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The Effects of Dietary Amino Acid Density in Broiler Feed on Carcass Characteristics and Meat Quality

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THE EFFECTS OF DIETARY AMINO ACID DENSITY IN BROILER FEED ON CARCASS CHARACTERISTICS AND MEAT QUALITY

By

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Research was conducted to evaluate the effect of dietary amino acid (AA) density (Deficient (D), Low (L), High (H), and Excessive (E)) on broiler breast and thigh meat quality. As expected, the feed conversion improved ($P<0.05$) as AA density increased. No differences ($P>0.05$) existed among treatments with regard to final pH, cooking loss, shear force, brine absorption proximate analysis, and average consumer acceptability of breast meat. The D AA diet yielded meat with less ($P<0.05$) moisture, less protein ($P<0.05$) and more fat ($P<0.05$) than all other treatments. Increasing AA density in the diet led to increased ($P<0.05$) concentrations of linoleic and linolenic acid in the thigh meat from the H and E treatments, thus making it more susceptible to oxidation ($P<0.05$) in comparison to the D and L treatments. Overall, data revealed that all four AA diets yielded high-quality breast and thigh meat with minimal product differences.

Key words: broiler, meat quality, consumer acceptability, amino acid density

DEDICATION

I would like to dedicate this thesis to my wife, Amanda Elizabeth Lilly, who stayed by my side late into the night as I read, studied, and worked my way through graduate school and all those chemistry tests.

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INTRODUCTION

Per capita consumption of broiler and poultry meat products has increased from 40.2 lb in 1970 to 86.5 lb in 2007 (USDA, 2009). This dramatic increase in per capita consumption has led to broiler products surpassing pork and beef in 1986 and 1993 respectively, to become the most consumed meat product in the United States (USDA, 2009). Increased consumption is partially due to an increased efficiency in broiler production that is due to genetics, selective breeding, disease control prevention, enhanced mass production techniques, management, processing, and enhanced broiler nutrition (Ensminger *et al.*, 2004). The increase efficiency in broiler production has dramatically lowered the price of broiler products, which increases consumption and availability of broiler products to all consumers, regardless of income. The deflated retail price of broilers has fallen by approximately 61% since 1955 (Martinez, 2000).

Feed efficiency has also increased significantly during the same time period. In 1944, broiler chickens required approximately 4.1 kg (9 lb) of feed for each 1 kg (2.2 lb) of weight gain. By 1994, broilers required only 1.8 kg (4 lb) of feed to produce a 1 kg (2.2 lb) weight gain (NRC, 1994). The broiler industry has become more efficient at producing meat, by using less feed to grow birds in a shorter amount of time. It is a remarkable feat of agricultural technology that the broiler industry can produce a 2 kg

bird using 3.6 kg of feed in six weeks (McGee, 2004). The reduction in the market age of the broiler is also significant because as the broiler ages, more feed is directed toward maintenance rather than adding weight to the broiler.

Broiler feed is important since it constitutes approximately 65% of the cost of commercial broiler production (Kerr and Agr, 1969; Qureshi, 1987; Kidd *et al.,* 2004; Corzo *et al.,* 2005a). Considering the high cost of broiler feed, it is important for a broiler production facility to optimize nutrition in order to maximize growth and meat yield of the broiler (Ensminger *et al*., 2004). In 1994, the National Research Council released Nutrient Requirements for Poultry and indicates that High amino acid densities are needed to support the rapid growth of a broiler (NRC, 1994). Therefore, adequate amino acid nutrition is important for a successful feeding program. However, a balance of amino acids must me maintained to make the diet economically and nutritionally feasible (NRC, 1994). Meeting amino acid requirements represents a large portion of the cost of a broilers diet. Over formulating is costly whereas under formulating may negate economic returns due to suboptimal growth and meat yields (Kidd *et al*., 2004, Corzo *et al.,* 2005a). Additionally, excessive amounts of amino acids have also been shown to decrease feed consumption, which directly influences the broilers supply of amino acids (Skomial *et al.*, 2002).

Furthermore, crude protein level directly affects broiler growth performance and carcass composition (Gu *et al.*, 2008). Researchers have reported that feeding high-density amino acid diets throughout the life of the broiler optimized breast meat yield and final broiler weight, while decreasing amino acid density caused reduced body weight,

breast meat yield, feed conversion and meat yields (Bartov and Plavnik, 1998, Kidd *et al*, 2004, Corzo *et al*., 2005a, Dozier III *et al*., 2008a). This research supports the theory that body weight and feed conversion depend above all, on the energy level in the feed mixture (Skomial *et al.,* 2002). It has been reported that reducing the crude protein content in broiler diets may reduce feed cost. However, it is not known whether a reduction in crude protein content will affect meat quality (Kidd and Kerr, 2006). Minimal research has been reported on the effects of amino acid density and crude protein level on broiler meat quality. Therefore, this research was conducted to evaluate the effects of amino acid density on broiler meat quality, which was evaluated through color, pH, water-holding capacity, tenderness and sensory acceptability of breast meat and fatty acid composition and lipid oxidation of thigh meat (McKee and Sams, 1997; van Laack *et al*., 2000; Schilling *et al*., 2008).

CHAPTER II

LITERATURE REVIEW

Introduction

From 2009 to 2050 the world's population is expected to increase by 25.4% to 9,149,795,000 people, the majority of which will occur in third-world countries (Coleman and Korver, 2004; UN, 2004). Less developed regions such as Asia, Africa and Latin America will grow 58 percent over the next 50 years accounting for 99 percent of the world's population growth in that period (UN, 2004). As third-world countries such as India and China become increasingly industrialized over the next decade, with a growing middle class, the demand for inexpensive animal products as a protein food source is expected to increase (Coleman and Korver, 2004). The poultry and broiler industry are ideally suited to meet the needs of a growing human population by producing inexpensive, high quality, nutritious meat products quickly and efficiently. Despite the recent economic downturn in the years 2007-2010, the demand for poultry and broiler meat products continues to remain strong due to its lower cost relative to pork and beef (USDA, 2009; USDA, 2010). Because of its higher feed conversion ratio and faster production process, the poultry industry is better able to adapt when feed costs are high and demand is low when compared to red meat industries (USDA, 2010).

Poultry exports are expected to rise as global economic growth resumes and the U.S. dollar depreciates making U.S. goods more competitive in the international market (USDA, 2010). The United States continues to be among the World's leaders in exports of broiler meat, exporting 2.7 million metric tons in 2007 to major foreign markets such as the Russian Federation, Mexico, Canada and China (American Meat Institute, 2009). The Russian Federation continues to be the greatest importer of U.S. broiler meat, importing 852,600 metric tons or 31.5% of total U.S. imports in 2007 (American Meat Institute, 2009). However, competition for worldwide exports is expected to remain strong due to Brazil's increasing worldwide market share (USDA, 2009). Due to the increasing worldwide demand for broiler meat products, poultry production in the United States has doubled in less than 20 years from 18 billion pounds in 1990 to over 36 billion pounds in 2010 (USDA, 2009). Lower prices and consumers preference for meat products that are convenient, nutritious and of high quality has caused an increase in production and consumption of broiler products (Martinez, 2000). This increase in U.S. broiler consumption and production has allowed it to surpass beef in the early 1990's as the most consumed and produced meat product in the United States and is expected to continue to increase at a faster rate than beef or pork (USDA, 2010).

Influence of Amino Acids on Broiler Development

In order to meet the growing demand for poultry products, feed conversion and weight gain of broilers must be optimized. The National Research Council (1994) suggests that feeding broilers essential amino acids is required in order to optimize feed

efficiency, utilization of the feed and optimize weight gain. A rapidly growing broiler needs a sufficient supply of nutrients in order to meet the requirements necessary for maintenance and growth of all components of the broiler (Coleman and Korver, 2004). In addition to affecting the growth rate of broilers, a sufficient supply of basic nutrients has also been shown to have a significant influence on carcass quality (Skomial *et al.,* 2002). In 1994, the National Research Council released nutrient requirements for poultry and suggested that high amino acid densities are needed to meet the rapid growth requirements of a broiler (NRC, 1994). The nutrient and amino acid requirement for a broiler is the concentration of amino acids in the diet that will generate the greatest growth response in the broiler (Gous, 1998). Previous research has supported the NRC (1994) recommendations, showing that high amino acid concentrations and protein levels in a broilers diet increases breast meat yield, carcass weight, and feed efficiency (Dozier III *et al.,* 2001; Kidd *et al.,* 2004; Corzo *et al.,* 2005a; Dozier *et al.,* 2008).

There are seven essential amino acids (Methionine, Lysine, Threonine, Arginine, Valine, Isoleucine, and Tryptophan) that are critical for muscle development in broiler chickens which are required in greater dietary concentrations than other amino acids in order to optimize feed efficiency (NRC, 1994; Corzo *et al*., 2005b; Pesti, 2009). However, high concentrations of both essential and non-essential amino acids are necessary to synthesize protein at an adequate rate (Pesti, 2009). Optimal balance between essential and non-essential amino acids is important for efficient utilization of dietary protein (NRC, 1994). An adequate balance among dietary amino acids is necessary in order to meet the nutritional requirements of the broiler (Applegate *et al.,*

2009; Peñaranda-Alie *et al.,* 2010). Imbalanced amino acid diets have been shown to cause a reduction in body weight, carcass yield and feed conversion efficiency (Peñaranda-Alie *et al.,* 2010). Imbalanced diets such as those that are low in lysine can limit breast meat yield (Kidd *et al*., 2004; Corzo, *et al.,* 2005a; Dozier III *et al.,* 2008a), which is important for U.S. poultry processors, as breast meat is one of their most valuable commodities (Corzo *et al*., 2005a).

Breast muscle protein is high in lysine and is therefore sensitive to dietary lysine amounts in the broiler diet (Coleman and Korver, 2004). Additionally, broiler feed that contains arginine at a level recommended by the National Research Council (1994) is necessary to support the immune system functions of broilers (Coleman and Korver, 2004). However, simply increasing amino acid density may not be the best solution in solving an amino acid imbalance in a broilers diet since research indicates that increasing dietary amino acid density may cause decreases in food intake which would further compound an imbalance in amino acid density (Acar *et al.,* 2001; Dozier III *et al.,* 2008b).

Since broiler feed constitutes approximately 65% of the cost of commercially raising a broiler, United States poultry processors have commonly reduced nutrient density as a way of reducing feed costs (Kerr and Agr, 1969; Kidd *et al.,* 2004; Corzo, 2005a; Peñaranda-Alie *et al.,* 2010). Broiler companies have reduced dietary protein in broiler diets as a means of increasing live production profitability. However, excessive decreases in dietary protein within broiler feeds has been shown to cause a reduction in

body weight, carcass weight, breast meat yield, and feed conversion efficiency (Bartov and Plavnik, 1998; Kidd *et al*, 2004; Corzo *et al.,* 2005a; Dozier III *et al.,* 2008b).

Conversely, a high to moderate excess in dietary crude protein and amino acids (i.e. Methionine, Lysine and Threonine), above the recommended levels established by the NRC (1994), optimizes breast meat yield and final broiler weight (Bartov and Plavnik, 1998; Kidd *et al.,* 2004; Corzo *et al.,* 2005a; Dozier III *et al.,* 2008b). Amino acid densities greater than the NRC recommendations (1994) are necessary to meet the nutritional needs of rapidly growing broilers that are grown in the current market (Saima *et al.,* 2010) For example, increasing lysine above NRC recommendations (1994) has been found to improve weight gain, feed efficiency and breast meat yield (Si *et al.,* 2004; Saima *et al.,* 2010). Therefore, broiler feed containing high concentrations of amino acids is needed in order to optimize feed efficiency (Kidd *et al.,* 2004; Corzo *et al.,* 2005a; Dozier III *et al.,* 2008b; Saima *et al.,* 2010).

