-I turkey genotype is more prone to producing PSE-like meat compared to the alternative homozygous α RYR1-II genotype. A third gene, Rendement Napole Gene, is responsible for the muscle's glycolytic potential (GP), a term used to describe the muscle's ability to prolong anaerobic glycolysis. Individuals with the recessive (rn^+) allele tend to produce meat that is substandard to the meat produced by the dominant (RN^-) allele (Le Roy et al., 1990). Although genetic predisposition plays an important role in the quality of meat, individuals that lack the genes responsible for producing pale, soft, and exudative meat are still at risk of

producing meat with poor quality attributes as a result of pre- and postmortem environmental factors.

Environmental Factors

Stress incurred during transportation, such as a dramatic temperature change, has been associated with inferior color and water holding capacity values (Froning et al., 1978). Non-halothane gene carrier swine subjected to intense pre-slaughter stress exhibit a strong relationship with the progression and eventual stability of color in pork over a twenty-four hour period (Rosenvold and Andersen, 2003). Independent of slaughter method, swine exposed to pre-slaughter stress had a 56% and 37% increase in drip loss compared to minimally stressed control swine at 24 and 48 hours respectively (Hambrecht et al., 2004).

In addition, low initial pH values in the muscle of broilers are a product of intense ante-mortem struggle (Bate-Smith and Bendall, 1949). Intense struggling quickly depletes the muscle of its glycogen reserve and accumulates lactic acid in the muscle, resulting in a lower initial pH (Berri et al., 2005; Froning et al., 1978). Currently, broiler industry electrically stuns birds prior to slaughter in an effort to reduce the amount of struggling and the subsequent diminishment of glycogen reserves that result in a rapid pH decline postmortem. Although electrical stunning is moderately effective at reducing the stress incurred by slaughter, evidence by Channon et al. (2002) suggests that CO₂ stunning results in a less rapid pH decline compared to electrical stunning suggesting that the rate of pH decline in US broilers, primarily stunned using electrical methods, has

the potential to fall less rapidly. The combination of stress and intense struggling acts to increase the bird's metabolic activity causing an increase in body temperature. High temperature conditions increase both the rate of pH decline and the amount of protein denaturation leading to undesirable meat (Bate-Smith and Bendall, 1949; Goldspink G, 1964; Penny, 1967).

Following slaughter birds are defeathered, chilled, and often deboned, all processes that have the potential to negatively impact the quality of meat from birds that would otherwise produce meat that fit consumer standards. Chilling is a process imperative to preserving the integrity and color of poultry meat while reducing the number of bacteria present in the meat. Studies investigating the effects of chill temperature and meat quality reveal that as temperature is reduced from 40°C to 0°C quality traits such the degree of paleness (L^*) and drip loss approach more desirable values (McKee and Sams, 1998; Molette et al., 2003; Sams and Alvarado, 2004). The depth of the breast muscle further complicates chilling poultry carcasses. The breast muscle cools more slowly than other muscles in the carcass (May et al., 1961), an important consideration when evaluating the effectiveness of various chilling methods. Producers' continued selection for larger breast muscle has the potential to make chilling methods less effective; as a consequence the thicker muscle is more likely to chill at a slower rate.

Meat Quality Characteristics

Color

Consumer preference has long dictated the need for poultry products to exhibit exceptional color. Traditionally, poultry meat that is light and yellow in color is associated with more desirable cooked products. Meat that deviates from the traditional consumer standard is a concern in the poultry industry. Muscle structure, a product of genetic selection and protein solubility, determines post-mortem color. Genetic selection of the broiler has focused on selection for high breast muscle yield, a trait correlated with paler meat (Le Bihan-Duval et al., 1999). It has been well established that muscle fiber number increases little over the course of a lifetime instead muscle growth occurs as a result of muscle fiber hypertrophy (Aberle and Doolittle, 1976; Remignon et al., 1995). Swatland (2002b), investigating the relationship of light to muscle fiber diameter, determined that light transmittance is a linear function. As fiber diameter increases a corresponding increase occurs in the transmittance of light through the fiber resulting in paler colored meat (Swatland, 2002a).

In addition, poor color associated with PSE is a result of sarcoplasmic protein precipitation from the muscle (McLoughlin and Goldspin, 1963). Seventy-one percent of the variation in the L^* values of breast muscle can be attributed to sarcoplasmic protein solubility (Joo et al., 1999). The L^* value has proven to be one of the best indicators of a muscle's potential to develop PSE. Wilkins and co-workers (2000) reported preliminary work in their lab that implies consumers

would not choose breast muscle fillets with L* values >57, a value associated in muscles with a high degree of paleness. When assessing L*, cut-off values are dependent on the conditions present for individual slaughter plants and may be as low as 50 in some plants (Barbut, 1997). Using an L* value of >54, it is estimated that up to 47% of chicken breast has the potential to be pale, soft, and exudative (Woelfel et al., 2002); although half of all chicken breasts exhibit L* values associated with PSE, many will not develop the negative characteristics of the condition. Although obvious signs of PSE may not be present in the current broiler industry, high L values indicate that there is room for improvement in today's flocks.

Water Holding Capacity and Texture

An increased ability to retain water has always been a desirable meat property but the advent of further processed meat has made this quality a necessity. Water holding capacity is the ability of muscle to retain the water present at the time of death, which averages approximately 75% of the muscle's weight (Offer et al., 1989). Water in the myofibril is found free between the filaments and bound to proteins. During the conversion of muscle to meat the myofibrils shrink in diameter causing a decrease in extra-cellular space between filaments resulting in the loss of free water from the meat (Heffron and Hegarty, 1974).

