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Effect of choline or betaine supplementation on broilers exposed to different temperature treatments

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To the Graduate Council:

I am submitting herewith a thesis written by Joseph D. Summers entitled "Effect of choline or betaine supplementation on broilers exposed to different temperature treatments." 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.

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Dedication

This thesis is dedicated to all my friends

who have enriched my life in so many ways.

Palmer, Charles, and Dominique

you have taught me to never take life for granted.

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Abstract

In this study, we looked at the effects of supplemental choline or betaine on broiler performance under different temperature conditions. In total there were eighty pens containing ten birds each for a total of 800 Cobb MX™ X Cobb 500™ (Cobb-Vantress, Incorporated, Siloam Springs, AR, USA). Each pen was randomly assigned to one of five dietary treatments in this study: Treatment 1, basal diet, Treatment 2, basal diet plus 500 methyl equivalents added choline, Treatment 3, basal diet plus 1000 methyl equivalents added choline, Treatment 4, basal diet plus 500 methyl equivalents added betaine, and Treatment 5 basal diet plus 1000 methyl equivalents added betaine. The pens were divided equally into two rooms. One room was assigned a treatment classified as thermoneutral (TN) while the second room was designated as high temperature (HT). Dietary treatments did not significantly impact performance ($p>0.05$), but temperature treatments did have a negative effect on feed intake and feed to gain conversion during days 21 through 42 ($p < 0.05$). The lack of effect found during this study may have been due to variance in the anticipated feed composition and the actual feed composition. The feed fed during this study contained more methionine, an amino acid known to minimize supplemental choline effects, than what was anticipated. This study showed that high temperatures have a negative impact on performance, and since our dietary treatments were high in methionine, it is still not known whether choline or betaine supplementation, above recommended amounts, to a broiler ration is beneficial or not.

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CHAPTER ONE

Literature Review

Introduction

The poultry industry produces two main products: eggs and broilers (meat birds). Although broilers are among the most efficient converters of feedstuffs to protein, there is still much room for improvement. As the poultry industry has modernized, new challenges have arisen. Production of broiler meat in the United States in 2012 was 37.039 billion pounds while per capita consumption was 80.3 pounds (USDA, 2012a). Both these figures are higher than the next competing meat product and reflect the trend since 1995. It has been estimated that world food consumption will double by 2050 and as more developing nations improve their economic status, per capita meat consumption will also increase (Godfray et al., 2010). If the broiler industry intends to keep up with this increasing demand, many challenges to efficiency, quality, and welfare must be faced.

The broiler industry has been able to achieve great efficiency through improved genetics, improved nutrition, and the advent of the confined animal feeding operation. Due to the confinement and rapid growth of today's broilers, heat stress is a major problem. Another factor, which contributes to the problem, is that the majority of the United States broiler operations are located in the southeast. Heat stress not only causes decreased production performance it also poses welfare issues, such as increased mortality, discomfort, and possibly foot pad dermatitis. Some problems associated with heat stress in broilers are: respiratory alkalosis (Teeter et al., 1985), decreased performance (Cooper and Washburn, 1998), decreased breast yield (Akşit et al., 2006) and slower growth rate (Howlider and Rose, 1989; Akşit et al., 2006). It was estimated in 2003 that heat stress cost the US broiler industry 120 million dollars annually (St-Pierre et al., 2003). Many studies have been conducted using different feed additives to abate heat stress symptoms. Heat stress is a definite problem that continues to present a challenge to broiler producers.

Another factor that prevents the industry from maximizing efficiency is the number of post-mortem condemnations. Average condemnation in a given month is about 1.07% by weight (USDA Economic Research Services, 2012). Condemnation can be the result of a number of factors, including contamination during the evisceration process. Contaminated carcasses are prime targets for microbial activity, which could result in food-borne illnesses. The integrity of the epithelial tissue that makes up the lining of the gastrointestinal tract is paramount in the ability of the gastrointestinal tract to withstand the evisceration process without breakage, thus lessening the possibility of carcass contamination.

Choline plays an essential role in several biological processes within the body. This includes being an essential nutrient for building and maintaining cell structure, primarily as a structural component of phospholipids. Choline has also been shown to have an enormous role in lipid metabolism in the liver thereby preventing abnormal accumulation of fat in that organ. Acetylcholine, the substance responsible for the transmission of nerve impulses, could not be formed without the presence of choline (Zeisel, 2012). In addition, very important to the poultry species, choline provides the labile methyl groups necessary for the formation of methionine from homocysteine, by being oxidized to betaine (Zhang et al., 2013). These facts point to the importance of this molecule in poultry production.

With broiler genetics and modern management practices constantly changing, it is important for researchers to reinvestigate the use of choline in poultry diets. In the past, many studies have looked at choline and betaine for their methionine sparing effects under ideal conditions (Kettunen et al., 2001; Pillai et al., 2006a; Pillai et al., 2006b; Waldroup et al., 2006; Rafeeq et al., 2011a; Rafeeq et al., 2011b), but it may also be important to examine choline for its primary function of supporting growth and not just as a methionine-sparing molecule. Choline may also have added benefits during heat stress, since bird physiology is altered in this condition.

Betaine, a methyl group donor, also functions in lipid metabolism by stimulating oxidative catabolism of fatty acids through carnitine synthesis. Thus, both choline and betaine may have an effect on carcass adiposity. Betaine is also an organic osmolyte and does not interfere with enzyme function or upset metabolism (Simon, 1999). The high permeability of cell membranes allows water to move across plasma membranes by the osmotic gradient. As an osmolyte, betaine may have a stabilizing function on cells subjected to osmotic stressors, such as coccidiosis (Klasing et al., 2002). Dehydration of cells occurs during infections and use of betaine may have the potential to improve this condition.

The world's population is constantly increasing, as well as the number of developed nations, meaning that the amount of animal protein produced will need to increase in order to meet demand in the future. This means that all production systems, even broiler production as efficient as it is, will need to improve efficiency. Improved efficiency will not be allowed to come at the expense of animal welfare, so improvements will have to be made carefully. Choline and its metabolite betaine may be able to improve broiler production efficiency as well as aid in improving animal welfare.

Broiler production

Broilers are chickens that are raised solely for meat, typically a four to six week process. The broiler industry has made vast improvements in the last seventy years, going from a mostly backyard industry to a vertically integrated industry. Genetic and nutritional improvements have greatly increased rate of gain, improved feed efficiency, and increased breast yield, as well as many other production traits. The industry has been able to make many of these improvements due to the vertically integrated nature of the industry, as well as the chicken's short generation intervals. These advancements have made chicken readily available at a lower cost than most other meats. All of this has led to chicken becoming the number one meat in the United States in 1995, and its popularity has increased ever since (USDA, 2012a). With the world's food needs ever increasing, as well as growth in developing countries, chicken, with its high efficiency and low space requirements, looks to be a key player in feeding the world in the future.

In 1925, it took 112 days and 11.75 pounds of feed to raise a two and a half pound broiler, with a mortality rate of 18 percent. By 1950 it took 70 days and 9.24 pounds of feed to raise a three pound broiler, with a mortality rate of eight percent, and today only it takes 47 days and 11.078 pounds of feed to raise a 5.8 pound broiler, with a 3.8 percent mortality rate (National Chicken Council, 2011). The modern broiler farm is much different than it was in the 1920's. Most chickens, in the United States in the early twentieth century, were contained in back yard flocks and were raised for eggs as well as meat. These birds also represented many different breeds. Today's broilers are raised in large houses, where a computer controls all feeding, water, and environmental factors. These houses hold thousands of birds with stocking densities of less than one square foot per bird. Modern broilers are bred by genetics companies as lines and are mostly some combination of Cornish X Rock crosses. These lines are sold as grandparent stock, and then their offspring are raised for harvest. Ownership of the industry has also changed from small family owned operations to vertically integrated corporate enterprises. Today's broiler farm is most likely locally owned, but the contract growers that raise the broilers never actually own the birds. The same company that bred, hatched, and will harvest as well as distribute them owns the birds. This vertical integration has given the boiler industry an advantage in efficiency over other meat producing industries.

Broiler production's many improvements would not have been possible without major improvements in nutrition. Today's broilers eat a total mixed ration, which is uniform in size, providing everything the birds need in each bite. Most rations are corn and soybean based with added vitamins, minerals, crystalline amino acids, and other feed additives that have been shown to improve health and performance. It has been shown that certain feed additives can benefit performance in heat stressed broilers (Sands and Smith, 1999; Smith et al., 2003; Attia et al., 2009; Willemsen et al., 2011; Ghazi Harsini et al., 2012; Hamano, 2012; Imik et al., 2012; Khan et al., 2012; Sandhu et al., 2012; Sohail et al., 2012), and improvements in broiler nutrition may help minimize the detriments caused by heat stress in the future.

Heat Stress

Heat stress is a condition that occurs when an animal is exposed to above optimal temperatures and humidity. Heat stress generally changes a bird's physiology and places stress on many systems thereby causing decreased performance, possible welfare concerns, and even increased mortality. This situation is a major challenge facing modern broiler production, in part because most broiler production in the United States is located in the southeastern region (USDA, 2012b). This region has a hot and humid climate, which during certain times of the year can wreak havoc on production. A decade ago it was estimated that heat stress costs the broiler industry 128 million dollars annually (St-Pierre et al., 2003). Although the normal broiler body temperature is 41 C, optimum growing temperature is 21 to 24 C, and heat stress can be elicited by conditions such as high temperature and high humidity depending on age (Teeter and Belay, 1996). The detrimental effects of heat stress have been shown to be alleviated by dietary means (Teeter et al., 1985; Smith and Teeter, 1993; Sands and Smith, 1999; Khan et al., 2012; Sandhu et al., 2012; Sohail et al., 2012), prior exposure to heat treatment (Arjona et al., 1988; De Basilio et al., 2001), chilled drinking water (Smith and Teeter, 1987a), fasting (Ait-Boulahsen et al., 1989), and house cooling measures (Teeter and Belay, 1996). Thus, it can be seen that heat stress is a costly problem for the broiler industry; however, nutritional improvements may be able to lessen its effects.