Numerous experiments have indicated that feeding high amino acid densities to broilers increases breast meat yield, final broiler weight and feed conversion efficiency (Kidd *et al.,* 2004; Corzo *et al.,* 2005a; Dozier III *et al.,* 2008b). However, discussions concerning if the benefits of feeding a higher amino acid diet is offset by the cost of the higher priced feed still remains. Kidd *et al.,* (2005) determined that feeding high to excessive levels of amino acids is costly but that feeding a low cost diet containing marginal amino acid levels may be even more costly due to a reduction in economic returns from a decrease in saleable meat. Research has supported this claim by revealing that broilers that were fed diets that were low in amino acid densities will experience a

reduction in growth rate, body weight, breast yield and feed conversion efficiency (Kidd *et al.,* 2004; Corzo *et al.,* 2005a; Dozier *et al.,* 2007). Additionally, feeding diets low or deficient in amino acids could potentially increase feed costs as broilers will over consume the feed in an attempt to meet its energy requirements (Gous, 1998; Kamran *et al.,* 2008). Despite research showing that a reduced crude protein diet causes a reduction in growth rate and breast yield, broiler producers are continuing their attempts to minimize crude protein content in order to control costs. Producers want to find the minimum amount of crude protein that will produce sufficient broiler yields.

Reducing dietary crude protein is not only desirable to producers from a cost perspective but also in terms of reducing nitrogen and ammonia emissions (Kidd and Kerr*,* 1996; Hussein *et al.,* 2001; Dozier III *et al.,* 2008b). Nitrogen and ammonia gas emissions can cause air quality, odor and environmental concerns to humans as well as adversely affect bird performance and health (Lacey *et al.,* 2004; Wathes, 1998; Miles *et al.,* 2004; Dozier III *et al.,* 2008b). A 1% reduction in crude protein has been found to cause a 7% reduction in nitrogen levels in broiler litter (Ferguson *et al.,* 1998; Hussein *et al.,* 2001). However, as stated previously, reducing amino acid density reduces growth potential, feed conversion and carcass yields. Therefore, producers need to explore opportunities to reduce crude protein and nitrogen excretion levels without affecting growth performance and carcass yields.

Previous research has suggested that the best solution for reducing crude protein levels, and reducing nitrogen excretion, while maximizing growth potential, is to have a low protein feed supplemented with a high concentration of essential amino acids (Kidd

and Kerr, 1996; Hussein et al., 2001; Corzo et al., 2005b; Payne, 2007; Kamran et al., 2008; Pesti, 2009). Si et al., (2004) reported that dietary protein could be reduced from 23 to 19% without affecting feed conversion ratio and performance of the broiler when diets were supplemented with lysine concentrations that are greater than the levels that are recommended by the NRC (1994). Kidd and Kerr, (1997) and Peñaranda-Alie *et al.,* (2010) reported improved breast meat yield in male turkeys when low protein diets were supplemented with amino acids such as lysine, threonine and tryptophan. Similarly, Payne (2007) concluded that crude protein levels could be reduced by 3 to 4 percents without negatively affecting yields, when supplemented with free amino acid concentrations that are equal to amino acid concentrations in the conventional diet. Corzo *et al.,* (2005b) and Pesti (2009) have reported that reducing crude protein and nitrogen excretion levels are possible with amino acid supplementation, without causing significant deleterious consequences on the live performance of broiler chickens. Kamran *et al.,* (2008) reported similar results. These authors indicated that nitrogen retention efficiency could be increased if low crude protein diets were supplemented with crystalline amino acids at an amount that was necessary for maintenance and tissue accretion.

Furthermore, knowing the time period in a broiler's life when amino acids are in greatest demand is critical for creating a feeding program that can be adjusted to determine the minimum protein level that can be fed to broilers and achieve maximum performance (Kidd *et al.,* 2005). High amino acid densities are critical early in broiler development (1 to 28 days) for optimum performance and meat yield (Dozier III, *et al.,*

2008). Broilers that are fed high amino acid diets early in development should have a favorable economic return on investment because feed intake is relatively low at this stage of the production cycle. Therefore, feeding high amino acid densities early in a broiler production cycle is economically advantageous as feed intake is low and the potential for growth improvements are high, which allows for maximum feed efficiency (Kidd *et al.,* 2005). The simultaneous increase in the knowledge of amino acids as it relates to broiler nutrition and genetic selection has allowed broiler feed conversion efficiency and growth rate to increase significantly over the last several decades (Coleman and Korver, 2004). During that time frame, broilers have become larger and more efficient with less feed necessary to reach a target weight. Despite the increased knowledge of broiler nutrition, research is limited on the relationship between broiler nutrition and growth rate on meat quality (Coleman and Korver, 2004),

Broiler Meat Quality

Consumer acceptance of meat depends primarily on quality, which is influenced by a series of factors ranging from the physical and chemical to the histological properties of and processing procedures for meat (Alvarado & Sams, 2004). Broiler breast meat quality is often evaluated in terms of color, pH, water-holding capacity, tenderness and sensory acceptability since consumers prefer meat that is juicy, tender and not too pale (McKee and Sams, 1997; van Laack *et al.,* 2000; Schilling *et al.,* 2003; Fletcher and Smith, 2006; Corzo *et al.,* 2009). Groom (1990) defines quality as it relates to meat as "the composite of those characteristics that differentiate individual units of a

product and which have significance in determining the degree of acceptability of that unit to the user". Concerns over broiler meat quality have increased in significance over the years as more poultry meat is blended into value-added products. (Denbow, 2003). In 1995, 63 percent of all broiler volume was cut-up and sold as parts and valued added products while an additional 11 percent was sold as further processed products such as chicken nuggets and patties (National Broiler Council, 1995; Martinez, 2000). Production of boneless broiler meat has increased over the last two decades due to high demand for whole fillet and restructured boneless products by the fast food industry and retail consumer (National Chicken Council, 2004; Castañeda *et al.,* 2005).

It is well established that amino acid density in the broiler diet affects feed conversion ratio, body weight, breast meat, and carcass yields. However, there is limited research on the effect of amino acid density on meat quality. Research has been conducted that reveals that diet can directly affect the fatty acid composition of broiler thigh meat since the fatty acids of the feed are deposited in the muscle (Wood and Enser, 1997; Corzo *et al.,* 2009). In addition, the degree of saturation of the triglycerides in the feed has been shown to affect the saturation level of the fatty acids in the meat tissue, which affects its oxidation potential and shelf life (Adams *et al.,* 1994; Cortinas *et al.,* 2004; Suksombat *et al.,* 2007; Pisulewski, 2005). Meat prone to oxidation may produce off-odors and flavor which causes decreased consumer acceptability of the product (Bou *et al.,* 2005). High amounts of polyunsaturated fatty acids have been known to influence lipid oxidation which influences oxidative stability during refrigeration as well as affects the color, flavor, texture and nutritional value of the meat (Pisulewski, 2005). However,

susceptibility of poultry meat to lipid oxidation can be controlled by the addition of antioxidants (Pisulewski, 2005).

Feeding small quantities of distillers dried grains with soluble to broilers have shown minimal effects on broiler meat quality. Corzo *et al.,* (2009) reported that the inclusion of 8% DDGS in the diets of broilers has minimal effects on the quality of broiler breast and thigh meat, which includes only slight differences in consumer acceptability of the breast and no differences in instrumental quality measurements. Similarly, Wang *et al.,* (2007) reported that broilers can be fed 15% DDGS without affecting carcass composition or growth. Bou *et al.,* (2005) indicated that feed supplemented with zinc and selenium created no differences in consumer acceptability or broiler breast meat quality.

Due to genetic selection, muscle growth, specifically in the breast, has increased dramatically over the last two decades through the production of a larger broiler with a greater percentage of muscle mass (Coleman and Korver, 2004). Broilers now have greater breast meat yield and less body fat, which is in response to consumer demand for leaner meat (Bihan-Duval *et al.,* 1999). Due to genetic selection, broiler breast muscle yield has increased in recent years when compared to the total carcass yield in broilers (Coleman and Korver, 2004). The selection of broilers for increased breast meat yield and lower percentages of body fat has been successful but limited research has been done on the impact it has on meat quality (Bihan-Duval, 1999). However, the research that has been performed has shown that large, rapidly growing broilers can have morphological abnormalities such as large fiber diameters and lower proteolytic potential in the muscles

(Solomon *et al.,* 1998; Dransfield and Sosnicki, 1999; Coleman and Korver, 2004). After processing, rapidly growing broilers may be more susceptible to the development of Pale, Soft and Exudative (PSE) characteristics such as more rapid rigor development, paleness in color and a reduction in water holding capacity (Coleman and Korver, 2004) suggesting that an increase in muscle fibers may detrimentally affect meat quality.

Effects of Broiler Stress on Meat Quality

During growth and transportation to processing plants, broilers are exposed to a variety of stressors such as heat, fasting, noise, social disruption and the withdrawal of water (Mitchell and Kettlewell, 1998). The catching, transporting, unloading and hanging of the broiler can reduce the quality and yield of broiler breast meat if performed improperly (Sams, 1999). The handling of the bird between farm and factory represents the greatest cause of downgrading of the quality of the broiler and is therefore responsible for the greatest financial loss (Groom, 1990). Antemortem stress and postmortem lactic acid production along with protein denaturation while the temperature is still high can result in meat quality defects such as poor texture, decreased water holding capacity, juiciness, and an increased incidence of PSE meat (McKee and Sams, 1997; Solomon *et al*., 1998; Sosnicki *et al*., 1998). Stressing of the broiler prior to processing may affect meat quality by causing a rapid post-mortem pH decline that can lead to myofibrillar and sarcoplasmic protein denaturation that may result in a decrease in tenderness, juiciness, and pale broiler breast meat (Schilling *et al.,* 2008; Battula *et al.,* 2008).

Myofibrillar and sarcoplasmic protein denaturation can result in low water holding capacity, pale color and an increased incidence of PSE-like meat (Lawrie 1966; Sams, 1999). The PSE condition refers to meat with pale color (paler than normal), undesirable texture (softer than normal), and low water holding capacity (lower than normal) (Remignon *et al.,* 2007). PSE meat was first identified in pork in the 1960's and later became a serious quality concern for the poultry industry in the 1980's and 1990's (Remignon *et al.,* 2007). After death, the broiler's muscle tissue undergoes anoxia and begins to produce ATP through the anaerobic metabolic pathway of glycolysis (Remignon *et al.,* 2007). Lactate begins to accumulate and acidification of the muscle tissue begins and the pH declines (Remignon *et al.,* 2007). PSE meat can develop from accelerated glycolysis and a rapid pH decline when the temperature of the carcass is still high, immediately after slaughter (Denbow, 2003; Battula *et al.,* 2008).

As pH declines, water-holding capacity decreases which leads to a dryer, less tender product that the consumer may find undesirable (Denbow, 2003). PSE poses a serious problem to further processed or cut-up meat products since decreased water holding capacity will cause a greater amount of purge within the package which consumers find unacceptable (Barbut, 1993; Denbow, 2003). In addition, PSE symptoms will cause protein denaturation that leads to a loss of protein functionality in processed meat products (Remignon *et al.,* 2007). Numerous researchers report that minimizing stress is necessary to decrease the incidence of PSE-like symptoms and maintaining acceptable meat quality. Remignon *et al*. (2007) reported the prevalence of PSE as occurring at 5 to 40% within a flock.