In addition to the effects of muscle shrinkage, the muscle's ability to retain water bound by proteins is a result of two factors: sarcoplasmic protein solubility

and myofibrillar protein solubility (Bendall and Wismer-Pedersen, 1962). Temperature and pH both play an important role in the solubility of proteins. Large decreases in sarcoplasmic and myofibrillar protein solubility have been reported in swine under conditions of high temperature and low pH at the start of rigor mortis, 55% and 75% respectively (Sayre and Briskey, 1963). Protein solubility decreases as the pH in the muscle approaches the isoelectric point, a pH of 5.5 (Zayas, 1997). At the isoelectric point the net charge of the protein is zero and the protein-protein attraction is greatest. The decrease in protein solubility indicates that the proteins have denatured and are unable to function in a normal capacity. Denatured proteins negatively affect meat texture resulting in abnormally soft meat that is unable to retain water.

PSE Counter Measures

Although both the poultry and swine industry have taken steps to decrease the occurrence of PSE, no single method has proved to be 100% effective. Pale, soft, and exudative is a condition comprised of causative components including nutrition, genetics, stress incurred during transport, pre- and post-slaughter environment, and biochemical properties of the muscle post-mortem. One component that can be easily and quickly manipulated is the nutritional status of the animal prior to slaughter. One potentially effective nutritional change is the addition of creatine supplements in the diet as reviewed by James et al. (2002).

Creatine

Form and Function

Creatine serves as an energy reserve in the muscle that alleviates the need for energy under anaerobic conditions, for example during short bursts of exercise or after death. Discovered in 1832, creatine is both a dietary and endogenous nitrogenous amine (Williams et al., 1999). The synthesis of creatine occurs in the liver, kidney, and pancreas (Borsook and Dubnoff, 1940; Gerber et al., 1962) and begins with the interaction of arginine and glycine in a transamidation reaction (Bloch and Schoenheimer, 1940) resulting in guanidinoacetate and ornithine (Bloch and Schoenheimer, 1941; Koszalka and Bauman, 1966). Methyltransferase catalyzes the transfer of a methyl group from S-adenosyl methionine to guanidinoacetate forming creatine (Du Vigneaud et al., 1941) (Figure 3). After formation, creatine is carried through the blood and enters the skeletal muscle against a concentration gradient via a Na⁺ and Cl⁻-dependent transporter (Daly and Seifter, 1980; Fitch and Shields, 1966; Garcia-Delgado et al., 2001; Guimbal and Kilimann, 1993; Ku and Passow, 1980; Loike et al., 1988). In the muscle, ATP phosphorylates creatine in a reaction catalyzed by creatine kinase (Cantoni and Vignos, 1954) in the presence of Mg²⁺ (Cohen, 1951). Phosphorylation of creatine continues until ATP and phosphocreatine match energy levels bringing the creatine kinase reaction into equilibrium. In skeletal muscle, phosphocreatine comprises 2/3 of the total creatine concentration, the remaining 1/3 is comprised of creatine (Figure 2).