Chickens have cooling mechanisms that allow them to survive at above optimal temperatures. The two main methods that chickens use are evaporative and non-evaporative cooling. Non-evaporative cooling occurs via convection and is enhanced by air movement in the house, while evaporative cooling occurs through the evaporation of water from the lungs. This method of heat dissipation is enhanced by low humidity and increased respiratory rates. It has been shown that non-evaporative cooling is more efficient than evaporative cooling (Wiernusz and Teeter, 1993; Teeter and Belay, 1996) and is therefore the first line of defense, but as the ambient temperature rises to near body temperature, this method becomes ineffective (Teeter and Belay, 1996) and evaporative cooling becomes necessary. As the ambient temperature gets closer to broiler body temperature, respiration rate will increase (Linsley and Burger, 1964) thereby initiating a higher rate of evaporative cooling (Wiernusz and Teeter, 1993; Teeter and Belay, 1996). High humidity will also compound the problem, since evaporative cooling rate is inversely related to humidity (Teeter and Belay, 1996). It is understood that chickens have means to cool themselves, but due to its ineffectiveness under certain climatic conditions, proper management is paramount.

When temperatures exceed the effective range for non-evaporative cooling, the way that chickens cool themselves causes certain physiological changes that further complicate the issue. Evaporative cooling is accomplished by increasing the respiratory rate (Linsley and Burger, 1964; Teeter and Belay, 1996; Toyomizu et al., 2005; Renaudeau et al., 2011), also known as panting, and this can lead to respiratory alkalosis (Linsley and Burger, 1964; Teeter et al., 1985; Ait-Boulahsen et al., 1989; Sandercock et al., 2001; Toyomizu et al., 2005; Steiss and Wright, 2008; Imik et al., 2013). Acid-base imbalance has been shown to cause electrolyte imbalance (Teeter et al., 1985; Belay et al., 1992; Borges et al., 2004) and increased electrolyte excretion (Smith and Teeter, 1987a; Belay et al., 1992). Acid-base imbalance and the resulting electrolyte imbalance, caused by heat-induced panting, may play a big part in the decreased performance (Dale and Fuller, 1980; Teeter et al., 1985; Howlider and Rose, 1989; Smith, 1993a; Teeter and Belay, 1996; Sands and Smith, 1999; Bartlett and Smith, 2003; Smith et al., 2003; Ahmad and Sarwar, 2006; Quinteiro-Filho et al., 2010; Willemsen et al., 2011; Ghazi Harsini et al., 2012; Quinteiro-Filho et al., 2012; Sohail et al., 2012), altered meat quality (Smith, 1993b; Akşit et al., 2006; Imik et al., 2012; Zhang et al., 2012), and increased mortality (Arjona et al., 1988) seen in heat stressed broilers.

Acid-base balance and electrolyte balance play key roles in broiler health and performance, and both can be drastically altered when chickens are heat stressed. Electrolytes may be supplemented in feed and water, and many studies have shown that the provision of additional electrolytes can increase performance (Teeter and Smith, 1986; Smith and Teeter, 1987a; Smith and Teeter, 1992; Smith and Teeter, 1993; Smith, 1994; Teeter and Belay, 1996; Ahmad et al., 2005), decrease mortality (Smith and Teeter, 1987b), and improve acid-base balance in heat stressed broilers (Teeter et al., 1985). While electrolytes have been shown to be of benefit to heat stressed broilers, increased electrolytes in broiler diets can cause unwanted side effects. It has been shown that excess electrolytes can increase excreta moisture, which then will increase litter wetness (Smith et al., 2000; Francesch and Brufau, 2004; Eichner et al., 2007; Abd El-Wahab et al., 2011; Youssef et al., 2011), a known cause of foot pad dermatitis in turkeys (Youssef et al., 2010a; Abd El-Wahab et al., 2011). Foot pad dermatitis is a welfare concern (Berg, 1998; Abd El-Wahab et al., 2011) as well as a production issue, since feet are a major poultry export product from some countries such as the United States of America. Many studies have shown benefits from the inclusion of non-electrolyte feed additives in broiler diets (Sands and Smith, 1999; Smith et al., 2003; Attia et al., 2009; Willemsen et al., 2011; Ghazi Harsini et al., 2012; Hamano, 2012; Imik et al., 2012; Khan et al., 2012; Sandhu et al., 2012; Sohail et al., 2012), one such non-electrolyte feed additive is betaine, which is a non-ionic organic osmolyte (Thorne Research Inc., 2003; Craig, 2004). This molecule has also been shown to improve the performance of heat stressed broilers (Enting et al., 2007; Attia et al., 2009; Sayed and Downing, 2011), but unlike electrolytes it has not been shown to increase litter wetness. Dietary additives, such as electrolytes, vitamins, certain fatty acids, and betaine, have been shown to help alleviate some of the symptoms of heat stress in broilers, but some of them have the unwanted side effect of increased litter wetness.

Heat stress is an expensive problem that the US broiler industry will always have to deal with, due to the fact that the primary geographic location is in the southeastern region of the United States (USDA, 2012b), a region known for its hot and humid climate. Heat stress causes physiological changes in broilers such as: acid-base imbalance (Linsley and Burger, 1964; Teeter et al., 1985; Ait-Boulahsen et al., 1989; Sandercock et al., 2001; Toyomizu et al., 2005; Steiss and Wright, 2008; Imik et al., 2013), electrolyte inbalance (Teeter et al., 1985; Belay et al., 1992; Borges et al., 2004), and increased respiration (Linsley and Burger, 1964; Teeter and Belay, 1996; Toyomizu et al., 2005; Renaudeau et al., 2011), which lead to decreased performance (Dale and Fuller, 1980; Teeter et al., 1985; Howlider and Rose, 1989; Smith, 1993a; Teeter and Belay, 1996; Sands and Smith, 1999; Bartlett and Smith, 2003; Smith et al., 2003; Ahmad and Sarwar, 2006; Quinteiro-Filho et al., 2010; Willemsen et al., 2011; Ghazi Harsini et al., 2012; Quinteiro-Filho et al., 2012; Sohail et al., 2012). Many feed additives have been tested and demonstrated positive effects on heat stressed broilers. These additives include electrolytes (Teeter and Smith, 1986; Smith and Teeter, 1987a; Smith and Teeter, 1992; Smith and Teeter, 1993; Smith, 1994; Teeter and Belay, 1996; Ahmad et al., 2005) and vitamins (Attia et al., 2009; Hamano, 2012; Khan et al., 2012; Imik et al., 2013), as well as betaine (Enting et al., 2007; Attia et al., 2009; Sayed and Downing, 2011). Since betaine has many physiological functions, and data from past research can be interpreted as inconclusive and conflicting, further research with betaine under heat stress conditions could be beneficial, especially if different levels of choline and methionine are tested as well. Heat stress is a costly problem for the US broiler industry (St-Pierre et al., 2003), and although it has been studied extensively, it is still not fully understood.

Footpad Dermatitis and Litter Quality

Footpad dermatitis is a condition that is characterized by hard scaly skin with increased keratin and can lead to lesions and necrosis (Mayne, 2005). Footpad dermatitis (FPD) is a contact dermatitis that is identified by lesions on the footpad (Berg, 1998), and it has been linked, in turkey poults and broilers, to dietary factors (Abbott et al., 1969; Jensen et al., 1970;

Chavez and Kratzer, 1974; Harms and Simpson, 1977; Eichner et al., 2007; Abd El-Wahab et al., 2011; Youssef et al., 2011), heating type (Abd El-Wahab et al., 2011) genetics (Kjaer et al., 2006), season (Berg, 1998), and litter quality (Harms and Simpson, 1977; Ekstrand et al., 1997; Mayne, 2005; Eichner et al., 2007; Mayne et al., 2007; Bilgili et al., 2009; Youssef et al., 2010a; Youssef et al., 2011). Footpad dermatitis presents welfare challenges as well as performance issues (Berg, 1998). Footpad dermatitis is evaluated by the amount of the pad involved in the lesion, and there are multiple scoring systems. Footpad dermatitis has become even more of an issue recently, with the advent of an export market to Asia. Since unhealthy chicken feet can pose a welfare concern, impair performance, as well as devalue the carcass, broiler producers are starting to take notice of footpad dermatitis.

Footpad dermatitis is a condition that is characterized by hard scaly skin with increased keratin and can lead to lesions and necrosis (Mayne, 2005). Most of the FPD research has been done with turkey poults, but the condition has been described as similar in the two species (Harms et al., 1977; Bruce et al., 1990; Ekstrand et al., 1997; Ekstrand et al., 1998; Mayne, 2005). Pass (1989) described it as an "irritant dermatitis" and described it as being found on the breast, plantar surface of the feet, as well as the hock, and correlated its incidence with the amount of time the skin was in contact with the litter. Hester (1994) suggested that FPD could have a negative impact on the gait of a broiler chicken.