Environmental Temperature Effects on Meat Quality

Temperature extremes have been proven to cause stress in broilers (Battula *et al*., 2008). With the decline in the cotton industry, the broiler production and slaughter capacity expanded into the south aided by the South's traditionally lower wages and absence of unions (Martinez, 2000). Southern states typically experience higher environmental temperatures and therefore may not be the ideal location to raise broilers since high environmental temperatures such as those during the summer months have been shown to adversely affect broiler growth rate, feed consumption and carcass yields (Dozier and Moran, 2001; Zaman *et al.,* 2008). Meat quality such as color, water holding capacity and texture can be affected during summer months, as heat stress just prior to slaughter can affect postmortem metabolism (Lambooij *et al.,* 1999; Warris *et al.,* 1999; Battula *et al.,* 2008).

Heat stress is one of the most prominent antemortem environmental factors that can cause a rapid early postmortem glycolysis (Mckee and Sams, 1997). Lee *et al*. (1976) reported that meat obtained from heat stressed broilers at 38ºC was less tender than meat from broilers obtained in cooler seasons. Additionally, cold extreme temperatures such as those at 2ºC have also been shown to negatively affect meat quality by increasing the shear values of broiler breast meat (Wood and Richards, 1975). Research indicates that poultry meat has increased L* (lightness) values during the summer months which has been known to correlate with decreased water-holding capacity, a lower pH and increased shear values (Coelho, 1994; Denbow, 2003). Breast meat has been shown to be more sensitive to hot environmental temperatures (Lin *et al.,* 2006), which is significant in the

United States where white meat is consumed more often than in other countries. High environmental temperatures are one of the major concerns for broiler producers, especially those in hot and humid regions (Gu *et al.,* 2008).

Since broilers have no sweat glands and are covered with feathers, the bird's thermoregulations are challenged during hot weather (Gu *et al.,* 2008). Previous research has shown the adverse effects that hot environmental temperatures have on broilers and meat quality such as reducing meat sensory quality, broiler growth rate, carcass yield and feed consumption (Dozier and Morgan, 2001; Gu *et al.,* 2008). Heat stress prior to processing has been known to cause a lower ultimate pH in breast meat, (Holm and Fletcher, 1997; Sandercock *et al.,* 1999; Battula *et al.,* 2008) which has been shown to reduce water holding capacity and increase toughness (Sandercock *et al.,* 1999, Petracci *et al.,* 2001; Battula *et al.,* 2008; Schilling *et al.,* 2008). Oxidative damage of tissues is another possible reason for the decrease in sensory quality of heat stressed birds (Gu *et al.,* 2008).

The nutrition requirements of broilers are altered as the environmental temperature changes since temperature affects feed intake (Gous, 1998). As a result, it has been suggested that broiler producers adjust protein and amino acid content in the feed during times of high environmental temperatures (Dozier and Moran, 2001; Gu *et al.,* 2008). Previous research has suggested that a low protein diet is recommended in order to reduce heat stress in broiler chickens (Musharaf and Latshaw, 1999). However, more recent studies have shown that increasing the dietary protein level could reduce the adverse effects that hot temperatures have on broiler performance (Gu *et al.,* 2008).

Dozier and Moran (2001) is one such study that suggests that increasing dietary protein level is one way to correct for the decreased feed consumption and protein intake of broiler chickens that occurs in hot environments. Temim *et al.,* (2000) reported that heat stressed birds that were fed high protein diets showed in an improvement in bird performance when compared to control broilers. These findings depict the significance of high protein diets not only in their ability to improve bird performance and carcass quality over low protein diets but also for its ability to reduce stress related symptoms in the broilers that are caused by extreme environmental temperatures (Gu *et al.,* 2008).

Objective Tenderness Measurements

Tenderness has been noted as the most important factor in consumer perception of quality of a meat product (Savell et al., 1989) and as the single most important quality attribute in determining consumer's ultimate satisfaction for a whole cut of poultry muscle (Fletcher, 2002). Furthermore, consumers have come to expect a high level of palatability, particularly tenderness, in the meat that they purchase (Kotula and Wang, 1994). Therefore, understanding the biological and physiochemical inter-relationships that influence tenderness is required to produce tender meat (Kotula and Wang, 1994). Many factors influence the ultimate tenderness of broiler breast meat including age, sex, location, deboning time, cooking method and pH (Goodwin, 1984; Denbow, 2003; Cavitt *et al.,* 2005; Chen *et al.,* 2007). Glycogen concentration within the muscle as well as the rate of ante and post-mortem glycolysis can influence the tenderness of the broiler meat (Kotula and Wang, 1994).

Accelerating glycolysis can cause an increase in accumulation of lactic acid in the muscle tissue and cause a more rapid drop in pH of the meat (Kotula and Wang, 1994). A rapid postmortem drop in pH has been found to cause a decrease in tenderness due to the changes in solubility of the protein and changes to the enzymes within the meat after slaughter (Koutula and Wang, 1994). A decreased pH leads to a decreased activity of calpains and Cathepsins, enzymes that break down the protein structure and reduces postmortem tenderization causing toughening of the meat (Dransfield, 1994; Dransfield and Sosnicki, 1999). With a faster pH decline, myosin will become more susceptible to denaturation (Dransfield and Sosnicki, 1999). pH decline has also been shown to decrease water-holding capacity, which creates a dryer less tender meat product that may be unacceptable to consumers (Denbow, 2003). The texture of PSE in poultry appears to be softer prior to cooking and tougher after cooking (Remignon *et al.,* 2007)

Additionally, stress and early onset of rigor mortis can also lead to decreased breast meat tenderness (Northcutt, 2009). Rigor development has a direct influence on tenderness since ATP is still present in prerigor samples and initiates muscle shortening upon deboning which creates a tougher meat product (Cavitt *et al.,* 2005). The time period between harvest and deboning (aging) in broiler processing is an important factor in meat tenderness (Schilling *et al.,* 2003; Battula *et al.,* 2008). The process of short aging, which involves deboning between 0 to 4 hours after slaughtering, has been determined to cause an undesirable decrease in tenderness (Lyon *et al.,* 1985; Battula *et al.,* 2008). Most poultry processors currently age broilers for 4 to 7 hours to allow for the

resolution of rigor and ensure that broiler breast meat is tender prior to deboning (Lyon and Lyon, 1990; Sams, 1999; Schilling *et al.,* 2003; Battula *et al.,* 2008).

Stunning method affects meat quality. For example, electrical stunning has been shown to cause defects in meat quality such as redness of the skin, wing hemorrhages, broken bones and blood blemishes in breast muscle (Bilgili, 1992; Lambooij *et al*., 1999; Wilkins *et al*., 1999). Gas stunning and low atmosphere pressure stunning have been shown to improve meat quality by reducing bloodspots, especially on the thigh and breast, and improving tenderness when compared to electrical stunning (Hoen and Lankhaar, 1999). Air pressure stunning also results in more tender breast meat than electrical whole body stunning by accelerating muscle glycolysis (Battula *et al*., 2008). In addition, marinating is commonly used to enhance meat tenderness and reduce aging time (Goodwin and Maness, 1984. A mixture of phosphates, salt, and spices can be used to enhance water holding capacity, tenderness and flavor of the meat product (Lyon and Lyon, 2000).

Tenderness has been noted as the most important sensory factor in consumer perception of palatability and quality of meat products (Deatherage, 1963; Savell *et al.,* 1989, Cavitt *et al.,* 2005). Several methods for measuring tenderness of meat have been established including instrumental, descriptive, sensory, and consumer analysis (Cavitt *et al.,* 2004). Instruments such as an Instron Universal Testing Center (3300, Instron, Norwood, MA) are commonly used to objectively evaluate the tenderness of broiler breast meat using either a Warner-Bratzler or Allow-Kramer Shear force blade to determine the amount of shear force to cut through chicken breast (Sams *et al.,* 1990).

The maximum peak force (shear force) indicates the amount of force that must be applied in order to cut though a sample and corresponds to the hardness of that particular sample (Battula *et al.*, 2008). A higher shear force value indicates a greater amount of force that must be applied to shear through the sample and correlates with the sensory attribute of toughness (Battula *et al.*, 2008). Schilling *et al.,* (2003) reported that consumers find chicken breasts between 1.1 and 4.0 kg shear force (using the Warner-Bratzler Shear Force Blade) to be acceptable as it relates to tenderness when 1 cm by 1 cm by 2 cm strips are used that are sheared perpendicular to the muscle fiber. It should be noted however that instrumental tenderness does not relate to the juiciness or other moisture related characteristics in meat that a panelist or consumer may perceive while chewing (Lyon and Lyon, 1997; Battula *et al.,* 2008). Additionally, when using these instruments, sample size, location within the muscle, and orientation of the muscle fibers to the shearing blade must be considered and consistent among all samples (Battula *et al.,* 2008).

The Effect of Breast Muscle pH on Meat Quality

pH is commonly used as an indicator of meat quality (Battula *et al.,* 2008). As rigor mortis develops within the cell after slaughtering, ATP concentration declines and lactic acid begins to be built up due to glycolysis, thus decreasing the overall pH of the muscle (Calkins *et al.,* 1982; Lawrie, 1991; Cavitt *et al.,* 2005). A rapid pH decline may cause protein denaturation, resulting in a decrease in tenderness and juiciness as well as a less intense (pale) color of the muscle (Solomon *et al*., 1998). A rapid decline in pH can

also decrease water-holding capacity creating a dryer less tender product that may be unacceptable to consumers (Denbow, 2003). In extreme conditions, these symptoms are known as PSE (Pale, Soft and Exudative) meat (Solomon *et al*., 1998). Sams (1999) has reported that as with PSE in swine, rapid development of rigor mortis at high postmortem temperatures results in PSE in poultry meat.

A rapid pH decline, pH <6.0 within 15-30 min postmortem, has been used as an indicator of meat that could potentially be PSE meat (incorrect reference, Kauffman studied pork, not chicken. Kauffman, et al., 1992). Research has also shown a correlation between muscle pH and muscle color (Fletcher, 1999). Darker muscles tend to have a higher pH and lighter muscles tend to have lower pH values (Fletcher, 1999). Bihan-Duval *et al.,* (1999) has shown that breast meat with a lower pH at 24 hr postmortem had lower water holding capacity. The muscular activity during stunning and slaughter has been found to have an accelerating effect on the rate of pH decline (Raj *et al.,* 1990). In addition, pH has been found to be the major determinant of L* value, moisture retention and cooking yield (Van Laack *et al.,* 2000). The 24 h pH in PSE broiler breast meat is lower than the pH of normal breast meat and is a major contributor to the paleness and the lower water holding capacity in PSE meat (Van Laack *et al.,* 2000). Therefore, procedures that limit pH decline or increase pH prior to processing may be effective at improving functionality and improving color and water-holding capacity (Van Laack *et al.,* 2000).

Color as a Measurement of Meat Quality

Poultry meat color is a critical food quality attribute and is significant for both the consumer's initial selection of the raw product at the purchasing location and the consumer's final determination of acceptability after cooking and prior to consumption (Fletcher, 1999). Raw poultry meat color is significant for consumers because they associate it with the products freshness, which they use when making their purchasing decisions (Northcutt, 2009). Differences in breast meat color have been linked to the preslaughter condition of the broiler and handling procedures of the poultry carcass (Allen *et al.,* 1998). The color of poultry meat can also be influenced a by age, sex, genetic strain, diet, intramuscular fat, and processing conditions of the broiler (Northcutt, 2009). Similarly, bruises, hemorrhages and poor exsanguination efficiency has been shown to negatively affect the meat and skin by causing unacceptable discoloration and a reduction in shelf- life (Griffiths and Nairn, 1984; Battula *et al.,* 2008).