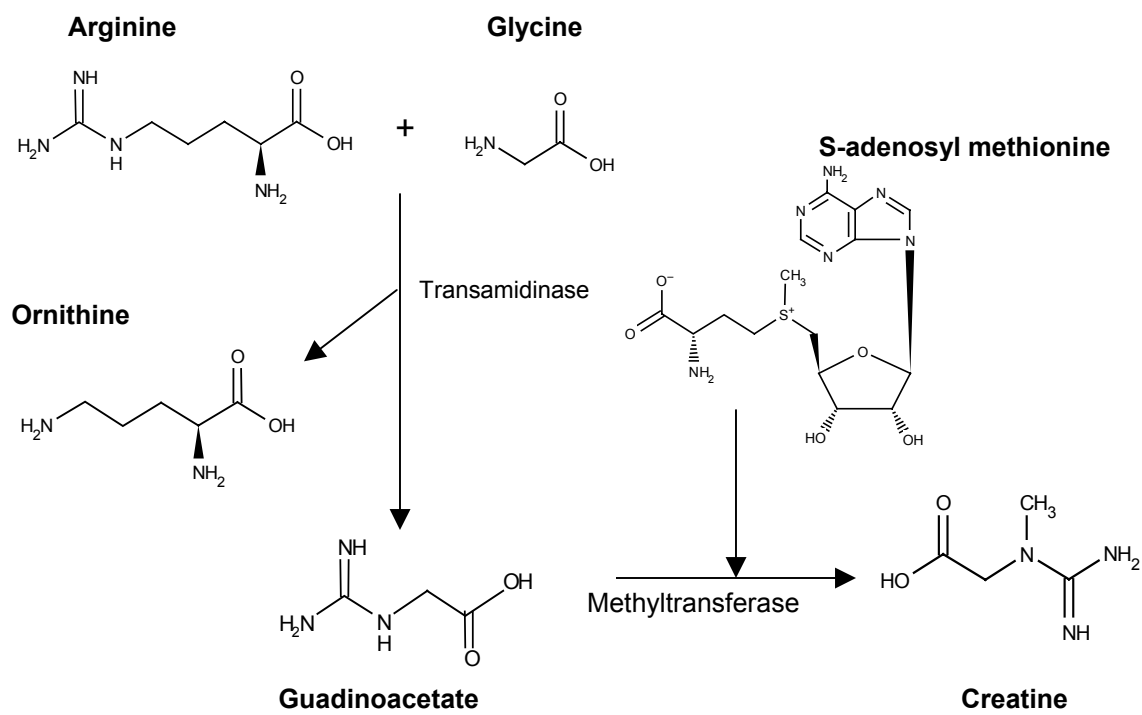


Figure 3: Pathway of Creatine Synthesis

Creatine Supplementation

Creatine has gained worldwide attention as an ergogenic substance used by competitive athletes to enhance their muscle building ability. Ergogenic substances are defined by Merriam-Webster as having the ability to enhance physical performance. Research investigating creatine's use as a nutritional supplement began as early as 1949, when Almquist et al. supplemented creatine to broiler chick diets and determined that creatine supplementation increased creatine concentration in the muscle and increases chick growth (Almquist et al., 1941). Increasing muscle creatine concentration is particularly important for athletes because creatine's phosphorylated form is responsible for supplying energy to the muscle when oxygen supply is low. Many athletes practice creatine loading, a supplementing method designed to increase the concentration of muscle creatine by nutritionally supplementing high doses of creatine monohydrate short term followed by a maintenance dose of a smaller amount. Hultman et al. (1996) discovered that creatine loading in men increased muscular concentrations of creatine by 20%, a concentration that was maintained over the course of 30 days through a maintenance dose.

Further studies investigating the potential for creatine supplementation to influence the concentration of creatine in the plasma and intramuscularly yielded mixed results. After humans were supplemented with a 30 gram dose of creatine once a day for five days, Snow et al. (1998) observed an increase in total creatine concentration intramuscularly, but failed to induce a change in the

muscle's anaerobic metabolism. Further studies cast more doubt on the ability of creatine supplementation to positively affect the muscle's ability to withstand fatigue (McKenna et al., 1999). Additional studies performed in humans indicate that individuals vary in their response to creatine supplementation based on pre-existing levels of plasma and intramuscular creatine, leading researchers to label the two groups as responders and non-responders (Brault et al., 2003).

Creatine Supplementation and Livestock Nutrition

The supplementation of creatine proposed as a method to improve meat quality was highlighted in a review by James et al. (2002). While creatine supplementation has little effect on improving carcass size and weight, evidence exists that supplementing creatine has a beneficial effect on post-mortem biochemical processes (Berg and Allee, 2001; Berg et al., 2003; Stahl et al., 2001; Stahl et al., 2007). Swine fed diets supplemented for five days with creatine exhibited meat with superior water holding capacity compared to controls (Berg et al., 2003). Further evidence exists to corroborate data found in human studies indicating that creatine supplementation effects vary between individuals who are responders and non-responders.

In swine, a genetic component related to breed is responsible for the different creatine supplementation effects in responders and non-responders (Young et al., 2007). A comparison of meat quality between Duroc and Landrace breeds indicate that Durocs are more likely to respond to creatine supplementation resulting in higher post-mortem pH values and higher water

holding capacity (Young et al., 2005). In an additional study, pork from creatine-supplemented Duroc swine had a higher creatine phosphate concentration in the muscle ante-mortem leading to a slower pH decline post-mortem and a lower L* value compared to the creatine supplemented Landrace swine (Lindahl et al., 2006b).

Alternatively, research exists that creatine supplementation has no effect on meat quality attributes (Nissen and Young, 2006; Stahl et al., 2007) and in some cases may result in decreased quality (Nissen and Young, 2006). Stahl and co-workers proposed that decreased quality of muscle attributes is a result of creatine supplementation exceeding a maximum number of five days (Stahl et al., 2001).

Conclusion

Consumer demand for poultry is expected to continue to rise over the next ten years, increasing the pressure on producers to rapidly grow heavily muscled birds, thus one can expect to see the incidence of PSE continue to occur. Pale, soft, and exudative meat results in large monetary losses for the poultry industry. Developing an effective method of combating the condition is essential to the poultry industry's future success.

Currently, creatine and its ability to increase energy availability to the muscle exist as a popular human sports enhancement supplement. The potential remains to utilize creatine supplementation in poultry diets as a means of increasing the energy available to the muscle under anaerobic conditions;

thus, delaying the post-mortem pH decline and reducing the occurrence of PSE. Further research is needed before creatine's capability, as a supplement, in animal production can be definitively determined.

Materials and Methods

Animals and Treatment

Three hundred, one-day-old RossxArbor Acre crossbred mixed-sex broiler chicks obtained from Tyson's Monteagle, TN hatchery were randomly allotted to fifteen pens resulting in a total of twenty birds per pen. Chicks were checked for physical deformities and a collective weight was measured for each pen. Each of the fifteen pens was randomly assigned one of three treatment diets so that each treatment was assigned to five pens and fed throughout the duration of the experiment. The diet was fed to each pen in three phases termed starter, grower and finisher on days one through fourteen, fifteen through twenty-four, and twenty-five through thirty-six respectively. Creatine was supplemented at .05% with all other parameters the same (Table 1, 2, and 3). Diets were formulated to meet recommendations set by the National Research Council.