Proper broiler house management goes a long way in preventing footpad dermatitis. It has been shown that litter quality and moisture content are the main causes of FPD in broilers and turkeys (Harms et al., 1977; Harms and Simpson, 1977; Martland, 1984; Martland, 1985; Eichner et al., 2007; Mayne et al., 2007; Youssef et al., 2010a; Youssef et al., 2010b; Youssef et al., 2011). Increased litter wetness has been shown to be increased by diet (Borges et al., 2003;

Ahmad and Sarwar, 2006; Abd El-Wahab et al., 2011). Therefore, dietary considerations are important when addressing the issue of footpad dermatitis. In addition, poor ventilation has been shown to increase litter wetness (Carr and Nicholson, 1980; Xin et al., 1996) but it can be decreased by certain heating systems (Abd El-Wahab et al., 2011; Abreu et al., 2012). Since FPD is a welfare and production issue, it would be in producer's best interest to make the management changes necessary to decrease the incidence of this condition.

It has been indicated that diet may play a role in FPD prevention. Litter wetness has been shown to increase when birds are given excess electrolytes (Borges et al., 2003; Ahmad et al., 2005) and increased litter wetness has been linked to FPD occurrence (Harms et al., 1977; Greene et al., 1985; Martland, 1985; McIlroy et al., 1987; Berg, 1998; Ekstrand et al., 1998; Martrenchar et al., 2002; Mayne, 2005; Eichner et al., 2007; Bilgili et al., 2009; Spindler et al., 2009). On the positive side, biotin supplementation has been shown to decrease FPD in broilers (Harms et al., 1977; Harms and Simpson, 1977), but it has been demonstrated that high levels of soy bean meal cause an increase in FPD in turkeys (Abbott et al., 1969; Jensen et al., 1970). Litter wetness is considered the main cause of FPD, and any diet that increases excreta moisture content is liable to cause an increase in footpad dermatitis.

Even though excess electrolytes are a likely cause of FPD, they have also been shown to be beneficial, especially in the diets of heat stressed broiler diets (Teeter and Smith, 1986; Smith and Teeter, 1987a; Smith and Teeter, 1992; Smith and Teeter, 1993; Smith, 1994; Teeter and Belay, 1996; Ahmad et al., 2005). So naturally, producers will add electrolytes to diets, especially in the summer. Electrolytes help maintain osmotic balance in cells, but they are not the only compounds that perform this task. There are other osmolytes, that are non-ionic, and one of them is betaine. Betaine has been shown to improve performance in heat stressed broilers, but it has not been linked to increased litter wetness (Enting et al., 2007; Attia et al., 2009; Sayed and Downing, 2011). It is possible that betaine could lower the amount of excess electrolytes needed in a broiler diet, thereby maintaining performance without increasing litter wetness.

Choline

Choline (2-hydroxy-N,N,N-trimethylethanaminium) is a water soluble, essential, quaternary amine (Blusztajn, 1998) which is biologically important for several reasons. This molecule is a critical part of acetyl choline as well as phosphatidylcholine, one of the most common phospholypids used in membrane formation (Zeisel, 2012). Choline is also utilized as a methyl donor to turn homocysteine to methionine (Zhang et al., 2013). While choline has been known to be an essential nutrient for quite some time, recently, multiple studies have investigated additional supplementation or partial replacement of methionine with choline in poultry diets.

Choline has been included in poultry diets as an essential nutrient for many years, but its value as a supplement has not been fully explored. Many studies, using poultry as a model, have examined choline effect on growth, feed efficiency, performance during coccidiosis, and other production qualities. Many of these studies have looked at choline as a methionine-sparing nutrient (Keshavarz and Austic, 1985; Waldroup et al., 2006). However, while choline is essential for its aforementioned roles, it is also beneficial because it can easily be oxidized to betaine, which functions as a methyl donor and an osmolyte. Some benefits attributed to choline are increased energy and protein utilization efficiency as well as decreased liver fat content in broiler breeders (Rao et al., 2001) and increased egg size in layers, fed methionine deficient diets

(Keshavarz and Austic, 1985). In addition, broilers have shown increase in growth performance (Dilger et al., 2007), decreased incidence of valgus and varus deformities and decreased folic acid needs (Ryu et al., 1995), improved breast yield and a positive effect on feed conversion (Waldroup et al., 2006), increased breast yield at day 42 (Waldroup and Fritts, 2005), and increased hepatic homocysteine remethylation (Pillai et al., 2006a). Not only have benefits been shown in poultry but humans as well, where an inverse relationship has been shown between choline consumption and breast cancer rates (Zhang et al., 2013). Multiple studies have shown choline to have benefits in many species, including broilers; however, choline metabolism is still not fully understood in the chicken, particularly under heat stress.

Choline is a charged hydrophilic cation and cannot pass into the cell by simple diffusion, which means that transport must be facilitated by some type of transporter. There are two main systems for choline transport (Lockman and Allen, 2002). The most common is a sodium independent low affinity transporter that primarily transports choline for phospholipid biosynthesis (Meyer Jr et al., 1982). This transporter is typically found on the cellular membranes of most cells. The other, less common transporter, is a sodium dependent high affinity transporter that is located on pre-synaptic cholinergic nerve terminals and is possibly used for acetylcholine synthesis (Barker and Mittag, 1975). Choline transport is similar to other molecule transport system in that it is inhibited by its structural analog hemicholinium-3 (Grassl, 1994). Choline transport is thought to be the rate-limiting step in acetylcholine synthesis (Simon et al., 1976). Due to the varied nature of the data on additional choline benefits in broiler rations, there is still much room for research in this area.

Choline has multiple functions in the body and therefore is metabolized in many ways. Choline’s main uses are for acetylcholine and phospholipid biosynthesis, but it is also oxidized to betaine and used in the synthesis of methionine. The feedback mechanisms that determine which pathway a choline molecule will take once it enters the body are still somewhat of a mystery. As stated previously, choline transport may play a role in this process, but there are still multiple pathways for metabolism once it enters a cell. Choline is acetylated to acetylcholine, phosphorylated to phosphorylcholine, and oxidized to betaine. It was once thought to be a direct methyl donor for methionine manufacture (Flower et al., 1972), however it has since been shown that choline has only a very minimal sparing effect of methionine (Baker et al., 1983). Increased methionine has been shown to lower a broiler's need for choline slightly (Baker et al., 1983). This indicates that despite methionine and choline having a slight interconnectedness, they are not fully interchangeable. This soft system of positive and negative feedback makes understanding choline metabolism difficult.

Choline's complexity has led to many poultry studies with conflicting results about the molecule and its benefits. In one study, Anderson and Dobson (1982) additional choline did not improve performance in broilers, but a second study, Dilger et al. (2007) showed increased growth performance in this type of poultry. Choline was shown by Waldroup et al. (2006) to not have a methionine sparing effect, but Pillai et al. (2006b) did show a methionine sparing effect while Keshavarz and Austic (1985) also showed a minor methionine sparing effect in layers. The conflicting views on supplemental choline benefits could be attributed to different supplementation levels or a lack of power in some cases (Simon, 1999). Many of choline's added benefits, in heat stressed broilers, have been thought to come from its ability to be readily oxidized to betaine, which is then used as a methyl donor to synthesize methionine, but since data promoting choline's methionine sparing effects are somewhat conflicting, choline, above essential levels, may not be as beneficial as once thought.

Even though choline is considered essential in poultry diets the degree of benefit exhibited by additional choline, particularly in heat stressed broilers, is not fully understood. Heat stress offers a way to evaluate choline benefits via its oxidation to betaine. Heat stress is a common problem in the broiler industry and it changes the physiology of the animal in question. Heat stress has been shown to up regulate betaine manufacture in the body (Craig, 2004), and betaine is a known osmolyte that is used by the body to maintain cell volume (Yancey et al., 1982; Craig, 2004; Lever and Slow, 2010). During heat stress, chickens cool themselves by panting (Linsley and Burger, 1964), and this type of evaporative cooling, while effective, leads to respiratory alkalosis (Teeter et al., 1985; Sandercock et al., 2001). It has been shown that providing additional electrolytes, which also aid in osmotic balance, can alleviate some of the adverse effects of heat stress (Teeter and Belay, 1996). Since heat stress leads to an osmotic imbalance and betaine is a known osmolyte, then a heat stress study, in which choline is fed, could shed some light on how readily it is oxidized to betaine. In order to fully understand the osmotic effect, performance data, blood parameters, and litter moisture would need to be assessed.

Betaine

Betaine, also known as trimethylglycine, is a zwitterionic quaternary ammonium compound. Betaine is found in many foods (Zeisel et al., 2003), and since it can be manufactured in the mitochondria, it is not considered essential (Craig, 2004). One of betaine's physiological functions is as an organic osmolyte (Craig, 2004). Osmolytes are osmotically active solutes that are able to help maintain cell volume (Yancey et al., 1982). Betaine is a non-ionic osmolyte, which allows it to accumulate in cells without disrupting normal function (Yancey et al., 1982). Osmolytes are especially important in broilers under heat stress. It has been shown that heat stress causes panting (Linsley and Burger, 1964) in broilers and this panting leads to respiratory alkalosis (Teeter et al., 1985; Sandercock et al., 2001). This causes imbalance in blood pH which then leads to excess K+ and Na+ excretion (Belay et al., 1992; Teeter and Belay, 1996; Sayed and Downing, 2011). Potassium and sodium are also osmolytes, and they have been shown to be beneficial when supplemented at high levels (Teeter and Smith, 1986), but because they are ionic there is a limit to how much can safely accumulate in the cell (Yancey et al., 1982). The osmotic effect of betaine is also used in other species (Yancey et al., 1982) to maintain cell volume.

Betaine's other function is as a methyl donor for the manufacture of methionine from homocysteine. Methionine is converted to S-adenosylmethionine (SAMe) which is used as a methyl donor for most methylation reactions in the body (Thorne Research Inc., 2003; Craig, 2004). When SAMe gives up its methyl group to a methylation reaction it then becomes homocysteine. Homocysteine can either be used for amino acid synthesis or be re-methylated to methionine (Thorne Research Inc., 2003; Craig, 2004), effectively recycling methionine, which is a limiting sulfur containing amino acid. Methionine re-methylation by betaine is a reaction catalyzed by betaine-homocysteine methyltransferase (BHMT) (Craig, 2004), which is osmoregulated (Schäfer et al., 2007), thereby linking betaine's roles as an osmolyte and a methyl donor. Methyl group donation is one of betaine's most important roles, as methyl groups are used throughout the body for various biosynthesis reactions.