Appearance of the meat product is the consumer's major criterion for purchase selection and initial evaluation of meat quality (Allen *et al.,* 1998). Meat color is dependent on the presence of the muscle pigments myoglobin and hemoglobin and discoloration of the meat can be related to the amount and chemical state of the pigments (Northcutt, 2009). A chroma meter is used to objectively measure color using L* (lightness), a* (redness) and b* (yellowness) values that have been standardized by the Commission Internationale de L'Eclairage (Denbow, 2003). Extreme color variations, such as very light to very dark have been shown to have significant effects on the function and chemical properties of broiler breast meat (Qiao, et al., 2001). A rapid pH

decline when the carcass temperature is still high can cause denaturation of the muscle causing the broiler meat to have a pale color, which is one of the indicators of PSE type meat (Murray, 1995; Denbow, 2003). Normal breast meat has an L* of approximately 55 and those that appear to be pale have L* values greater than 60 (Van Laack *et al.,* 2000; Schilling *et al.,* 2008). In normal breast meat, the pH is far above the isoelectric point of the myofibrillar proteins, which allows more space for water molecules to exist in the meat matrix and allows more light to be absorbed by the muscle, which makes the meat appear darker in color with a lower L* value (Kauffman and Marsh, 1987; Cornforth, 1994; Battula *et al.,* 2008).

Pale meat has been attributed to lower water holding capacity, poor functionality in processed products, lower pH and greater cook loss when compared to normal meat (Fletcher, 1999; Denbow, 2003). The lightness of the broiler meat appears to be highly correlated with the pH at 24 hr postmortem (Bihan-Duval *et al.,* 1999). Additionally, research has suggested that lightness is highly heritable and selecting for lower L* values could lead to higher pHu which could in turn lead to poultry meat with better water holding capacity (Bihan-Duval *et al.,* 1999). Fletcher (1999) reported that there are variations in breast meat color within poultry from commercial producers and that there is a strong relationship between breast meat color and muscle pH with darker muscle having a higher pH and lighter muscle having a lower pH.

Water-Holding Capacity and Cooking Loss

Cooking loss is a very important characteristic for the processing industry as water retention is a main point of profit (Van Laack *et al.,* 2000). The main determinants of water-holding capacity of meat are pH and protein denaturation (Offer and Knight, 1988; Van Laack *et al.,* 2000). The isoelectric point of the major water binding protein, myosin is 5.1-5.3 and at this pH, water binding will be minimal (Offer and Knight, 1988; Van Laack *et al.,* 2000). A low pH has also been associated with an increase in cook and drip loss (Northcutt *et al.,* 1994; Allen *et al.,* 1998). Water holding capacity can be increased by the addition of polyphosphates to meat that works in by increasing the ionic strength and increasing the pH so that it is further away from the isoelectric point (Young *et al.,* 1992; Yang and Chen, 1993). The combination of sodium chloride and polyphosphates have been shown to synergistically improve moisture absorption and water holding capacity, and reduce drip and cook loss (Young *et al.,* 1987; Allen *et al.,* 1998). Tumbling the poultry breast meat has also been found to increase tenderness and the amount of soluble protein (Maki and Froning, 1987), which enhances the binding properties of the poultry meat making it useful for processed meat products (Froning, 1966; Allen *et al.,* 1997). Since decreased water holding capacity is one indicator of PSE meat (Denbow, 2003), researchers have used drip loss and water holding capacity along with L* values and pH decline as characteristics to evaluate meat quality (Schilling *et al.,* 2008). A high L* value and a low ultimate pH (5.7<) have been found to be indicative of broiler breast meat that was both pale in color and low water-holding capacity (Van Laack *et al.,* 2000; Schilling *et al.,* 2008).

CHAPTER III

MATERIALS AND METHODS

Treatments

All broilers were fed standard starter (day 1 to day 13) and grower (day 14 to day 27) poultry diets until the finishing phase. During the finishing phase (days 28 to 42), the broilers were given pellets of four corn-soybean meal based diets differing in increasing concentrations of amino acid and crude protein densities. Treatment 1 consisted of a diet deficient in amino acid concentration (D), treatment 2 consisted of a diet low in amino acid concentration (L), treatment 3 consisted of a diet high in amino acid concentration (H) and treatment 4 consisted of a diet excessive in amino acid concentration (E). Nutritional information of the dietary treatments is included in Table 1 to demonstrate how amino acid density and crude protein concentration varied among treatments.

Bird Husbandry Four hundred eighty Ross x Ross 308 male broiler chicks were obtained from a commercial hatchery and distributed equally across 40 floor pens so that each treatment was replicated 10 times with 12 broilers in each pen (0.09 m^2/bird). Chicks were vaccinated at the hatchery for Marek's disease, Newcastle disease, and infectious bronchitis. Each pen was equipped with a hanging feeder, a nipple drinker line and built-up litter (previously used soft-wood shavings). Birds consumed feed and water on an *ad*

libitum basis, and experimental diets were provided in pellet form. An ambient temperature program was maintained at 33°C at placement until 4 d of age, 32°C from 5 to 9 d of age, 29°C from 10 to 14 d of age, 27°C from 15 to 23 d of age, 25°C from 24 to 28 d of age, 23°C from 28 to 35 d of age, and 20°C from 35 to 42 d of age. The Photoperiod followed a continuous schedule with lighting intensities of 30 lux from 0 to 7 d of age, 10 lux from 7 to 22 d of age, and 3 lux from 22 to 42 d of age, and light intensity was verified at bird level (30 cm) using a photometric sensor with NIST-traceable calibration (Extech Instruments, Waltham, MA, USA). The University's Institutional Animal Care and Use Committee approved all animal procedures.

Sample Preparation

At 42 d of age, 8 broilers from each of the 4 treatments within 10 replications (total of 80 birds per treatment) were randomly selected for harvesting and whole breast and thigh removal at 4 hr postmortem. Broilers were hung by their feet in steel shackles and were electrically stunned by manually placing their heads in a saturated saline bath (11.5 volts, < 0.5 mA AC to DC current for 3 sec). The shackle line speed was constant and set so that approximately 22 broilers were stunned per minute. Unilateral neck cutting was manually performed immediately after stunning, and bleeding lasted for 140 sec. Upon completion of exsanguination, the broilers were scalded at 53.3^{o} C for 191 sec, picked for 35 sec using a rotary drum picker (Baader-Johnson, Kansas City, Kansas) and then mechanically eviscerated. After harvest, all broiler carcasses were stored in ice water in metal containers (173 cm in length, 85 cm in width, and 68.5 cm in depth), and

later transferred to rubber containers (142 cm in length, 81 cm in width, and 50.8 cm in depth) for sampling and sorting of breast and thigh muscles.

At 4 hr postmortem, breast (boneless and skinless) and thigh (bone-in) muscles were removed from the carcass. A total of 320 whole breasts and thighs were placed into individually labeled Ziploc bags (Ziploc brand freezer bags, S.C. Johnson & Son, Inc., Racine, WI), brought to the Food Processing Plant (Department of Food Science, Nutrition and Health Promotion, Mississippi State University), and cooled (2^{o}C) over night. At 24 hr postmortem, each whole breast was separated into right and left halves. Within these samples, eight breast samples (right side of carcass) per treatment within ten replications (total of 320 breast samples) were evaluated for color and pH.

Breast samples were then individually vacuum-packaged (Turbovac 320-ST-S, Inject Star of the Americas, Inc, Brookfield, CT) in 15.2 × 20.3 cm, 3 mil vacuum pouches (item # 75001815, Rebel Butcher Supply Co. Inc, Flowood, MS) and frozen (-23^{o}C) until proximate analysis (n=10 with 4 subsamples per treatment), cook loss (n=10 with 4 subsamples per treatment) and shear force determinations (n=10 with 4 subsamples per treatment) could be performed. The breasts from the left side of the carcasses from the same treatment within each replication were bagged (4 breasts per bag), vacuum packaged (40.64 × 50.8 cm, 4 mil vacuum pouch; item # 75001987; Rebel Butcher Supply Co. Inc, Flowood, MS) and frozen (-23^{o}C) until consumer sensory acceptability tests could be performed. Thigh meat samples were placed into labeled Ziploc bags (Ziploc brand freezer bags, S.C. Johnson and Son Inc.) and frozen (-23^{o}C)

until proximate analysis, fatty acid and lipid peroxidation (TBARS) tests could be performed.

Proximate Analysis

Four broiler breast and two thigh meat samples from each of the four treatments within the ten replications (n=10 broiler breast with 4 subsamples, and n=10 for broiler thighs with 2 subsamples) were used to measure fat, protein, and moisture percentage using a Near Infrared Spectrometer (FoodScan Lab Analyzer Model 78800, FOSS Analytical) that is AOAC approved (AOAC 2007-04). Frozen samples were thawed for 24 hrs at 2°C and then ground using a meat grinder (Cabelas PRO 450, Sidney, NE 69160) that was fitted with a 3 mm (1/8") cutting plate. Ground samples were packed tightly (10 to 15 mm thick) in a 140 mm sample compartment prior to analysis.

pH Measurement

Two broilers per replication (n=10 with 2 subsamples per treatment) were used to measure pH decline at 30, 60 and 240 minute intervals postmortem using a pH meter (Model Accumet 61a, Fisher Scientific, Hampton, NH) by inserting the pH probe (Model FlexipHet SS Penetration tip, Cole Palmer, Vernon Hills, IL) into the *pectoralis* muscle at approximately 2.5 cm from the top of the breast and 2.5 cm from the breast bone. When pH decline measurements were not being recorded, samples were stored in ice water in metal (173 cm in length, 85 cm in width, and 68.5 cm in depth) and rubber containers (142 cm in length, 81 cm in width, and 50.8 cm in depth) to mimic the chilling process in

a poultry plant. At 24 hours postmortem, ultimate pH (pH_u) measurements for each sample (n=10 with 8 sub samples 320) were recorded using the same pH meter in the same anatomical location as the pH decline measurements.

Color Measurement

Instrumental color measurements were taken for each breast within each treatment (n=10 with 8 subsamples) using a chroma meter (Chroma meter Model CR-400, Minolta Camera Co., LTD., Osaka, Japan Serial No C8202489) that was calibrated using a standard white calibration plate (Model No 20933026, Japan). Three measurements were taken at three different locations for each breast on the medial portion of the pectoralis major muscle and values were averaged. Color for each sample was expressed in terms of CIE values for lightness (L*), redness (a*), and yellowness (b*).

Cooking Loss

Frozen breast samples (n=10 with 8 subsamples) were thawed at 2°C for 24 hrs. The thawed samples were weighed and baked in an oven (JBP25DOJ2WH, General Electric, Louisville, KY) to a final internal temperature of 77°C. Internal chicken breast temperatures were determined using thermocouples and a data logger (UWTR, Omega Engineering, Stamford, CT). Cooked breasts were cooled to ambient temperature (20°C), patted dry with 1 paper towel (1-ply), and reweighed. Cooking loss was reported as a percentage and calculated as (initial weight – final weight) / (initial weight) x 100.

Warner-Bratzler Shear Force Determination

Tenderness was assessed using an objective texture procedure described by Meek *et al*. (2000). Breasts that were used for cooking loss determinations (n=10 with 4 subsamples) were used for Warner-Bratzler shear force determinations. Four to 6 adjacent 1 cm (width) x 1 cm (thickness) x 2 cm (length) strips were cut from the cooked breast, parallel to the direction of the muscle fibers. Each strip was sheared once and the mean shear force value (N) was calculated for each breast. Samples were sheared perpendicular to the muscle fibers using a Warner-Bratzler shear attachment mounted on an Instron Universal Testing Center (3300, Instron, Norwood, MA) using a 50-kg load transducer and a crosshead speed of 200 mm/min.