Birds were grown for thirty-six days and feed and water were fed free choice. Each pen was provided a feed bin labeled with the corresponding pen number color-coded by assigned treatment. Feed was weighed when added or removed from feeders in each pen. A record sheet was attached to each pen and color-coded based on treatment assigned. Each record sheet allowed for the weekly recording of feed weight and number of birds per pen; in addition the record sheet included a date and explanation note for birds that were permanently removed from the pen during the week. Once per week for six weeks, collective pen weights were measured and feed consumption recorded.

Table 1: Composition of Starter Diet

Ingredient	Diet		
	1	2	3
	%		
Corn	53.62	53.62	53.62
Soybean Meal (48.5%)	34.09	34.09	34.09
Corn Gluten Meal (60.0%)	1.05	1.05	1.05
Fat	7.01	7.01	7.01
Limestone	0.90	0.90	0.90
Dicalcium phosphate	2.44	2.44	2.44
Iodized Salt	0.29	0.29	0.29
DL-methionine	0.30	0.30	0.30
Vit-TM premix	0.25	0.25	0.25
Sand	0.05	-	-
Creatine H ₂ O	-	0.05	-
Creatine HCl	-	-	0.05
Total	100.00	100.00	100.00
Calculated Nutrient Composition			
ME (kcal/kg)	3630.42	3630.42	3630.42
CP %	25.03	25.03	25.03
Methionine %	0.70	0.70	0.70
Methionine and Cystine %	1.09	1.09	1.09

Table 2: Composition of Grower Diet

	Diet		
	1	2	3
Ingredient	%		
Corn	59.02	59.02	59.02
Soybean Meal (48.5%)	29.73	29.73	29.73
Corn Gluten Meal (60.0%)	0.39	0.39	0.39
Fat	7.00	7.00	7.00
Limestone	0.80	0.80	0.80
Dicalcium phosphate	2.16	2.16	2.16
Iodized Salt	0.30	0.30	0.30
DL-methionine	0.30	0.30	0.30
Vit-TM premix	0.25	0.25	0.25
Sand	0.05	-	-
Creatine H ₂ O	-	0.05	-
Creatine HCl	-	-	0.05
Total	100.00	100.00	100.00
Calculated Nutrient Composition			
ME (kcal/kg)	3692.41	3692.41	3692.41
CP %	22.72	22.72	22.72
Methionine %	0.67	0.67	0.67
Methionine and Cystine %	1.02	1.02	1.02

Table 3: Composition of Finisher Diet

Ingredient	Diet		
	1	2	3
	%		
Corn	63.33	63.33	63.33
Soybean Meal (48.5%)	25.81	25.81	25.81
Corn Gluten Meal (60.0%)	0.51	0.51	0.51
Fat	7.00	7.00	7.00
Limestone	0.69	0.69	0.69
Dicalcium phosphate	1.89	1.89	1.89
Iodized Salt	0.22	0.22	0.22
DL-methionine	0.25	0.25	0.25
Vit-TM premix	0.25	0.25	0.25
Sand	0.05	-	-
Creatine H ₂ O	-	0.05	-
Creatine HCl	-	-	0.05
Total	100.00	100.00	100.00
Calculated Nutrient Composition			
ME (kcal/kg)	3755.15	3755.15	3755.15
CP %	21.04	21.04	21.04
Methionine %	0.60	0.60	0.60
Methionine and Cystine %	0.93	0.93	0.93

Slaughter

On day thirty-five, fifteen birds were randomly selected from each pen. Of the fifteen birds selected, special attention was given to maintaining close to an equal ratio of males to females. Each bird was leg banded and the corresponding numbers were recorded for each pen. Ten birds from each pen were allotted to part 1 of the experiment leaving the five remaining birds allotted to part 2. Feed was withdrawn 12 hours prior to slaughter.

On day thirty-six birds leg banded the previous day were brought to the kill floor one hour prior to slaughter. Birds were individually suspended by leg shackles and stunned and exsanguinated by an electrical knife. After exsanguination birds were scalded, defeathered, and eviscerated. Each carcass was weighed and weights were used to determine dressed carcass as a percent of live weight. The whole carcass from five birds in each pen were chilled and subsequently frozen. The breasts of the remaining ten carcasses were removed and placed on a foam tray and immediately refrigerated at 4°C. Each tray was previously labeled with the number corresponding to each bird's leg band.

Experiment Part 1

pH and temperature

A ½ inch cut was made in the cranial portion of the right pectoralis major muscle. A handheld dual pH/temperature silicon probe pH meter (IQ Scientific Instruments model IQ150) was inserted into the muscle. Special care was made to ensure contact between the muscle and probe was maintained in order to

receive an accurate reading. Four measurements were taken and recorded at 30 minutes, four hours, seven hours and twenty-four hours postmortem.

Color Measurement

Color was evaluated using a color measurement device and was assessed on a numeric scale for light to dark (L/L^*), red to green (a/a^*), and yellow to blue (b/b^*). The three measurements can be assessed using a HunterLab color scale or the International Commission on Illumination (CIE) color scale, CIE values are denoted with an asterisk. The Hunter scale was developed in 1966 and was improved upon in 1976 and called the CIELAB scale. Both scales are still in use and considered to be valid measuring systems. When needing to assess color value in terms of consumer preference, the CIELAB scale is better able to place a value on color as seen by the consumer (HunterLab, 2001).

In order to assess the muscle in terms of outward appearance, color measurements were recorded within 30 minutes of slaughter by a portable spectrophotometer, HunterLabs Miniscan XE Plus (Model number 45/0-L with parameters set at light source D65, and standard observer of 10). The Miniscan was placed flush with the cranial aspect of the left breast muscle, the thickest portion, in order to reduce discoloration from background color (Bianchi and Fletcher, 2002). Values for lightness, redness, and yellowness were recorded using the International Commission on Illumination scale (L^* , a^* , and b^*).