Betaine, with its dual function, has been tested as a feed or water additive in many broiler studies. Betaine has been evaluated for its methionine sparing, choline sparing, performance enhancing, acid-base balancing, osmolytic, and many other effects. The results show that betaine has many benefits to the broiler industry including: improved carcass yield (Esteve-

Garcia and Mack, 2000; Neoh and Ng, 2012), increased carcass yield and breast percentage as well as decreased abdominal fat (Jahanian and Rahmani, 2008), and decreased mortality (Lukic et al., 2012). Other studies have indicated that betaine can cause improved growth, feed conversion efficiency and breast yield when supplemented in a methionine deficient diet (Rao et al., 2011), improved feed conversion as well as breast yield regardless of methionine levels (Waldroup et al., 2006), improved performance under heat stress (Sayed and Downing, 2011), and improved dressing percentage in 42 day old male broilers (Waldroup and Fritts, 2005). Some other benefits include: improved weight gain and feed efficiency (Pillai et al., 2006b; Honarbakhsh et al., 2007), increased re-methylation of homocysteine through methionine synthase (Pillai et al., 2006a), and methionine and choline sparing effects as well as increased profit margins (Patil et al., 2007). Most of the benefits are related to betaine's methyl donor functions and its choline sparing effects. Even though many studies support betaine benefits, there have been many that show no significance for these effects. Esteve-Garcia and Mack (2000) showed no significance for gain and feed efficiency, while Lukic et al. (2012) showed that betaine could not be substituted for methionine without negative effects (Waldroup et al., 2006; Rafeeq et al., 2011a). Rafeeq et al. (2011a) showed that increased betaine did not significantly increase performance (Pillai et al., 2006a), and Waldroup and Fritts (2005) showed no positive effects for weight gain, efficiency, or mortality. Some of the conflicting data could be explained by differing sample sizes causing difference in power, but it is also indicative of a lack of thorough understanding of the feedback mechanisms for betaine metabolism and therefore how it benefits the animal.

In order to understand what benefits betaine truly offers to the broiler industry, further research needs to be conducted. Betaine is a known osmolyte (Thorne Research Inc., 2003;

Craig, 2004), but its osmolytic effects in chickens has not been well defined. Heat stress could be one way to see if betaine is a beneficial osmolyte, as heat stress causes increased excretion of electrolytes, which also happen to function as osmolytes (Belay et al., 1992). If heat stress causes greater excretion of electrolytes, than an osmolyte, if absorbed in its osmolytic form, should have beneficial effects to birds that are being heat stressed. Some parameters that could be used to measure betaine's osmolytic effects are water consumption versus excretion, dehydration parameters, acid base balance, and tissue dry matter percentage.

Betaine, Choline, Methionine and Their Interactions

Betaine, choline, and methionine are all nitrogen containing nutrients that have interwoven paths of metabolism (Figure 1). Choline and methionine are considered essential nutrients in poultry diets, and although betaine is not considered essential, it is an important molecule in cellular function. Betaine provides a methyl group that is used to recycle methionine in the homocysteine-methionine cycle (Simon, 1999; Thorne Research Inc., 2003; Craig, 2004). The cycle of interaction is completed, because methionine can form S-adenosylmethionine, which can be used to synthesize phosphatidylcholine, which when hydrolyzed forms choline (Zeisel, 1990; Craig, 2004). Due to choline, betaine, and methionine's interactions, these nitrogen-containing molecules may be able to substitute for each other, in least-cost livestock diets, based on current prices for each compound. These nutrients may also provide benefits

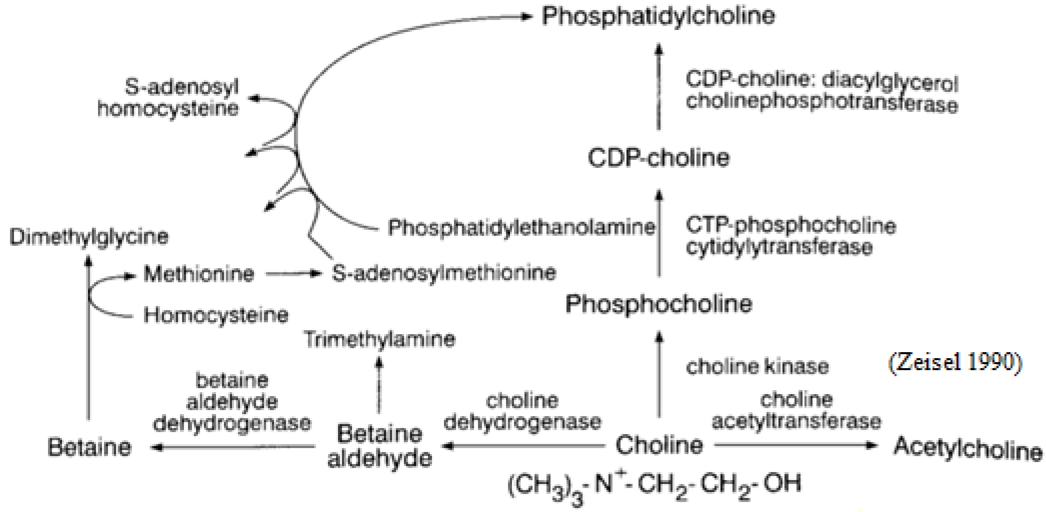

Figure 1. The interaction pathway for betaine, choline, and methionine (Zeisel, 1990; Simon, 1999).

when supplemented above minimum levels that would increase performance in the broiler industry.

The first step in the choline-betaine-methionine cycle is choline oxidation to betaine, which is a two-step process. First, choline is oxidized to betaine aldehyde by choline dehydrogenase, which can then either be metabolized into trimethylamine or further oxidized, by betaine aldehyde dehydrogenase, to form betaine (Zeisel, 1990; Simon, 1999). This is very important, because the body is able to form an osmolytic and methyl donating molecule, thus increasing choline effects beyond phospholipid and acetyl choline biosynthesis (Thorne Research Inc., 2003; Craig, 2004). Betaine formation has been shown to increase under heat stress and high salt environments (Craig, 2004). This may, in part, be due to its osmoprotective effects (Yancey et al., 1982; Simon, 1999; Thorne Research Inc., 2003; Craig, 2004; Schäfer et al., 2007; Metzler-Zebeli et al., 2009; Ratriyanto et al., 2009; Lever and Slow, 2010). Choline oxidation to betaine is a process that not only increases its functions, but also, due to its regulation, allows the body to adjust to varying conditions.

The second step in the choline-betaine-methionine cycle involves betaine's methyl donor role in the homocysteine-methionine cycle, which recycles methionine that has been oxidized (Thorne Research Inc., 2003; Craig, 2004). Betaine is an important methyl donor for cellular function, but its methyl donation is thought to be limited to one reaction: the methylation of homocysteine to form methionine (Thorne Research Inc., 2003). In the cell, S-adenosylmethionine (SAMe) is the primary methyl donor for many reactions (Thorne Research Inc., 2003), and this molecule is formed when an adenosyl group, from adenosine triphosphate (ATP), binds with the sulfur atom on a methionine molecule (Berg et al., 2007). When a SAMe molecule donates its methyl group, it forms S-adenosylhomocysteine, which is then hydrolyzed to homocysteine (Thorne Research Inc., 2003; Craig, 2004; Berg et al., 2007). Homocysteine can be methylated by betaine to form methionine, in a reaction catabolized by the enzyme betaine-homocysteine methyltransferase, effectively recycling methionine (Miller and Kelly, 1997; Millian and Garrow, 1998; Miller, 2003; Thorne Research Inc., 2003; Craig, 2004). Homocysteine remethylation rate has been shown to increase when choline or betaine are supplemented to broilers fed a methionine deficient diet (Pillai et al., 2006b). Betaine's ability to provide methyl groups for many reactions, via SAMe, by the homocysteine-methionine cycle, and the fact that choline is oxidized to form betaine, indicate that these are valuable molecules for their roles outside their direct physiological functions.

Finally, the choline-betaine-methionine cycle is closed by the formation of phosphatidylcholine from phosphatidylethanolamine, by phosphatidylethanolamine methyltransferase, using methyl groups from SAMe, which is formed from methionine and ATP (Simon, 1999; Thorne Research Inc., 2003; Berg et al., 2007). Phosphatidylcholine, when metabolized, forms many metabolites including choline. This step completes this cycle and creates a situation where all three molecules can play interchangeable roles in the body.