Allo-Kramer Shear Force Determination

Tenderness was assessed using an objective texture procedure described by Sams *et al*., (1990) and Cavitt *et al.,* (2005). Breasts that were used for cooking loss determinations were used for Allo-Kramer shear force determinations (n=10 with 4 subsamples). Allo-Kramer shear force value (kg force per gram of meat) was determined on two adjacent 4 cm (length) x 2 cm (width) x 0.7 cm (height) strips cut from the medial area of the cooked breast, parallel to the direction of the muscle fibers and then weighed to approximately 6.5 grams. Shear value (N) was measured using an Instron universal testing machine (3300, Instron, Norwood, MA) equipped with a 10 blade Allo-Kramer shear compression cell using a 500-kg load cell with a load range of 200 kg and a crosshead speed of 500 mm/min (Sams *et al*, 1990; Cavitt *et al*, 2005). Each strip was

sheared once and the mean of the two strips from each sample was calculated. The average shear force per gram was determined by dividing the mean shear value (N) by the mean weight (g). Average total energy was then computed (N/g) for each sample.

Sensory Analysis

Three consumer based sensory panels (n=60 panelists per a replication) were conducted to evaluate the acceptability of chicken breast meat from broilers fed diets with different levels of dietary amino acid densities. Participants were recruited by an advertising sign, emails and though word of mouth. Chicken breasts were thawed at 2ºC for 24 hours before sensory testing and were vacuumed packed using Cryovac 10K OTR vacuum bags (Cryovac, Duncan, SC). Samples were *sous vide* cooked for approximately 15-20 minutes in 98ºC pot of water until an internal temperature of 77ºC using meat thermometers (78631, Farberware, Westbury, NY) to verify temperature. The chicken breasts were then cooled for approximately 15 minutes at room temperature and then cut into 2.5 x 2.5 cm cubes, and kept warm (60ºC to 70ºC) in a 8-quart chafer dish (53042, Polarware Co., Kiel, WI), until panelists evaluated the samples.

Random 3-digit numbers were assigned to identify the four samples. Sample order was randomized to account for sampling order bias. Each panelist was asked to evaluate 4 coded chicken breast samples; 1 sample from each treatment (D, L, H, E) for appearance, aroma, texture, flavor, and overall acceptability using a nine point hedonic scale, where 1 = dislike extremely, 5 =neither like nor dislike, and 9 =like extremely (Meilgaard *et al.,* 2007). Acceptability of texture was defined as product liking in respect

to tenderness. Acceptability of appearance was defined as product liking in respect to color and moisture. Acceptability of flavor was defined as product liking in respect to chicken flavor (taste). Acceptability of aroma was defined as product liking in respect to chicken aroma (smell). Water and unsalted crackers were provided and panelists were asked to expectorate and rinse their mouths between each sample.

Fatty Acid Profile

Fatty acid profiles were conducted on broiler thigh meat samples from each of the 4 treatments. (n=6 per treatment). Lipids were extracted in ether as described by the AOAC (2000A, 996.06). The extracted lipids were converted to methyl esters as described by AOAC (2000B, 969.33) and analyzed for individual fatty acids (C14:0 and C20.4) using a gas chromatograph (3400 Varian Inc., Walnut Creek, CA) that is fitted with a flame ionization detector. Gas chromatography parameters were as follows: the column temperature was 50°C for 3 minutes and then increased to 220°C at 4°C/min and was held for 15 minutes. The injector temperature was 200°C, and the detector temperature was 250°C. The flow rates of the carrier gases (hydrogen and oxygen) were 30 and 300 mL/min, respectively. Identification and quantification of individual fatty acids were completed using a standard fatty acid methyl ester mixture (2010, Matreya Biochemicals LLC, Pleasant Gap, PA).

Lipid Oxidation (Thiobarbituric Acid)

Thiobarbituric acid (TBA) levels, expressed as milligrams of Malonaldehyde (MA) per kilogram of sample were determined using the direct chemical-extraction method described by Spanier and Traylor (1991). One broiler thigh meat sample was randomly selected from each of the 4 treatments for 9 replications (n=36) to determine lipid peroxidation changes as a function of storage (4°C) time at 1, 3, and 5 days after thawing. Thighs were thawed for 24 hours at 4°C before storage.

Statistical Analysis

A randomized complete block design (replications as blocks) with 10 replications (n=10) was used to test the effects of different dietary amino acid density regimes on pH decline, ultimate pH, color, cooking loss, and shear force and total energy of broiler breast meat and the peroxidation (TBARS) and fatty acid profile of broiler thigh meat (version 9.1, SAS Institute, Cary, NC). In addition, a factorial structure was utilized in the TBARS analysis since samples were analyzed over time for each diet. When significant differences ($P< 0.05$) existed among treatments, the Fisher's least significant difference (LSD) test was used to separate treatment means. A randomized complete block design (replications and panelists as blocks) with 3 replications was utilized to test the treatment effects ($P< 0.05$) of diet on the sensory acceptability (appearance, texture, flavor, aroma) and overall acceptability of the chicken breasts.

Agglomerative hierarchical clustering using Wards Method (XL Stat, 2006) was performed to group panelists together based on their preference and liking of broiler breast meat. A dendrogram and a dissimilarity plot were used to determine how many

clusters should be utilized to group the panelists. After separating the data into clusters, the entire data set was evaluated to confirm that the data for each panelist was relatively close to the means of the treatments that were within the cluster that they were grouped into. After conducting agglomerative hierarchical clustering, randomized complete block designs (panelists as blocks) were performed within each cluster, and the LSD test was utilized to separate treatment means within a cluster when significant differences ($P<0.05$) occurred among treatments.

CHAPTER IV

RESULTS AND DISCUSSION

Body Weight, Feed Conversion and Carcass Characteristics

As expected, feed conversion efficiency increased ($P<0.05$) as amino acid density increased (Table 3) In addition, the Excessive amino acid density treatment yielded heavier ($P<0.05$) body weights than the Deficient and Low treatments, and the High treatment yielded heavier ($P<0.05$) body weights than the Low treatment (Table 3). The High and Excessive amino acid density treatments also yielded heavier breast and thigh weights when compared to the Deficient treatment, but no differences ($P<0.05$) existed among treatments with regard to carcass and thigh yields. However, there were numerical trends for increased carcass weight and breast yield as amino acid density increased from Deficient to Excessive with the Excessive and High amino acid densities producing higher yields ($P<0.05$) than the Deficient amino acid density treatment.

Results of the study were in agreement with previous literature that indicated decreased amino acid density diets resulted in reduced body weight and a less efficient feed conversion ratio (Kidd *et al*., 2004; Corzo *et al*., 2005a). Inversely, high amino acid diets have been shown to maximize growth performance, meat yields, feed conversion, and body weight (Kidd *et al*., 2004; Corzo *et al*., 2005a; Dozier III *et al.,* 2008b). Feeding broilers high amino acid diets are costly, due to marginal improvements in yield and a large increase in feed costs. Conversely, low cost feed with low amino acid densities has

been shown to be even more costly because of reduced economic returns brought upon by a reduction of saleable meat due to reduced breast and carcass yield and reductions in feed efficiency (Kidd *et al*., 2005). Therefore, high amino acid density diets that meet the amino acid needs of broiler chickens are necessary in order to optimize breast meat yield, body weight, carcass weight, and feed conversion (Dozier III *et al*., 2001; Kidd *et al*., 2004; Corzo *et al*., 2005a; Dozier III *et al.,* 2008b).

Proximate Analysis

No differences existed ($P>0.05$) among breast meat with respect to fat, protein and moisture from broilers fed diets that differed in amino acid density (Table 4). In contrast, thigh meat from broilers that were fed the deficient amino acid diet had higher ($P<0.05$) fat and a lower ($P<0.05$) moisture percentage than the other AA treatments. Thigh meat from the deficient amino acid diet also had a lower ($P<0.05$) protein percentage than the high or excessive AA diets (Table 4). These results were in agreement with previous literature revealing that carcass parameters such as fat decrease when amino acid density increases (Corzo *et al*., 2005a). Although no differences existed in proximate composition of breast meat, yields were still lower ($P<0.05$) in the low and deficient amino acid treatments, indicating a low protein content that is likely caused by the deficient and low concentrations of amino acids in the diet. In addition, proximate analysis values of the thigh and breast portions of the broiler from the current study were similar to those reported previously by Schilling *et al*., (2010) for the same strain of broiler that were fed varying levels of DDGS in the diet.

pH

At 60 min, 240 min and 24 h post-mortem, no differences existed ($P>0.05$) among treatments with respect to breast meat pH (Figure 1, Table 5). At 30 minutes post-mortem, the excessive amino acid diet had a lower ($P<0.05$) pH than the High AA treatment. This could potentially yield lower quality meat, due to increased protein denaturation, especially if the live animal has been exposed to stressors. pH is an indicator of meat quality (Fernandez *et al*., 1994; Alvarado *et al*., 2007). A product that is of low quality such as Pale, Soft, Exudative (PSE) meat is usually associated with a more precipitous drop in pH and a lower ultimate pH value than normal poultry meat (Murray, 1995; Van Laack *et al*., 2000; Denbow 2003; Remignon *et al.,* 2007). Because pH is an indicator of meat quality, minor concerns exist with the excessive amino acid treatment since breast meat from this treatment experienced a more rapid ($P<0.05$) pH decline by 30 minutes post-mortem than breast meat from the High amino acid diets.

However, only breast meat with either a very rapid pH decline (pH<6.0 within 15-30 min postmortem) or a pH_u of less than 5.7 is considered poor meat quality due to low water-holding capacity and paleness (Corzo *et al*., 2009). Since breast meat from all the dietary treatments had pH_u above 5.7 and pHs at 15-30 min were above 6.0, breast meat from all treatments are considered to be of good quality (Fernandez *et al*., 1994 Alvarado *et al*., 2007). Results of this study are similar to those of previous studies who reported breast meat pHs between 5.8 and 6.0 at 24 hr. postmortem (Schilling *et al*., 2008; Battula *et al*., 2008; Corzo *et al*., 2009; Schilling *et al*., 2010).

Color

CIE L* is used as a measurement of meat quality and an indicator of PSE meat in broiler breast since it is a measure of lightness and paleness of the broiler breast meat (Barbut, 1998; Van Laack *et al*., 2000; Woelfel *et al*., 2002, Corzo *et al*., 2009). No differences existed (P>0.05) among breast meat from broilers fed different amino acid densities with respect to CIE L*, a* and b* (lightness, redness, yellowness) (Table 5). L*, a* and b* values for all treatments were characteristic of normal breast meat at 24 hrs post-mortem indicating that all broiler breast meat is of good quality (Van Laack *et al*., 2000; Woelfel *et al*., 2002; Schilling *et al*., 2008; Battula *et al*., 2008; Corzo *et al*., 2009; Schilling *et al*., 2010). Results of the study were similar to those of Schilling *et al.* (2008) Battula *et al.* (2008), Corzo *et al.* (2009) who all reported L* (lightness) values around 55 and a* (redness) values around 1.6. However, b* (yellowness) values were similar to Battula *et al*. (2008) and Schilling *et al*., (2010) at 2.0 but less than Schilling *et al.* (2008) and Corzo *et al*., (2009) who reported values ranging from 4.9 to 6.0.