Drip Loss

A 1 to 2 g sample of the cranial portion of the left pectoralis major breast muscle was removed and weighed. Each sample was tied on a fishing line and suspended inside a scintillation vial. Samples were stored at 4°C for twenty-four hours. Each sample was cut from the fishing line and removed from the scintillation vial. A post-24 hour weight was recorded for each sample. Drip loss was calculated using the formula below.

$$\text{Percent Loss} = \left\{ \frac{(\text{Initial Sample Weight} - 24 \text{ hour Sample Weight})}{\text{Initial Weight}} \right\} * 100$$

Expressible Moisture

A 2-3 g sample was removed from the cranial end of the left pectoralis major muscle. Each sample was placed into a Whirl-Pak and immediately placed into a freezer. After freezing each sample was thawed for twenty-four hours and ground in a small Kitchen-aid food processor. Expressible moisture was determined using a procedure described by Wardlaw, 1973. Weighed samples were incubated in a centrifuge tube with .6 M NaCl Solution for four hours.

After incubation, each sample was centrifuged at 12,000g for ten minutes. Following centrifugation, samples were removed from the solution and re-weighed. Expressible moisture was calculated using the formula below.

$$\text{Expressible Moisture} = \frac{\text{Volume of Solution Added} - \text{Volume of Solution Retained}}{100 \text{ g}}$$

Experiment Part 2

Carcasses were thawed for 48 hours at 4°C. After thawing each carcass was ground, placed in a bag labeled according to leg band number and frozen. Ground carcasses were thawed for 48 hours and mixed by hand. An approximately 90 g sample was taken from each ground carcass, weighed and spread ½ inch thick on a weighing tray. Samples were placed in an oven at 55°C until dry. After drying, samples were removed from the oven and weighed. Dried samples were ground into small particles in a food processor. After processing, samples were stored to be later used in dry matter, total protein, ash, and fat determination.

Dry Matter

Approximately 2g of dried and ground sample were weighed and placed in pre-weighed crucibles. Samples were further dried in a 100° C oven overnight. The following day crucibles and samples were cooled in desiccators and re-weighed to determine 100° dry matter.

Crude Protein

Percent nitrogen was determined using combustion method 39.1.16 validated by the AOAC (1997). The analysis was performed by placing approximately .3g of the dried sample in a Leco FP200 Nitrogen Analyzer for five minutes (the furnace temperature was set at 1050°C and ethylenediaminetetraacetic acid was used to calibrate). Samples were run in duplicates. Crude protein was determined as the product of multiplying 6.25 by percent nitrogen.

Fat

Using an AOAC (1997) validated indirect method of lipid extraction, approximately .3g of dried sample was submerged in petroleum ether for 14-16 hours. After submersion, the ether was allowed to evaporate off the samples before placing samples in an oven set at 100°C for one hour. Samples were performed in duplicates.

$$\text{Fat Percent} = \left(\frac{\text{Weight of extraction pouch after drying} - \text{weight of pouch prior to extraction}}{\text{Sample Weight}} \right) * 100$$

Ash

Approximately 2 g of dried sample were further dried at 600° C. Ash was determined according to standard procedure validated by the AOAC (1997).

Statistical Analysis

Results were analyzed using an ANOVA proc mixed model in SAS 9.1. Least squares means were used to identify differences between treatments. A log transformation was used to normalize data for drip loss and expressible moisture. The quadratic effect of time on post-mortem muscle pH change was analyzed using an orthogonal contrast.

Results

Growth Parameters

Gain to Feed

The least square means describing the amount of weight gained as a result of feed consumed each week are displayed in table 4. Adding creatine to the diet did not produce any statistically important differences in the gain to feed ratio of birds.

Dressed Carcass Percent Evaluation

Dressed carcass percents did not change as a result of adding creatine in the diet. Table 5 displays the least square means of the dressed carcass percents observed for two hundred twenty-two birds.

Body Composition Analysis

Percent nitrogen and percent ash in the carcasses were not altered by the addition of creatine in the diet. The addition of creatine H₂O in the diet of birds increased percent fat (16.31%) compared to the percent fat (15.29%) of birds fed the control diet (P<.05). Least square means are displayed in Table 6.

Table 4: Conversion of Feed into Weight Gained

		Diet^{1,2}		
		Control	Creatine H ₂ O	Creatine HCl
		Gain: Feed³		
Week	1	0.5962	0.5362	0.5706
	2	0.7737	0.8120	0.7729
	3	0.4633	0.4398	0.4315
	4	0.5382	0.5261	0.5545
	5	0.5574	0.5442	0.5507

¹ Creatine was supplemented as .05% of the diet

² Data are least square means and show no significant differences. Means square errors are listed in numerical order by week and are as follows: .02053, .02295, .01713, .01650, and .008678.

³ Weights were recorded as a whole pen weight consisting of 20 birds each.

Table 5: Dressed Carcass Percent Evaluation

		Diet^{1,2}		
		Control	Creatine H ₂ O	Creatine HCl
Dressed Carcass³		67.91	68.01	67.90

¹ Creatine was supplemented as .05% of the diet.

² Data are least square means with no significant differences. The least squares errors are .4121, .4065, and .4038 for the Control, Creatine H₂O, and Creatine HCl diets respectively.