Due to the interconnected nature of betaine, choline, and methionine, many studies have investigated the substitution of one molecule for another in broiler diets with mixed results. Dilger et al. (2007) showed that betaine could partially spare choline in low choline diets, while Rafeeq et al. (2011a) showed that betaine was a better substitute for methionine than additional choline, but indicated it was not as good as methionine. Waldroup et al. (2006) demonstrated no methionine sparing effect by choline or betaine on gain or intake, while Esteve-Garcia and Mack (2000) showed that betaine did not have a methionine sparing effect in broilers. When methionine was fully substituted with betaine, body weight gain, feed consumption, and feed efficiency decreased, but mortality rates also decreased (Lukic et al., 2012). Supplementation of methionine-deficient broiler diets with betaine improved growth performance (Rao et al., 2011). Baker et al. (1983) concluded that betaine was not very effective at sparing choline in broiler rations, but methionine did have a minimal sparing effect of choline, while Augspurger et al. (2005) also showed methionine to have a choline sparing effect in New Hampshire X Columbian Plymouth Rock male chicks. It was demonstrated that turkey poults performance was increased by choline supplementation when diets contained adequate levels of methionine, however when dietary methionine levels were increased, the effect of the supplemental choline was eliminated (Harms and Miles, 1984). In an earlier study, Miles et al. (1983), these researchers showed that sulfate increased choline's ability to spare methionine, however Pesti et al. (1980) showed that choline or betaine could be substituted for some methionine in a broiler diet without significant detrimental effects. It has been shown that choline or betaine supplementation of methionine deficient diets causes increased homocysteine methylation (Pillai et al., 2006a; Pillai et al., 2006b), which could be indicative of why these molecules have some methionine sparing effect. Betaine is more readily absorbed through the intestinal wall of broilers than choline (Rafeeq et al., 2011b), which could explain why betaine has been shown to be a better methionine-sparing agent than choline. Based on previous research, one can conclude that if choline or betaine spares methionine, this effect is minimal at best. The conclusion, by Pesti et al. (1980), that no difference existed when choline or betaine were substituted for methionine, can be questioned, because performance was decreased in the methionine deficient groups, and the lack of significance was probably due to a lack of power (Simon, 1999).

Even though each of the three molecules can play a part in the formation of the other two through a series of reactions to form either of the other two, this does not mean that that is the most efficient way for an animal to meet its needs. In order for choline to be formed from methionine, phosphatidylcholine, which requires the cleavage of three ATP molecules, must be formed (Simon, 1999; Craig, 2004; Berg et al., 2007). This process uses three SAMe molecules (Simon, 1999; Craig, 2004; Berg et al., 2007). Adenosine triphosphate is a major source of energy in any cell, and ATP usage will increase energy needs and decrease performance. This fact is based on the *Second Law of Thermodynamics, which states that the total entropy of a system plus that of its surroundings is always increasing.* This means that a spontaneous reaction will release energy or a non-spontaneous reaction takes energy, in some form, to occur (Berg et al., 2007), and since no reaction is one-hundred percent efficient, every additional reaction in a system will decrease the efficiency of that system. Thus, although choline has been demonstrated to spare methionine, the additional reactions needed will have a cost in efficiency. However, one benefit derived from this relationship is that it allows cells to cope with sudden changes in needs, such as heat stress increasing the need for betaine's osmotic function, but complete substitution is probably not an efficient way to meet the long-term needs of a broiler chicken.

Conclusion

There are many challenges facing today's broiler industry such as heat stress and footpad dermatitis. Nutritional enhancements have provided many solutions to these challenges, but there is still much room for improvement. Choline and its metabolite betaine, provide many benefits including methyl donation and osmolytic function, as well as having been shown, in certain situations, to increase performance, in broiler chickens. This experiment, described in the subsequent pages, will look at choline and betaine supplementation, to heat stressed broilers, in addition to a diet that meets minimum choline and methionine requirements.

CHAPTER TWO

Materials and Methods

Birds and Housing

All animal procedures were reviewed and approved by the University of Tennessee Institutional Animal Care and Use Committee. In this study 800 day-old Cobb MX™ X Cobb 500™ (Cobb-Vantress, Incorporated, Siloam Springs, AR, USA) mixed sex (straight run) broiler chicks were obtained from Pilgrim's Corporation hatchery in Cohutta, GA. The birds were vaccinated at the hatchery against coccidiosis (Coccivac® -B, Merck Animal Health, Summit, NJ, USA), Newcastle disease and infectious bronchitis (Newhatch-C2-M®, Merck Animal Health, Summit, NJ, USA), fowl pox (Vectormune® FP LT, Ceva, Lenexa, KS, USA), and *Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* (Garasol®, Merck Animal Health, Summit, NJ, USA). The *E. coli,* fowl pox, salmonella, and pseudomonas vaccines were administered inovo by Embrex ® (Pfizer Animal Health, New York City, NY, USA), on day 18 of incubation, while the coccidiosis and newcastle vaccines were administered by spray post-hatch. These are standard health practices in the broiler industry.

Birds were randomly assigned to 80 identically furnished pens in two different rooms, with 40 pens per room, on the day of acquisition. The rooms were located in the Johnson Animal Research and Teaching Unit (JARTU) at the East Tennessee Research and Education Center. Each pen was 1.525X1.219 m (5X4 ft) and housed 10 birds. Each pen was randomly assigned a treatment group and replicate number. One room was assigned a treatment classified as thermoneutral (TN) while the second room was designated as high temperature (HT). Both rooms were on a 23 hours of light and 1 hour of dark daily schedule, and the temperature in each room started out at 35 C and decreased gradually to 24 C over the first three weeks of the study. At the end of week three, a high temperature cycle was implemented in the HT room. Daily the temperature in the HT room was allowed to cycle between 24 C and 34 C, while temperature in the TN room was maintained at 24 C, throughout the last four weeks of the study. The HT birds were exposed to 24 C from 0:00 to 12:00. At 12:00, the temperature was steadily raised until it reached 34 C, and then at 18:00 the temperature was steadily decreased to 24 C (Figure 2). Food was provided for ad libitum consumption by gravity feeders hung in each pen. Water was provided for ad libitum consumption by three automatic nipple/cup waterers in each pen. Birds were weighed and feed consumption was calculated weekly by pen. When the birds were one week old, they were identified with wing bands placed in the wing web of the right wing on each bird.

Dietary Treatments

Each pen was randomly assigned to one of five dietary treatments in this study: Treatment 1, basal diet, Treatment 2, basal diet plus 500 methyl equivalents added choline, Treatment 3, basal diet plus 1000 methyl equivalents added choline, Treatment 4, basal diet plus 500 methyl equivalents added betaine, and Treatment 5 basal diet plus 1000 methyl equivalents added betaine. Other than the additional choline or betaine, a standard corn-soybean meal based broiler diet was provided to all birds. As is standard in the broiler industry, the birds were fed in phases across all treatments. Broilers are fed in phases to supply the energy and protein levels that most accurately meet their needs at that stage in growth. For the first two weeks the birds were given a starter diet (Table 1), weeks two through four the birds were on a grower diet was

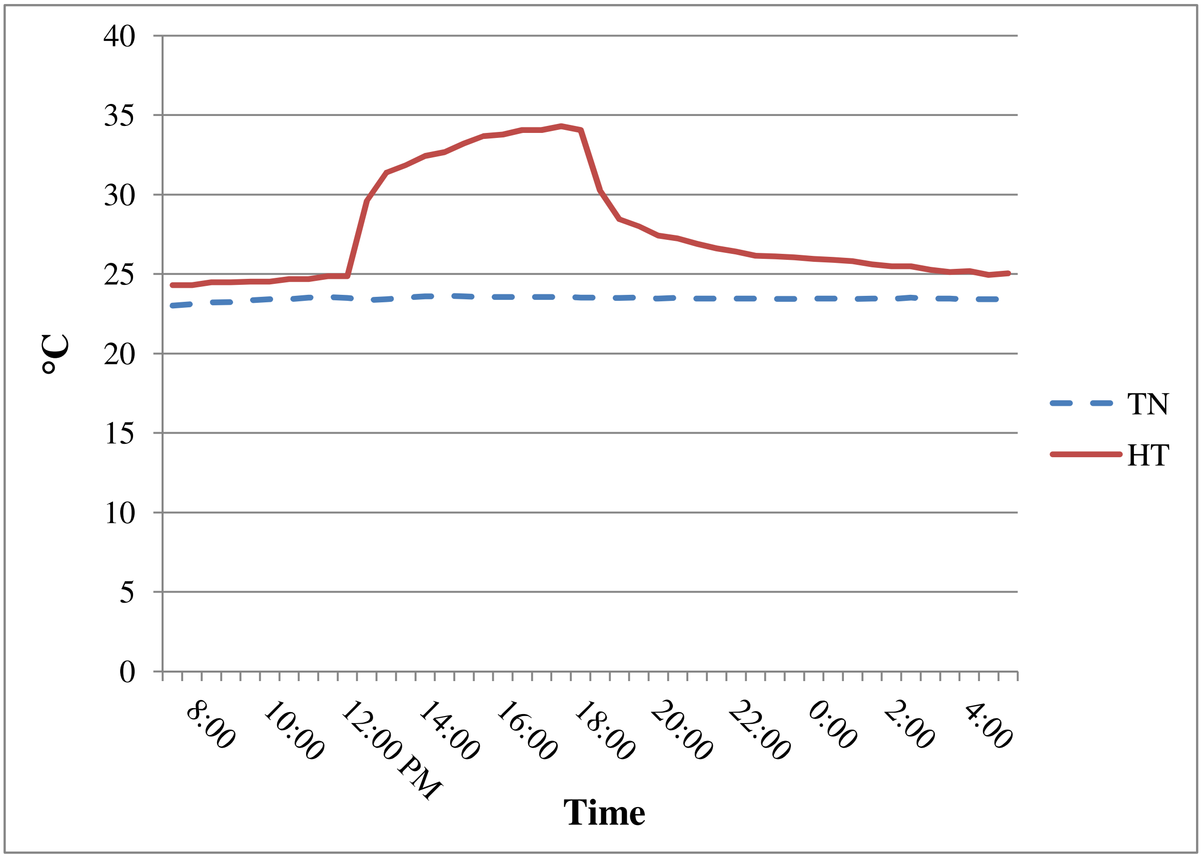

Figure 2. The daily temperature cycle for the thermoneutral and high temperature rooms. Ambient temperature in the high temperature room (HT) cycled from 24.3 to 34.3 C and in the thermoneutral room (TN) it stayed at 23±1 C.