Cooking Loss and Brine Absorption

There were no differences (P>0.05) in cooking loss of breast meat from broilers fed diets that differed in amino acid densities (Table 5). Cooking loss ranged from 21.5 % for the excessive treatment to 22.3 % for the Low treatment which is within the parameters of the expected cooking loss for chicken breasts reported by Meek *et al*., (2000) and Battula *et al*., (2008) but slightly higher than Schilling *et al*., (2008), Corzo *et al*., (2009) and Schilling *et al*., (2010) who reported a lower cooking loss of around 20%.

In addition, no differences existed (P>0.05) among treatments regarding brine absorption with breast meat from each treatment increasing by 12-13 % in weight when it was vacuum tumbled with a 15 % brine solution (meat weight basis).

Instrumental Tenderness

There were no differences (P>0.05) in instrumental tenderness of breast meat from broilers fed diets that differed in amino acid density (Table 5). Statistically, the data indicated that increasing amino acid density had no influence on Warner-Bratzler shear force, Allo-Kramer shear force or Total energy and would therefore not be likely to have a significant effect on meat quality or sensory acceptability. Warner-Bratzler shear force values were in the range of 14.4 to 15.9 N which were similar to the values reported by Corzo *et al*., (2009) and Schilling *et al*., (2010) but slightly lower than Schilling *et al*., (2008) and Battula *et al*., (2008). On average, all amino acid diets produced chicken breasts that required less than 30 N to shear through using the Warner-Bratzler shear device, which indicates that the breast meat is very tender and would be highly acceptable to a large percentage of consumers (Schilling *et al*., 2003; Battula *et al.*, 2008, Corzo *et al*., 2009 Schilling *et al.*, 2010).

Consumer Acceptability

No differences existed (P>0.05) in appearance, aroma, texture and overall acceptability of broiler breast from the different amino acid treatments (Table 6). However, the excessive diet yielded breast meat that scored lower (P<0.05) in flavor

acceptability than breast meat from the deficient and low amino acid density diets. This suggests that increasing amino acid density may slightly decrease the consumer's acceptability of flavor. On average, acceptability scores ranged between "like slightly" to "like moderately" for all treatments with respect to appearance, aroma, flavor, texture and overall acceptability, indicating that consumers have a high degree of acceptability of chicken breasts regardless of amino acid density in the diet.

Since consumers vary significantly in their perception of acceptability, cluster analysis was performed and a dissimilarity plot was used to group the panelists into 4 clusters based on acceptability ratings and preference of broiler breast that was yielded from broilers that were fed the various dietary treatments (Table 7). Cluster 1 (n=72, 39.1% of panelists) contained the highest proportion of panelists. These panelists scored all treatments between "like moderately" to "like very much" with breast meat from the low AA diet receiving a higher ($P<0.05$) acceptability rating than the excessive treatment. No other differences existed ($P>0.05$) among treatments within this cluster. Cluster 2 (n=37, 20.1%) scored all treatments either between "like slightly" and "like moderately" or between like moderately" and "like very much" with breast meat from the deficient and excessive AA treatments receiving higher ($P<0.05$) mean acceptability ratings than the low and high AA diets. Cluster 3 (n=32, 17.4%) scored breast meat from Deficient and Excessive AA treatments between "neither like nor dislike" and "like slightly and scored breast meat from Low and High AA diets between "like slightly" and "like moderately" with breast meat from the low and the high AA diets receiving higher ($P<0.05$) mean values than the deficient and excessive treatments. Cluster 4 (n=43,

23.4%) scored all treatments “dislike slightly” to “neither like nor dislike” with no differences ($P>0.05$) between the treatments.

Three of the four clusters (76.6% of the panelists) liked chicken breast. However, in these three clusters, all panelists scored chicken breast from broilers fed Low or High AA diets at least like slightly, but only 59 % of the panelists scored breast meat from broilers that were fed Deficient and Excessive AA densities a value of like slightly or higher. However, these panelists (59 %) also scored breast from broilers fed the Deficient and Excessive A.A. densities between like moderately and like very much, indicating that consumers with the highest liking of the chicken breast samples had a high degree of liking for these treatments. Results of the current study are similar to sensory acceptability results of breast meat from those in previous research when a 9-point hedonic scale was used. (Schilling *et al.,* 2003; Battula *et al.,* 2008; Corzo *et al.,* 2009; Schilling *et al.,* 2010).

Fatty Acid Analysis

Differences existed ($P<0.05$) in palmitoleic, linoleic, linolenic (ω3), monounsaturated and unsaturated fatty acid percentages in thigh meat from broilers that were fed different amino acid densities in their diet. Thigh meat from broilers that were fed the Excessive AA diet had increased ($P<0.01$) percentages of linoleic acid in comparison to thigh meat from broilers that were fed diets that were Deficient and Low in AA density (Table 8). Similarly, linolenic acid concentrations were also elevated ($P<0.01$) in thigh meat from broilers that were fed the Excessive AA diet when compared

to thigh meat that was yielded from the Low or Deficient AA treatments. This indicates that increasing AA density in broiler diets increases ($P<0.01$) the percentage of linoleic (18:2 *cis*) and linolenic (18:3ω3) fatty acids in the lipid portion of the thigh meat.

Linoleic (18:2 *cis*) and linolenic (18:3ω3) are significant fatty acids because of their degrees of unsaturation, which makes them more susceptible to oxidation and a shorter shelf life. The increase in linoleic (18:2 *cis*) and linolenic (18:3ω3) fatty acids is not due to the fatty acid composition of the feed since High and Excessive amino acid density treatments have less corn oil than the Deficient and Low treatments, and therefore less unsaturated fatty acids in the feed. Consequently, these increases in linoleic and linolenic acids are probably due to metabolic processes in the broilers and this mechanism(s) has yet to be elucidated.

Lipid Oxidation (TBARS)

No differences existed ($P>0.05$) among treatments with respect to TBARS values after 1 and 3 days of storage. All TBARS values at day 3 were relatively low indicating only slight oxidation. After 5 days of storage, thigh meat from the Excessive amino acid diet had higher ($P<0.05$) TBARS values than thigh meat from the Deficient and Low AA treatments. As expected, oxidation increased as storage time progressed. However, the level of oxidation increased faster ($P<0.05$) in thigh meat from the Excessive amino acid treatment when compared to the Deficient or Low amino acid diets (Figure 2). The data indicates that increasing amino acid density in the diet increases the susceptibility of the thigh meat to oxidation and that broilers fed High and Excessive amino acid density diets

may be more susceptible to oxidation than thigh meat from broilers fed a low or deficient AA diet.

These findings support previous research that the broiler's diet directly affects the fatty acid composition in broiler thigh meat since the fatty acids in the feed are deposited in the muscle (Wood and Enser, 1997; Schilling *et al*., 2010). This increase in oxidation is likely due to the increase in polyunsaturated fatty acids from the feed such as linoleic (18:2 *cis*) and linolenic (18:3ω3) acids, which are more susceptible to oxidation due to their unsaturated double bonds. These results are similar to previous literature that has reported that triglyceride saturation levels in the diet affects the saturation level of the fatty acids in the thigh meat and therefore influences a products susceptibility to oxidation (Adams *et al*., 1994; Cortinas *et al*., 2004; Suksombat *et al*., 2007; Schilling *et al*., 2010).

The increase in essential fatty acids of linoleic (ω6) and linolenic (ω3) increases the nutritional qualities of the thigh meat, although it is potentially detrimental to meat quality since it increases the products susceptibility to oxidation. Nevertheless, if differences in oxidation at storage times of 5 days or longer were not a concern then increasing the nutritional qualities of the broiler breast meat by adding essential fatty acids such as linoleic and alpha- linolenic (omega 3 fatty acid) would be nutritionally beneficial. Furthermore, the susceptibility to oxidation could be minimized by adding dietary antioxidants to the broiler feed (Wang *et al*., 2009).

Table 1

Experimental Composition of Broiler Finisher–phase (28-42 d) Diets

Ingredients	Deficient AA	Low AA	High AA	Excessive AA
Corn	77.349	72.723	63.577	56.748
Soybean Meal	15.782	19.644	27.31	33.04
ProPlus[1]	2.0	2.291	3.817	4.972
Poultry oil	1.526	2.0	2.0	2.0
Dicaulcium phosphate	1.231	1.212	1.174	1.145
Limestone	0.981	0.973	0.957	0.946
Salt	0.472	0.471	0.468	0.466
Premix[2]	0.25	0.25	0.25	0.25
L-Lysine-HCl	0.194	0.186	0.146	0.098
DL-Methionine	0.131	0.176	0.248	0.286
Coccidiostat[3]	0.05	0.05	0.05	0.05
Choline chloride	0.033	0.023	0.004	-
Calculated composition				
CP, %	15.5	17.1	20.2	22.5
Ca, %	0.80	0.80	0.80	0.80
Av P, %	0.40	0.40	0.40	0.40
AME, kcal/kg	3,200	3,200	3,200	3,200
Na, %	0.22	0.22	0.22	0.22
Lys, %	0.90	1.00	1.18	1.30
TSAA, %	0.67	0.75	0.89	0.98
Thr, %	0.59	0.65	0.77	0.86

[1] Pro-Plus: animal by-product blend, with CP content of 60% (H. J. Baker & Bros., Inc.; Little Rock, AR).

[2] The vitamin and mineral premix contained per kg of diet: retinyl acetate, 2,654 µg; cholecalciferol, 110 µg; dl-α-tocopherol acetate, 9.9 mg; menadione, 0.9 mg; B_{12}, 0.01 mg; folic acid, 0.6 µg; choline, 379 mg; d-pantothenic acid, 8.8 mg; riboflavin, 5.0 mg; niacin , 33 mg; thiamin, 1.0 mg; d-biotin, 0.1 mg; pyridoxine, 0.9 mg; ethoxiquin, 28 mg; manganese, 55 mg; zinc, 50 mg; iron, 28 mg; copper, 4 mg; iodine, 0.5 mg; selenium, 0.1 mg.

[3] Provided 60 grams of salinomycin Na / 907.2 kg of diet to prevent intestinal coccidia from developing.