³ Dressed carcass is presented as percent of carcass weight to live bird weight.

Table 6: Body Composition Analysis

		Diet^{1,2}		
		Control	Creatine H ₂ O	Creatine HCl
Component³	Nitrogen	2.86	2.91	2.94
	Ash	2.30	2.37	2.44
	Fat	15.29B	16.31A	15.69AB

¹ Creatine was supplemented as .05% of the diet.

² Data are least squares means. Means without a common subscript are significantly different (P<.05). Means square errors are .000580, .000586, and .003327 for nitrogen, ash, and fat respectively.

³ Data are expressed as percent on a live basis.

Breast Muscle Quality Characteristics

pH Decline

As shown in table 7 and figure 4, pH declined in the breast muscle of birds in all treatment groups as a result of the quadratic effect of time ($P < .0001$). The rate of pH decline was slower in the muscle from birds fed the control diet compared to muscle from birds fed diets containing creatine ($P < .05$). Overall, the difference in rate of pH decline can be attributed to the interaction of the quadratic effect of time and the addition of creatine into the diet ($P < .0001$).

Breast Muscle Color

Least squares means of breast muscle color are presented in Table 8. The addition of creatine to the diet resulted in paler less red breast muscle but had no effect on the observed yellowness of the breast muscle. The palest ($L^* = 51.04$) breast muscles were observed in birds fed the diet containing creatine HCl whereas the darkest ($L^* = 49.81$) breast muscles were observed in control birds ($P < .05$). The degree of redness observed was highest ($a^* = 6.40$) in the breast muscle of birds fed the control diet compared to the lowest measurement ($a^* = 5.98$) observed in the breast muscle of birds fed the diet containing creatine H₂O ($P < .05$).

Table 7: Diet Effect on pH

		Diet^{1,2}		
		Control	Creatine H ₂ O	Creatine HCl
		pH		
Time (Hours)	<.5	6.28A	6.15BC	6.21AB
	4	6.11CD	6.04DE	5.97E
	7	5.64F	5.58F	5.63F
	24	5.38G	5.33GH	5.28H

¹ Creatine was supplemented as .05% of the diet.

² Data are least squares means. Means without a common subscript are significantly different (P<.0001). The mean square error is .03070.