Table 1. Starter basal diet formulation.

Feed Ingredient	% of Ration
Corn, grain	63.3
Soybean meal	30.49
Vitamin Premix	0.064
DL Methionine	0.3
Salt	0.35
Limestone	1.1
Dical phosphate	2.1
Trace Min. Premix	0.1
Fat, animal	1.9
Lysine	0.1

mixed (Table 2), and the last three weeks the birds were fed a finisher diet (Table 3). All feed in 227 kg (500 lb.) batches in a feed mixer. Vitamins and minerals were pre-mixed in a smaller container before being added to the larger mixer. Once all ingredients were in the feed mixer, the mixer was allowed to run for 20 minutes. Feed was provided for ad libitum consumption and the amount consumed, by the birds in each pen, was calculated weekly. When feed was added to a pen its weight was measured and recorded. Weekly feed consumption was calculated by measuring the remaining feed in a pen and then subtracting that from the total amount of feed provided during the previous seven days.

Growth and Carcass Traits

During this study, bird weight was measured by pens on a weekly basis. Weekly weight gains were calculated and these figures were used with the weekly consumption to determine feed conversion ratios. On days 42 and 49 of the study, three birds, randomly selected from each pen, were euthanized and processed into market halves. Birds were stunned by three seconds of electric shock and then killed by exsanguination following severing of the jugular vein and carotid artery. Once a bird had been allowed to adequately bleed out; it was scalded, by submerging in hot water at 59.5 C, to ease feather removal. Following removal from the scalding vat, the birds were placed in a plucking machine to remove the feathers. After defeathering, the birds were eviscerated and the head and feet were removed. During the evisceration process, abdominal fat and liver were excised and weighed. Dressed birds were weighed to obtain a dressed carcass weight. Carcasses were cut into a breast half, which included the whole breast and both wings, and a leg half, which included both legs and both thighs. Breast weights and leg weights were measured, and carcass part percentages were calculated.

Table 2. Grower basal diet formulation.

Feed Ingredient	% of Ration
Corn,grain	62.3
Soybean meal	31.1
Vitamin Premix	0.064
DL Methionine	0.3
Salt	0.35
Limestone	1.1
Dical phosphate	1.69
Trace Min. Premix	0.1
Fat, animal	2.7
Lysine	0.1

Table 3. Finisher basal diet formulation.

Feed Ingredient	**% of Ration**
Corn,grain	67.4
Soybean meal	26
Vitamin Premix	0.064
DL Methionine	0.3
Salt	0.35
Limestone	1.1
Dical phosphate	1.69
Trace Min. Premix	0.1
Fat, animal	2.7
Lysine	0.1

Litter Wetness and Foot Scores.

Throughout the duration of the study, a shredded, recycled paper product was used for litter. The litter was not changed during the duration of the study except in the case of a waterer leaking. When a leak occurred, litter had to be replaced, and the pen was excluded from moisture data analysis. On days 41 and 48 litter samples from the same location in each pen were collected in Whirl-Pak® bags (Nasco, Fort Atkinson, WI, USA) and placed in a freezer (-20 C) for moisture content to be analyzed at a later time. In order to calculate moisture content, frozen litter samples were thawed and then weighed. The samples were then placed in a drying oven at 100 C and allowed to dry for 48 hours. The dried samples were then weighed and the percent moisture was calculated. On day 48 of the study, all birds remaining were scored for footpad dermatitis. Foot scores range from a zero, which has little or no signs of dermatitis, to a three, which is where the majority of the footpad has dermatitis (Figure 3).

Intestinal Integrity

On day 42, during evisceration, the small intestine was removed and a section, of approximately 10 cm with the diverticulum in the middle, was cut and placed in plastic bag with phosphate buffered saline (PBS). The samples were placed in the refrigerator for later analysis. The following day the samples were analyzed, for tensile strength, on a food texture analyzer. The sample was cut to an exact length of 10 cm with the diverticulum in the middle. The sample was placed in clamps, on the top and bottom of the analyzer, and the sample was slowly pulled taught. The test was initiated with the texture analyzer which is fully automated. It slowly pulled the sample apart until the first break occurred. Breaking strength was measured as the amount of

Figure 3. The scoring system for foot pad dermatitis (Mello et al., 2011).

force in grams required to compromise the membrane. A macro for noodles was used since noodles are similar in shape and size to chicken intestines.

Experimental Design and Statistical Analysis

Body weight gain, litter moisture data, conversion, and consumption data were analyzed as a completely randomized design with repeated measures across weeks and evaluated on a per pen basis. Carcass traits, intestinal integrity, foot score and was analyzed as a completely randomized design with dietary treatment nested within temperature treatment. This data was measured on a per bird basis and not by pen. In pens where birds expired during the trial, data was adjusted by dividing by the number of birds left and then multiplying the per bird average by ten. All results were reported as means, and all data was analyzed using analysis of variance (ANOVA). Least square means were compared and treatment differences were evaluated to determine significance. Direct effects as well as interaction effects were observed. Significance was set at a value of $P < 0.05$. All statistical analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC).

CHAPTER THREE

Results

Growth and Performance

Feed intake, body weight gain, and feed conversion are three of the major parameters used by the broiler industry. Feed intake during the first three weeks, when no temperature treatments were applied, did not differ among dietary treatments (Figure 4). During weeks four to six, feed intake differed among dietary treatments, with treatment five having the highest average consumption and treatment one having the lowest (Figure 5). Feed intake was also affected by temperature treatment, during weeks four to six, with feed intake lower for the HT treatment during weeks four and six (Figure 6). Weight gain was not affected by dietary treatments during the first three weeks (Figure 7) or for the second three weeks (Figure 8). Temperature did not have an effect on weight gain during weeks four to six (Figure 9). Feed conversion was the same for all dietary treatments weeks one through three and weeks four through six (Figure 10 and 11); however, feed conversion was higher ($P = 0.0163$) for the HT treatment group during weeks four through six (Figure 12). High temperature treatments effect on feed conversion was significantly ($P = 0.0330$) different from TN treatment group during week six (Figure 13). Performance, measured as intake, gain, and feed conversion, was not affected by dietary treatments, but it was affected by high temperatures.

Litter Moisture and Foot Scores

Litter moisture differed across dietary treatments, on day 41, with treatments four and five having the highest percent moisture and treatment one having the lowest percent moisture (Figure 14). However, on day 48, litter moisture did not differ amongst dietary treatments

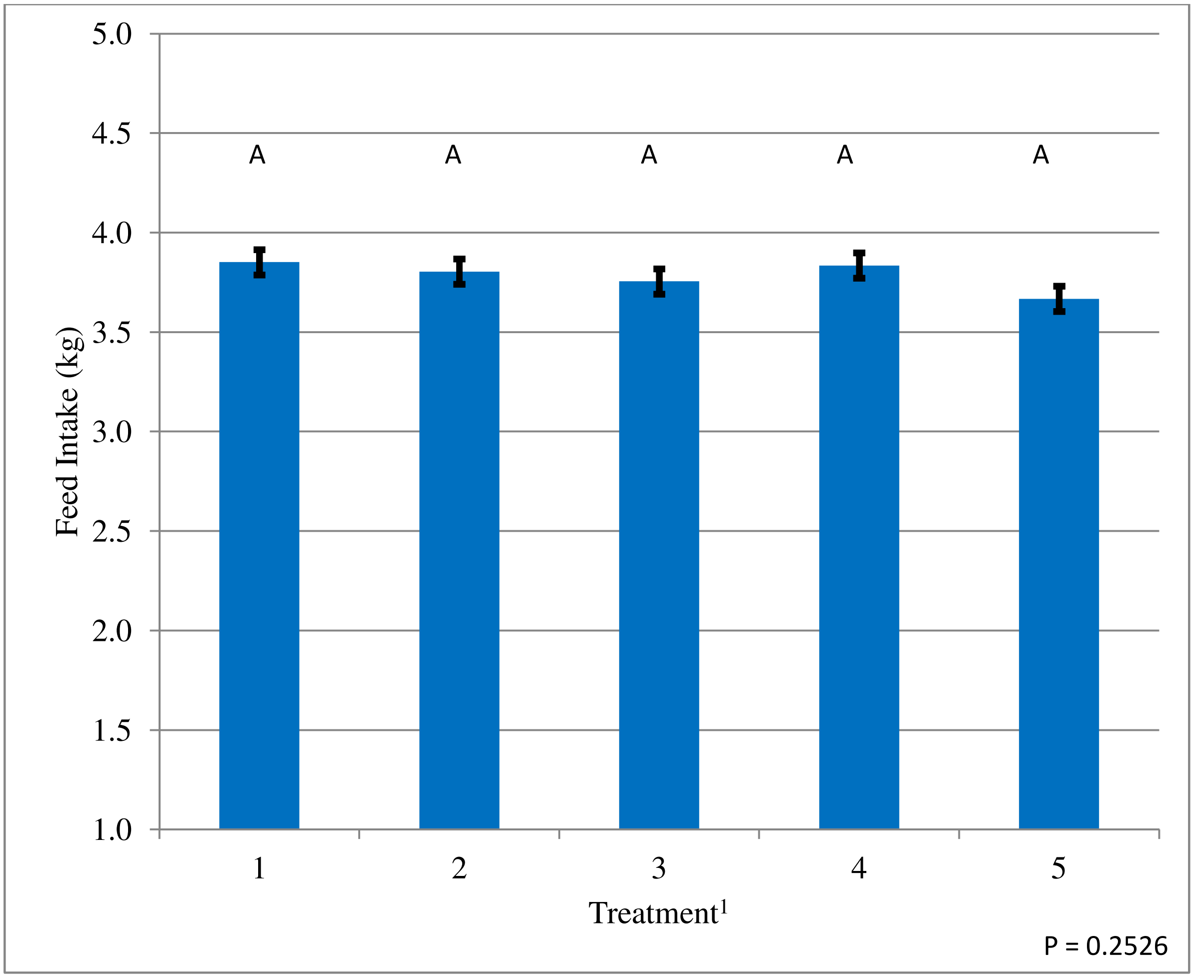

Figure 4. Feed intake for days 1-21 across dietary treatments.