Table 2

Nutrient Requirements of Broilers as Percentage or Units per Kilogram (Kg) of Diet (90 Percent Dry Matter)

Nutrient	Unit	0 to 3 Weeks[a]; 3,200[b]	3 to 6 Weeks[a]; 3,200[b]	6 to 8 Weeks[a]; 3,200[b]
Protein and amino acids				
Crude protein	%	23	20	18
Arginine	%	1.25	1.1	1
Glycine + serine	%	1.25	1.14	0.97
Histidine	%	0.35	0.32	0.27
Isoleucine	%	0.8	0.73	0.62
Leucine	%	1.2	1.09	0.93
Lysine	%	1.1	1	0.85
Methionine	%	0.5	0.38	0.32
Methionine + Cys	%	0.9	0.72	0.6
Phenylalanine	%	0.72	0.65	0.56
Phe + Tyr	%	1.34	1.22	1.04
Proline	%	0.6	0.55	0.46
Threonine	%	0.8	0.74	0.68
Tryptophan	%	0.2	0.18	0.16
Valine	%	0.9	0.82	0.7
Fat				
Linoleic acid	%	1	1	1
Macrominerals				
Calciumd	%	1	0.9	0.8
Chlorine	%	0.2	0.15	0.12
Magnesium	mg	600	600	600
Nonphytate phos	%	0.45	0.35	0.3
Potassium	%	0.3	0.3	0.3
Sodium	%	0.2	0.15	0.12
Trace minerals				
Copper	mg	8	8	8
Iodine	mg	0.35	0.35	0.35
Iron	mg	80	80	80
Manganese	mg	60	60	60
Selenium	mg	0.15	0.15	0.15
Zinc	mg	40	40	40
Fat soluble vitamins				
A	IU	1,500	1,500	1,500
D_3	ICU	200	200	200
E	IU	10	10	10
K	mg	0.5	0.5	0.5
B_{12}	mg	0.01	0.01	0.007
Biotin	mg	0.15	0.15	0.12
Choline	mg	1,300	1,000	750
Folacin	mg	0.55	0.55	0.5
Niacin	mg	35	30	25
Pantothenic acid	mg	10	10	10
Pyridoxine	mg	3.5	3.5	3
Riboflavin	mg	3.6	3.6	3
Thiamin	mg	1.8	1.8	1.8

Source: National Research Council (1994)

Table 3

Feed Conversion Ratio, Body Weight, Carcass Yield, Carcass Weight, Breast Yield, Breast Weight, Thigh Yield and Thigh Weight of Broilers (n=10) Fed Diets Differing in Amino Acid Density (AA)

Treatment	Feed Conversion Ratio (kg/kg)	Body Weight (kg/Bird)	Carcass Yield (%)	Carcass Weight (kg)	Breast Yield (%)	Breast Weight (kg/Bird)	Thigh Yield (%)	Thigh Weight (kg/Bird)
Deficient AA	1.80[a]	2.55[a]	68.5	1.78[a]	19.51[a]	0.508[a]	12.07	0.315[a]
Low AA	1.75[b]	2.63[ab]	68.9	1.85[ab]	19.86[ab]	0.535[ab]	12.17	0.328[ab]
High AA	1.69[c]	2.70[bc]	68.3	1.89[b]	20.14[b]	0.561[b]	11.94	0.332[b]
Excessive AA	1.65[d]	2.73[c]	68.7	1.91[b]	20.23[b]	0.563[b]	12.25	0.34[b]
SEM	0.01	0.04	0.24	0.03	0.01	0.01	0.18	0.01
P-value	<0.01	<0.01	0.19	<0.05	<0.01	<0.01	0.72	<0.01

Means with the same letter within each column are not significantly different (P>0.05)

Table 4

Proximate Composition of Thigh and Breast Meat from Broilers Fed Diets Differing in Amino Acid Density (AA)

	Thigh			Breast		
Treatment	Fat (%)	Protein (%)	Moisture (%)	Fat (%)	Protein (%)	Moisture (%)
Deficient AA	7.6[a]	19.1[a]	71.5[a]	1.0	23.1	74.1
Low AA	6.4[b]	19.3[ab]	72.3[b]	0.9	23.4	73.9
High AA	6.4[b]	19.6[b]	72.4[b]	0.9	23.2	74.1
Excessive AA	6.0[b]	19.7[b]	72.6[b]	1.1	23.3	74.1
SEM	0.38	0.16	0.26	0.10	0.19	0.11
P-value	<0.05	<0.05	<0.05	0.30	0.76	0.67

Means with the same letter within each column are not significantly different (P>0.05)

Table 5

pH, Color, (24 hrs) (n=8 per treatment) Cooking Loss, and Shear Force Values of Breast from Broilers Fed Diets Differing in Amino Acid Density (AA)

Treatment	24 h pH	CIE L* (Lightness)	CIE a* (Redness)	CIE b* (Yellowness)	Cooking Loss (%)	Warner-Bratzler Shear Force (N)	Allo-Kramer Shear Force (N/g)	Allo-Kramer Total Energy (J/g)
Deficient AA	5.9	54.8	1.5	2.5	22.2	14.4	44.5	3.4
Low AA	5.9	54.3	1.5	2.1	22.3	15.5	43.9	3.6
High AA	5.9	55.1	1.6	2.1	22.2	14.9	43.7	3.5
Excessive AA	5.9	54.9	1.6	1.8	21.5	15.9	43.6	3.4
SEM	0.02	0.36	0.09	0.23	0.45	0.37	1.45	0.08
P-Value	0.37	0.41	0.42	0.14	0.57	0.24	0.99	0.67

Means with the same letter within each column are not different ($P<0.05$)
N = Maximum peak force (N) required to shear through the sample

Table 6

Consumer Acceptability of Breast Meat (n=184) from Broilers Fed Diets Differing in Amino Acid Density (AA) Using a 9-point Hedonic Scale

Treatment	Appearance	Aroma	Flavor	Texture	Overall Acceptability
Deficient AA	6.9	6.9	6.6[a]	6.7	6.6
Low AA	7.1	6.9	6.6[a]	6.7	6.7
High AA	6.8	6.8	6.5[ab]	6.7	6.6
Excessive AA	6.9	6.8	6.3[b]	6.5	6.5
SEM	0.30	0.26	0.34	0.39	0.32
P-value	0.08	0.29	<0.05	0.66	0.18

Means with the same letter within each column are not different (P>0.05)
Hedonic scale was based on a 9-point scale (1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely)
Texture acceptability refers to tenderness and was defined as how much the product is liked in respect to tenderness.

Table 7

Mean Hedonic Scores for Overall Consumer Acceptability of Broiler Breast Meat from Broilers Fed Diets Differing in Amino Acid Density (AA) According to Different Clusters of Consumer Segments

Treatment	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Deficient AA	7.7[ab]	7.4[a]	5.9[a]	4.9
Low AA	7.9[a]	6.6[b]	6.8[b]	4.9
High AA	7.7[ab]	6.2[b]	6.5[b]	5.3
Excessive AA	7.5[b]	7.3[a]	5.6[a]	4.9
n	72	37	32	43
Percentage Of Panelists	39.1	20.1	17.4	23.4

Means with the same letter within each column are not different (P>0.05)
Hedonic scale was based on a 9-point scale (1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely)
Texture acceptability refers to tenderness and was defined as how much the product is liked in respect to tenderness.

Table 8

Fatty Acid Profile of Chicken Thighs (n=24) from Broilers Fed Diets Differing in Amino Acid Density (AA).

Fatty Acid	Deficient AA	Low AA	High AA	Excessive AA	SEM	P-value
Myristic (C14:0)	0.67	0.62	0.63	0.65	0.03	0.73
Pentadecanoic (C15:0)	0.09	0.09	0.09	0.12	0.01	0.69
Palmitic (C16:0)	25.9	25.2	25.1	24.6	0.27	0.09
Heptadecanoic (C17:0)	0.14	0.16	0.12	0.11	0.02	0.62
Stearic (C18:0)	6.20	6.21	6.64	6.40	0.12	0.18
Myristoleic (C14:1)	0.26	0.26	0.19	0.22	0.02	0.24
Palmitoleic (C16:1)	7.85^{a}	7.39^{a}	6.62^{b}	7.26^{ab}	0.18	<0.01
Oleic (C18:1 *cis*)	39.7^{a}	39.8^{a}	38.7^{ab}	37.8^{b}	0.35	<0.05
Linoleic (C18:2 *cis*)	16.4^{a}	17.4^{ab}	18.8^{bc}	19.5^{c}	0.46	<0.01
Linolenic (C18:3ω6)	0.21	0.23	0.29	0.25	0.02	0.20
Linolenic (C18:3ω3)	0.67^{a}	0.72^{a}	0.82^{b}	0.84^{b}	0.02	<0.01
Eicosenoic (C20:1)	0.26	0.28	0.29	0.30	0.02	0.67
Eicosadiennoic (C20:2)	0.10	0.08	0.10	0.14	0.01	0.09
Eicosatrienoic (C20:3ω6)	0.30	0.30	0.34	0.34	0.02	0.61
Arachidonic (C20:4)	1.26	1.26	128	1.57	0.04	0.17
Saturated (%)	32.9	32.6	32.3	31.9	0.43	0.30
Monounsaturated (%)	48.0^{a}	47.7^{a}	45.7^{b}	45.5^{b}	0.44	<0.01
Polyunsaturated (%)	18.9	25.0	21.7	22.6	1.75	0.33

Means with the same letter within each row are not different (P>0.05)
Values are expressed as a percentage of the total fatty acid concentration

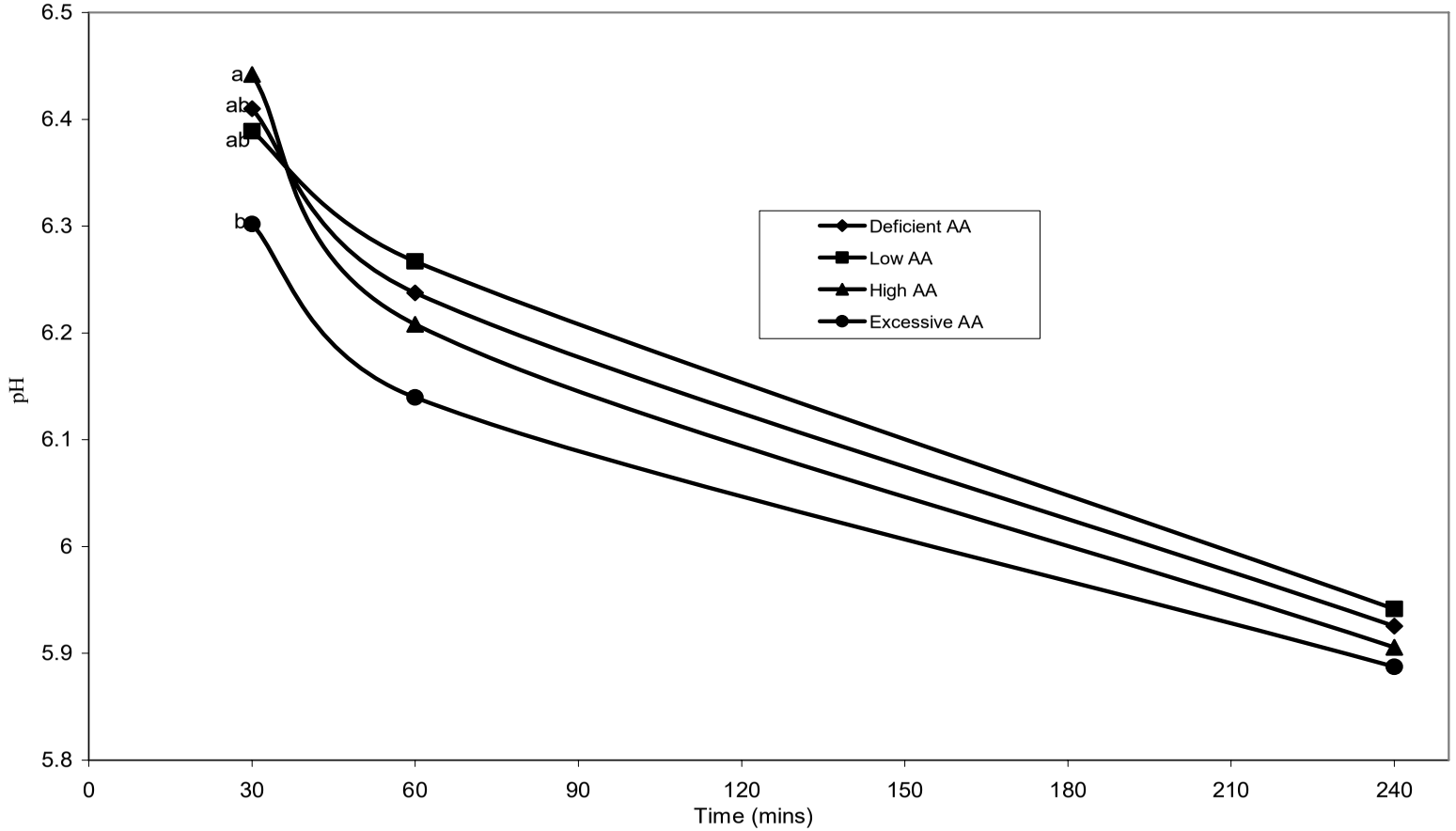

Figure 1

pH Decline of Broiler Chickens (n=10) Fed Diets Differing in Amino Acid Density (AA) at 30, 60, and 240 Minute Intervals.