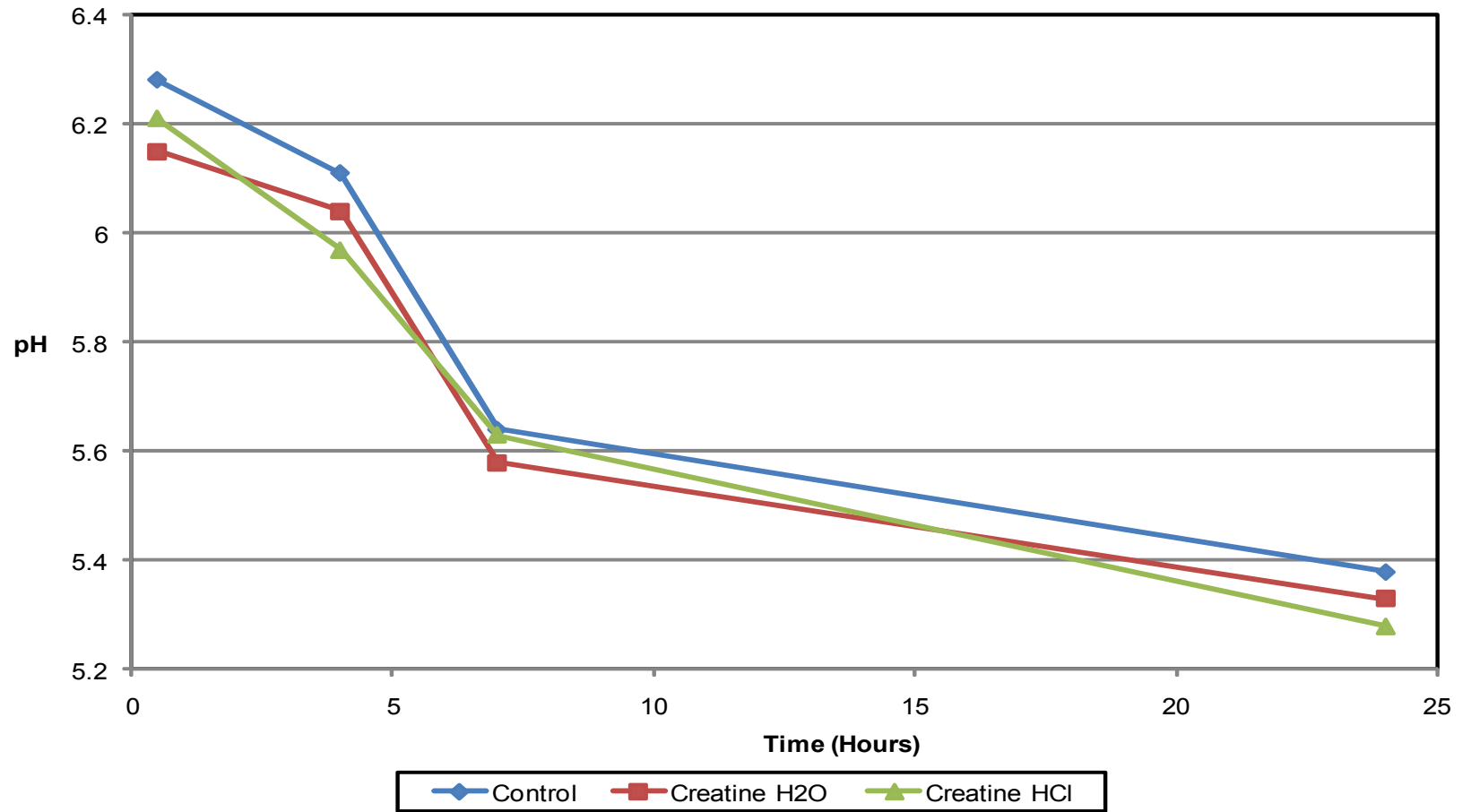


Figure 4: Rate of Post-mortem pH Change in the Muscle

Table 8: Evaluation of Breast Muscle Color

		Diet^{1,2}		
		Control	Creatine H ₂ O	Creatine HCl
Color Values³	L*	49.81 B	50.36AB	51.04 A
	a*	6.40 A	5.68 B	5.98AB
	b*	16.66	16.26	16.85

¹ Creatine was supplemented as .05% diet.

² Data are least squares means. Means without a common subscript are significantly different (P<.05); The mean square errors are .3921, .1691, and .2595 for L*, a*, and b* respectively.

³ Values are measured on the CIE lab scale; L*=degree of paleness, a*=degree of redness, and b*=degree of yellowness

Water Holding Capacity

The negative affects of creatine-containing diets on water holding capacity are displayed in Table 9. Drip loss from breast muscle over a twenty-four hour period increased as an effect of the addition of creatine in the diet ($P<.05$). Consequently, the addition of creatine in the diet resulted in breast muscle with lower expressible moisture values indicating an inability to retain moisture ($P<.05$).

Table 9: Water Holding Capacity

		Diet^{1,2}		
		Control	Creatine H₂O	Creatine HCl
Method	Drip Loss³	4.99 B	6.89 A	5.62 AB
	Expressible Moisture⁴	7.95 A	5.70 B	6.31 B

¹ Creatine was supplemented as .05% of the diet

² Data are least squares means following a log transformation. Means without a common subscript are significantly different (P<.05); The mean square errors are ordered by diet (Control, CreatineH₂O, and CreatineHCl) with .18383, .20883, and .18800 and .15725, .13335, and .13976 for drip loss and expressible moisture respectively.

³ Drip loss is expressed as percent loss over a twenty-four hour period.

⁴ Expressible moisture is expressed in ml/100g of solution retained.

Discussion

Breast Muscle Quality Characteristics

The results of pH decline verify that the post-mortem decline in pH occurs as a result of time. Unexpectedly, the results indicate that the supplementation of creatine actually increased the rate at which pH declines in the muscle following death. This is in contrast to a series of experiments performed on swine that concluded that creatine may delay early pH decline post-mortem (Berg and Allee, 2001; Berg et al., 2003; Stahl et al., 2001) and to previous work completed by Lindahl (2006b) that determined supplementing creatine in the diet will lead to positive results in relation to muscle quality characteristics. One explanation for this discrepancy is the length of time that birds in this experiment were supplemented. Birds in this experiment were fed creatine-supplemented diets for thirty-five days. It should be noted that Stahl (2001) indicated that dietary creatine supplementation of greater than five days may lead to negative effects on quality characteristics.

Previous evidence from Nissan and Young (2006) indicated that creatine supplementation might result in negative muscle quality characteristics but in this case, creatine was tested in conjunction with glucose supplementation. By supplementing both creatine and glucose, the researchers were not able to explain the extent to which each supplement contributed individually to the rapid pH decline and negative quality characteristic results. The results from the

current study indicate that dietary creatine supplementation alone will lead to a more rapid rate of pH decline.

Color value is an important quality characteristic of poultry meat that is affected by pH. Qiao (2001) determined that low pH was negatively correlated (-.9610) with L* value. In addition, low pH has been associated with higher L* values (Wilkins et al., 2000). Our results support the previous observations that pH affects breast muscle color. Birds that exhibited the fastest rate of pH decline had higher L* values and lower a* values, meaning the breast muscles were lighter and less red. This evidence is corroborated by previous work from Lindahl (2006a), who determined that the decline of pH in the muscle has considerable influence on the muscle's color unrelated to genetic predisposition. As a result of the increased rate of pH decline, the birds fed a creatine-supplemented diet had higher L* values indicating that adding creatine to the diet may induce color values in breast muscle associated with PSE.

The distinction between pale and normal breast muscle is not a standard value. The range of L* values considered pale extends from approximately 50 to 60. A standard value to determine pale breast muscle from normal breast muscle does not exist because the conditions that impact meat development in individual slaughter plants are not uniform (Barbut, 1997). Therefore, when analyzing the color value results, greater emphasis was placed on determining a significant difference between the control and creatine-supplemented diets rather

than focusing on the numerical value of L^* and comparing our L^* value results to the results obtained in previous studies.

The second quality characteristic associated with PSE is water-holding capacity (Van Laack et al., 2000). Low pH has been implicated as a major factor in the development of poor water holding capacity (Offer et al., 1989). Water-holding capacity can be determined in a variety of ways including drip loss and expressible moisture. Adding creatine to the diet resulted in a faster rate of pH decline, and as a consequence, higher drip loss values were observed in the breast muscle of birds fed a creatine-supplemented diet. Creatine uptake facilitates the increase of water uptake into the muscle (Berg and Allee, 2001; Young et al., 2005). An increased amount of water in the muscle of birds fed a creatine-supplemented diet is likely to explain the increased drip loss observed in those birds assuming that the diameter shrinkage of the myofibrils remained relatively the same and independent of diet.