Values are expressed as mean ± SE. Means with different letters differ ($P < 0.05$).

[1] Treatment 1=control, treatment 2=low choline, treatment 3 equals high choline, treatment 4=low betaine, and treatment 5=high betaine.

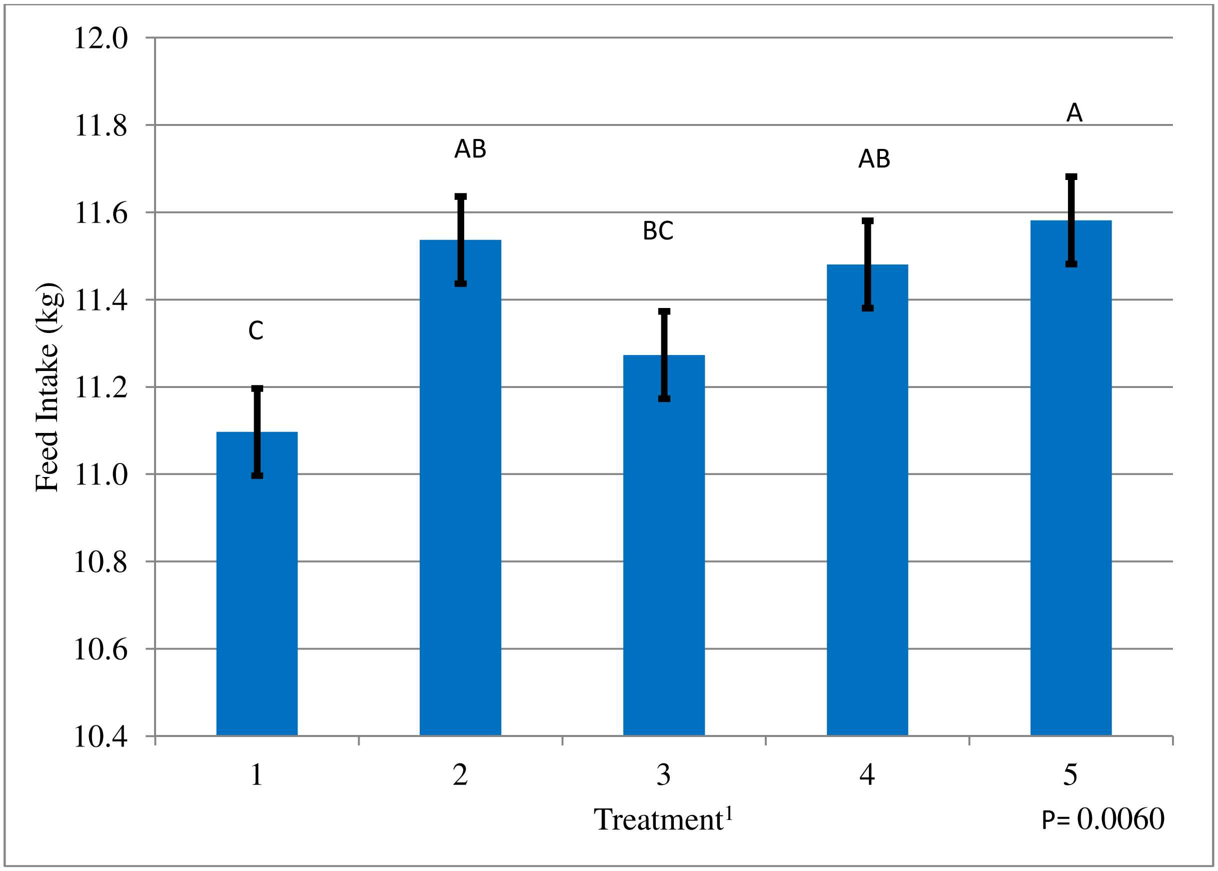

Figure 5. Feed intake for days 21-42 across dietary treatments.

Values are expressed as mean ± SE. Means with different letters differ ($P < 0.05$).

[1] Treatment 1=control, treatment 2=low choline, treatment 3=high choline, treatment 4=low betaine, and treatment 5=high betaine.

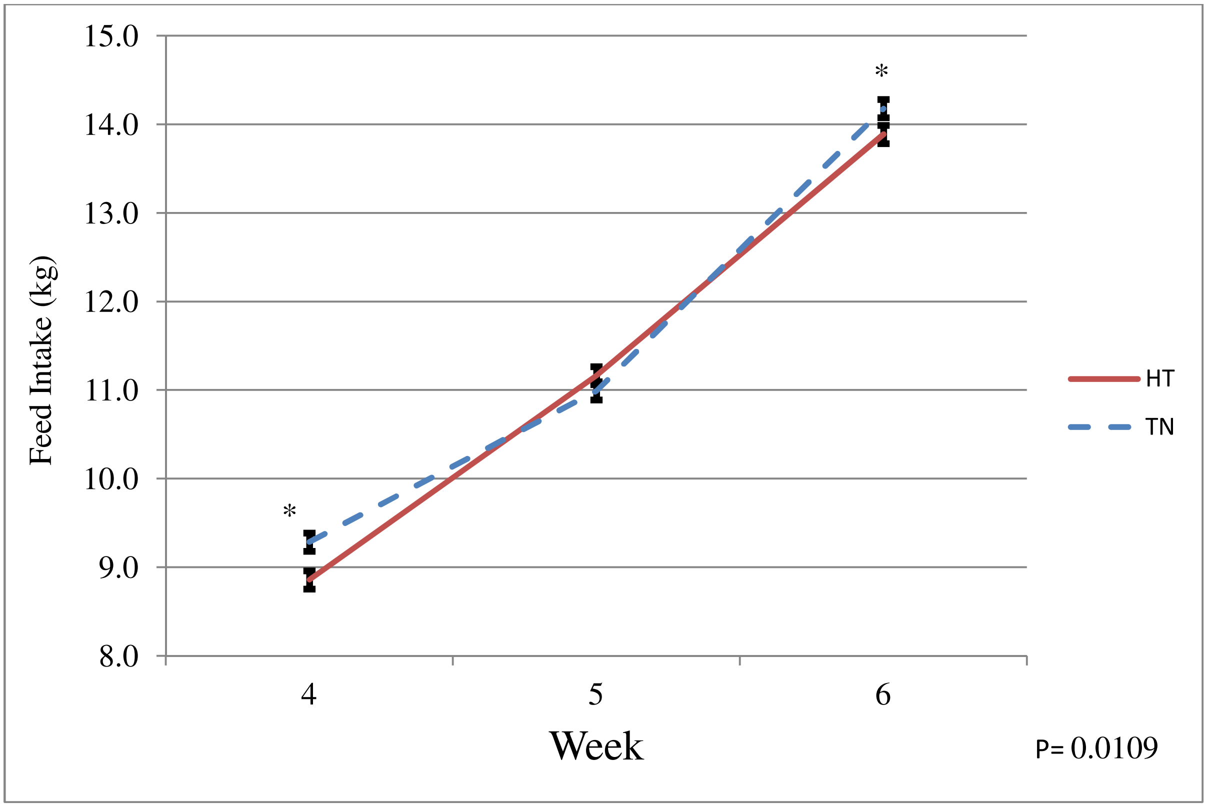

Figure 6. Feed consumption for temperature by week interaction during weeks four five and six.

Values are expressed as mean ± SE. Means that differ between temperature groups for a week ($P < 0.05$) are indicated by (*).