For Each Treatment Time (Minutes), Means with Different Letters are Significantly Different ($p<0.05$).

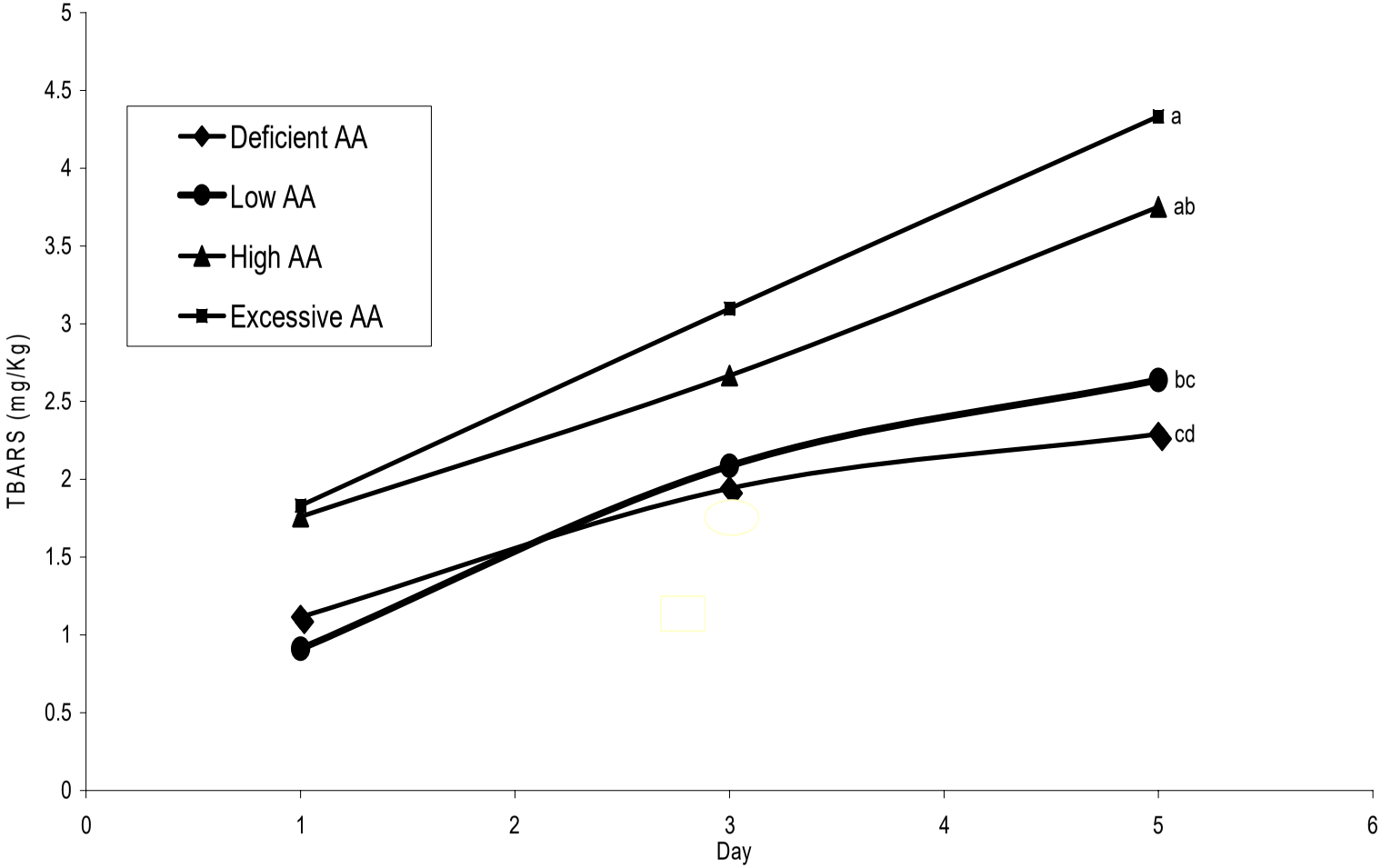

Figure 2

Thiobarbituric acid reactive substances (TBARS) values (mg/kg) of chicken thighs (n=36) from broilers fed diets

For Each Treatment (Day), Means with Different Letters are Significantly Different ($p<0.05$).

CHAPTER V

CONCLUSIONS

High and Excessive Amino Acid densities (AA) in the diet maximized breast yield and feed conversion efficiency. Minimal differences existed in breast meat quality among all AA density treatments. The use of Low and High AA diets caused a slight increase in sensory acceptability based on cluster analysis. Increasing AA density increased unsaturated fatty acid composition in the thigh portion of the broiler making it slightly more susceptible to oxidation. The high AA density diet resulted an increase in meat quality with only slight increases in oxidation. Additional research should be conducted to determine if varying amino acid densities in the diet affects fatty acid composition in breast meat and volatile flavor compound composition in breast and thigh meat In conclusion, the results from the present study suggest that feeding broilers differing levels of amino acid densities will have a minimal effect on the acceptability of broiler breast meat with all four amino acid diets receiving high consumer acceptability scores. However, a higher percentage of consumers liked breast meat from the Low and High AA treatments, indicating that there may be a slight advantage to feeding Low or High AA densities versus Deficient and Excessive AA diets with respect to sensory quality.

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APPENDIX A

IRB APPROVAL LETTER

MISSISSIPPI STATE UNIVERSITY

July 24, 2009

Reid Lilly
FSNHP
Mail Stop 9805

RE: IRB Study #09-148: Dietary Amino Acid Density Regimes and its Influence on Meat Characteristics and Consumer Acceptability of Broiler Chickens

Dear Mr. Lilly:

The above referenced project was reviewed and approved via administrative review on 7/24/2009 in accordance with 45 CFR 46.101(b)(6). Continuing review is not necessary for this project. However, any modification to the project must be reviewed and approved by the IRB prior to implementation. Any failure to adhere to the approved protocol could result in suspension or termination of your project. The IRB reserves the right, at anytime during the project period, to observe you and the additional researchers on this project.

Please note that the MSU IRB is in the process of seeking accreditation for our human subjects protection program. As a result of these efforts, you will likely notice many changes in the IRB's policies and procedures in the coming months. These changes will be posted online at http://www.orc.msstate.edu/human/aahrpp.php. The first of these changes is the implementation of an approval stamp for consent forms. The approval stamp will assist in ensuring the IRB approved version of the consent form is used in the actual conduct of research. You must use copies of the stamped consent form for obtaining consent from participants.

Please refer to your IRB number (#09-148) when contacting our office regarding this application.

Thank you for your cooperation and good luck to you in conducting this research project. If you have questions or concerns, please contact me at jmiller@research.msstate.edu or call 662-325-2238.

Sincerely,

Jonathan Miller, CIP
IRB Officer and Assistant Director

cc: Wes Schilling
SPA (325030)

APPENDIX B

CONSENT FORM

Informed Consent Form – Chicken Breast
(You must be over 18 in order to participate)

Title of Study: DIETARY AMINO ACID DENSITY REGIMES AND ITS INFLUENCE ON MEAT CHARACTERISTICS AND CONSUMER ACCEPTABITLITY OF BROILER CHICKENS

Study Site: Department of Food Science, Nutrition and Health Promotion, Garrison Sensory Evaluation Laboratory

Name of Researcher(s) & University affiliation: Reid A. Lilly (Graduate Student), Dr. M. Wes Schilling (Faculty Advisor, Associate Professor), Viodelda Jackson (Research Associate II) and Vijayakumar Radhakrishnan (Graduate Student)

What is the purpose of this research project? To determine the effects of feeding broilers diets that differ in levels of crude protein and amino acid densities on the sensory (consumer) acceptability of chicken breast meat.

How will the research be conducted? You will be provided with chicken breast samples to taste. You will then be asked to record your responses on the provided score sheets. Samples consumed are considered safe under USDA requirements.

Are there any risks or discomforts to me because of my participation? There are no anticipated risks or discomforts. You may discontinue your participation at any point.

Does participation in this research provide any benefits to others or myself? This research will provide information that will help optimize poultry production and quality of broiler breast meat.

Will this information be kept confidential? Yes. Personal information, including your name will not be released. Only the data obtained from the testing will be provided to the contracting company.

Who do I contact with research questions? If you should have any questions about this research project, please feel free to contact Dr. M. Wes Schilling at 662-325-2666. For additional information regarding your rights as a research subject, please feel free to contact the MSU Regulatory Compliance Office at 662-325-5220.

What do I do if I am injured at a result of this research?
In addition to reporting an injury to Dr. M. Wes Schilling (662-325-2666) and to the Regulatory Compliance Office (662-325-5220), you may be able to obtain limited compensation from the State of Mississippi if the injury was caused by the negligent act of a state employee where the damage is a result of an act for which payment may be

made under §11-46-1, et seq. Mississippi Code Annotated 1972. To obtain a claim form, contact the University Police Department at *MSU UNIVERSITY POLICE DEPARTMENT, Stone Building, Mississippi State, MS 39762, (662) 325-2121.*

What if I do not want to participate?
Please understand that your **participation is voluntary**, your **refusal to participate will involve no penalty or loss** of benefits to which you are otherwise entitled, and you **may discontinue your participation** at any time without penalty or loss of benefits. Additionally, you may skip any portion of the taste evaluation process.

If you agree to participate in this research study, please sign the signature sheet provided and keep this form for your records.

Chicken Breast Consumer Acceptance

SIGNATURE CONSENT FORM

DATE _____

	Print	**Signature**
1.	_____	_____
2.	_____	_____
3.	_____	_____
4.	_____	_____
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34.	_____	_____
35.	_____	_____

APPENDIX C

SCORE SHEET

CONSUMER ACCEPTANCE TEST

Samples: Chicken Breast
Date:_____

You have been provided with a tray containing four (4) coded samples. Please follow the instructions as indicated:

1. **Taste each sample starting with the number on the left and continuing to the right.**
2. **Rate each sample in each of the five categories listed and place a check mark to indicate your choice.**
3. **Expectorate the sample in the cup provided and rinse with the water provided.**
4. **Each column will need a check mark if you choose to evaluate all samples.**
5. **Thank you for your participation.**

478	237	159	946	APPEARANCE
				Like extremely
				Like very much
				Like moderately
				Like slightly
				Neither like nor dislike
				Dislike slightly
				Dislike moderately
				Dislike very much
				Dislike extremely

478	237	159	946	AROMA
				Like extremely
				Like very much
				Like moderately
				Like slightly
				Neither like nor dislike
				Dislike slightly
				Dislike moderately
				Dislike very much
				Dislike extremely

478	237	159	946	TEXTURE
				Like extremely
				Like very much
				Like moderately
				Like slightly
				Neither like nor dislike
				Dislike slightly
				Dislike moderately
				Dislike very much
				Dislike extremely

478	237	159	946	OVERALL FLAVOR
				Like extremely
				Like very much
				Like moderately
				Like slightly
				Neither like nor dislike
				Dislike slightly
				Dislike moderately
				Dislike very much
				Dislike extremely

478	237	159	946	OVERALL ACCEPTABILITY
				Like extremely
				Like very much
				Like moderately
				Like slightly
				Neither like nor dislike
				Dislike slightly
				Dislike moderately
				Dislike very much
				Dislike extremely