Birds fed a creatine-supplemented diet are likely to have had breast muscles that exhibited a greater ability to take-up water. Therefore, high drip loss values are a result of more water being present in the myofibrils at the time of slaughter resulting in increased drip loss values as the fibers shrink. The high L^* values observed in the muscle of birds fed the creatine-supplemented diet is a direct result of the increased size of myofibrils. This conclusion is supported by Swatland (2002b) who reported evidence that an increased amount of water in the muscle cause the myofibrils to swell, resulting in an increase of the

transmittance of light through the muscle thus high drip loss values in conjunction with high L* values.

The expressible moisture results are further proof that water-holding capacity was negatively affected by the addition of creatine in the diet. After soaking in sodium chloride solution, the expressible moisture method was designed to measure the amount of sodium chloride solution absorbed and retained by the meat after placing the meat under a centrifugal force (Wardlaw, 1973). Meat from birds with the slowest rate of pH decline was found to have the greatest ability to absorb and retain the solution under centrifugal force. Both drip loss and expressible moisture results in this study indicate that birds fed a creatine-supplemented diet will produce meat with a reduced capacity to hold water, a detrimental effect to the meats usability in further processing.

Growth Parameters

As a consequence of being an ergogenic substance, creatine supplementation is most effective in conjunction with an increase in physical activity. The ergogenic value of creatine has been evaluated and verified repeatedly in human studies (Hultman et al., 1996; McKenna et al., 1999; Snow et al., 1998). Conversely, livestock are not subjected to increased levels of exercise and as a consequence do not experience positive effects on growth parameters as a result of creatine supplementation. As a result, in this study creatine was not expected to change the birds' ability to exceed conventional growth parameters.

The results of this study are consistent with the current literature that creatine supplementation in the diet of livestock is of no value in terms of growth parameters. The gain to feed ratio of birds fed a creatine-supplemented diet did not differ from birds fed a control diet. The means obtained from the calculation and analysis of dressed carcass percent provide more evidence that growth parameters were not altered by the addition of creatine in the diet. Analysis of the body composition included nitrogen, ash, and fat determination. Of the components analyzed, the fat content was higher in both creatine-supplemented diets but was only markedly higher in the creatine monohydrate supplemented diet.

As documented by Pond et al. (1995), water and fat are the two most variable components in the body of broiler and share an inverse relationship. The results of this study are contrary to the inverse relationship that exists between water and fat. Muscle from birds fed the creatine monohydrate supplemented diet had the highest amount of fat at 16.31% and should have the lowest amount of drip loss as a consequence of muscle fibrillar shrinkage. Given that the highest drip loss percent (6.89%) was observed in the birds that exhibited the highest amount of fat, then the drip loss observed can not only be attributed to muscle fibrillar shrinkage. If fibrillar shrinkage was the only factor that contributed to drip loss, birds with the highest muscular drip loss would expect to be birds with the highest amount of water in the body and the lowest amount of fat. The most likely reason that this study appears to contradict the

inverse relationship that has been established between water and fat as a percent of total body composition is twofold. One, while the bodies of birds fed a control diet may have been comprised of more water overall, the amount of water found in the muscle of birds fed a creatine-supplemented diet was likely to be higher (Berg and Allee, 2001; Young et al., 2005) as increased fat deposition occurs in the sub-dermal and visceral tissue not the muscle. Second, birds fed a creatine-supplemented diet experienced a more rapid rate of pH decline in the muscle, a factor that is known to increase protein solubility (Bendall and Wismer-Pedersen, 1962) thus increasing the amount of drip loss and decreasing the muscles ability to retain water.

Conclusion

As a result of higher consumer demand for cut-up and further processed parts, the breast muscle quality characteristics of today's broilers are particularly important. PSE decreases the usability of meat in a market that is already in high demand. This research was performed to determine the possibility of using dietary creatine as a method of reducing the incidence of PSE affected meat.

Contrary to the original hypothesis of this experiment, birds fed creatine-supplemented diets produced inferior meat compared to birds fed a control diet. Creatine affected the biochemical factors of post-mortem muscle physiology. More specifically, the addition of creatine in the diet appeared to increase the rate at which pH declined in the muscle following death. Increasing the rate of pH decline in the muscle after death led to undesirable breast muscle quality characteristics. The breast muscle of birds fed a creatine-supplemented diet exhibited lighter redder color and presumably poorer texture, given the substandard water holding capacity.

At present the research investigating creatine's benefit or lack thereof is insufficient. The results of this experiment indicate that creatine supplemented at a concentration of .05% for a six-week duration proved to be detrimental to the quality of the meat produced. Using creatine as a method to decrease the incidence of PSE in broiler breast meat may still be feasible. Future experiments

are necessary to determine if the concentration in the diet or length of time supplemented can be manipulated to provide positive affects on the breast meat quality characteristics.

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Vita

Amy Chandler was born in Knoxville, TN on April 13, 1983. In August 2005, she received a Bachelor of Science degree in Animal Science with a concentration in Production and Business from the University of Tennessee, Knoxville. She re-entered the University of Tennessee in August 2005 and completed her Master of Science degree in Animal Science in August 2008.