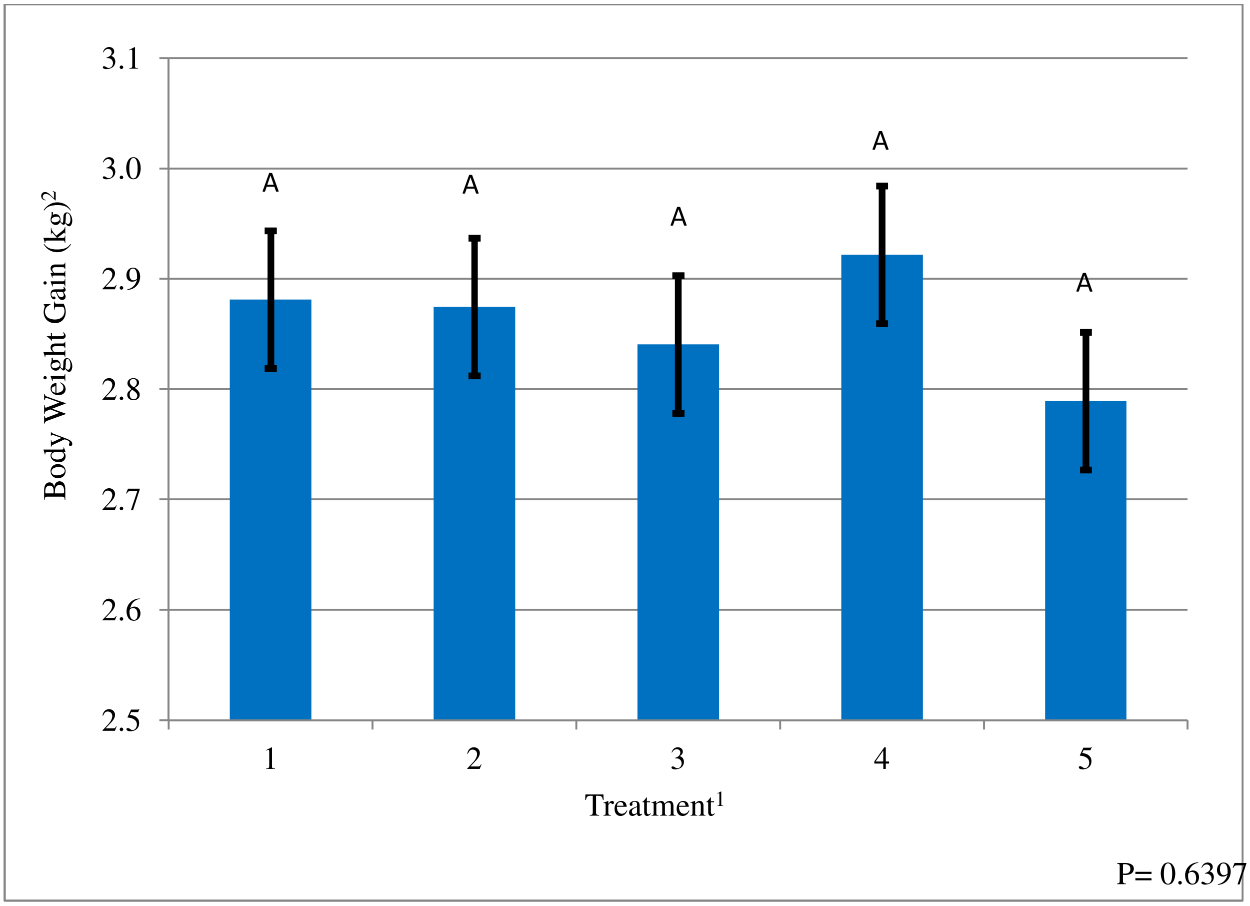

Figure 7. Body weight gain for day 1-21 amongst dietary treatments.

Values are expressed as mean ± SE. Means with different letters differ ($P < 0.05$).

[1] Treatment 1=control, treatment 2=low choline, treatment 3=high choline, treatment 4=low betaine, and treatment 5=high betaine.

[2] Gain was measured by pen.

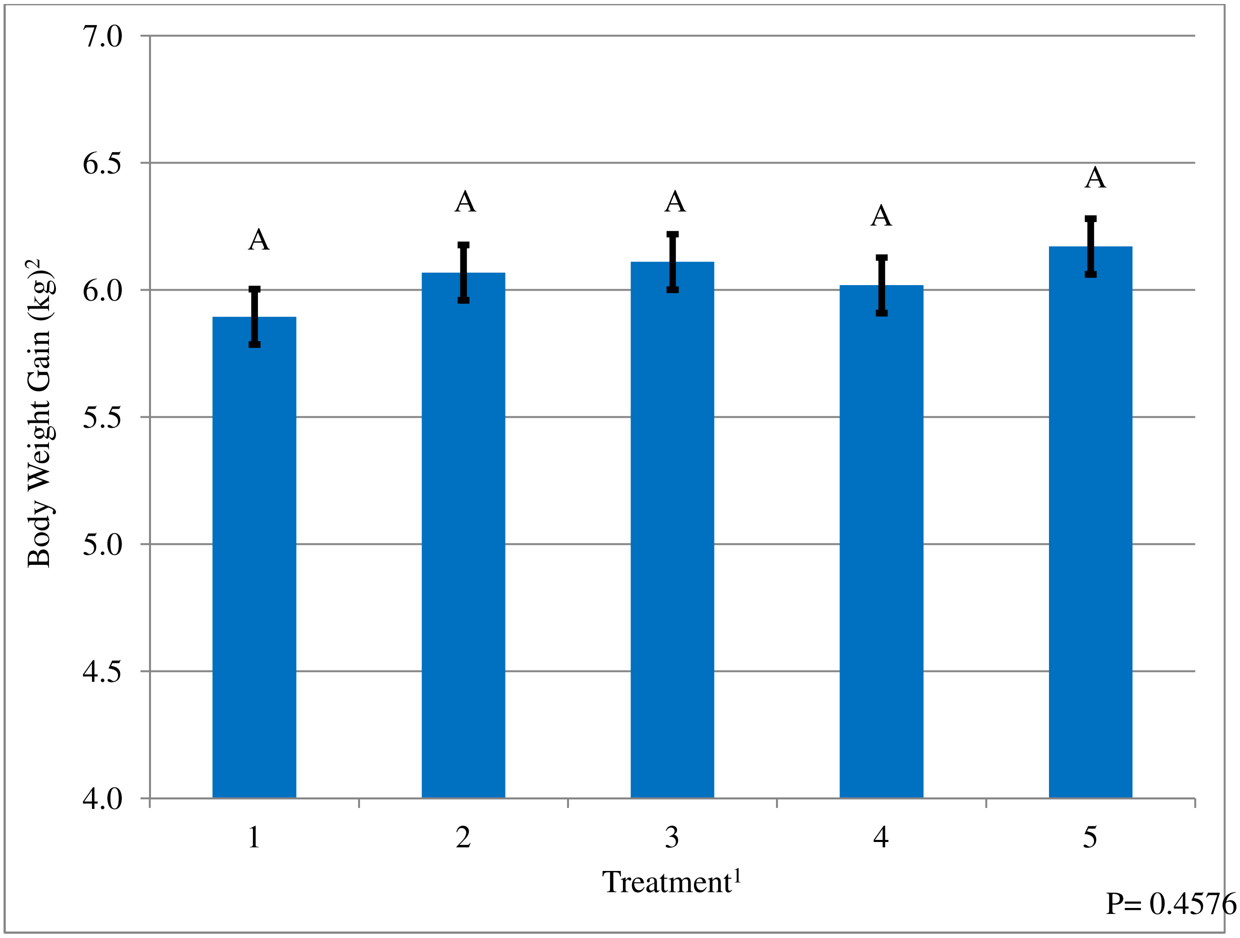

Figure 8. Body weight gain for days 21-42 amongst dietary treatments.

Values are expressed as mean ± SE. Means with different letters differ ($P < 0.05$).

[1] Treatment 1=control, treatment 2=low choline, treatment 3=high choline, treatment 4=low betaine, and treatment 5=high betaine.

[2]Gain was measured by pen.

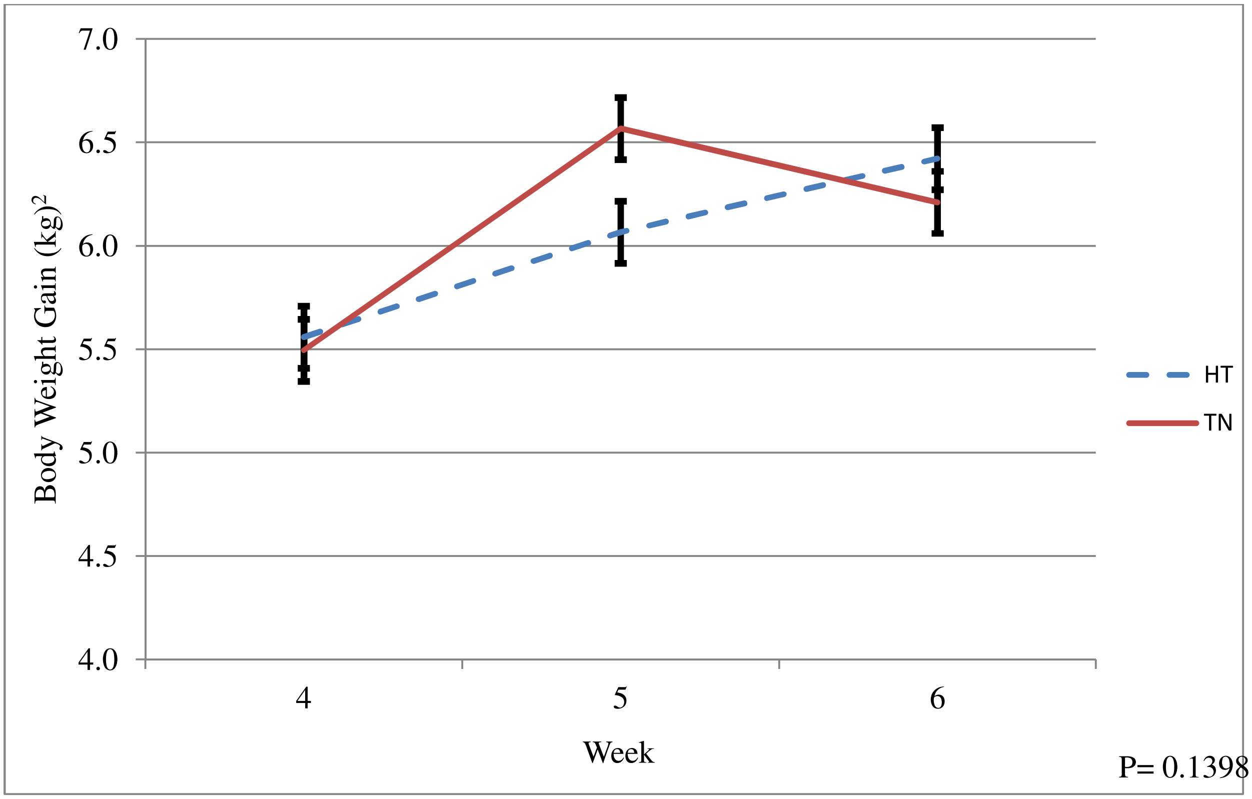

Figure 9. Body weight gain for temperature by week interaction during weeks four five and six.

Values are expressed as mean ± SE. Means that differ between temperature groups for a week ($P < 0.05$) are indicated by (*).

[2]Gain was measured by pen.

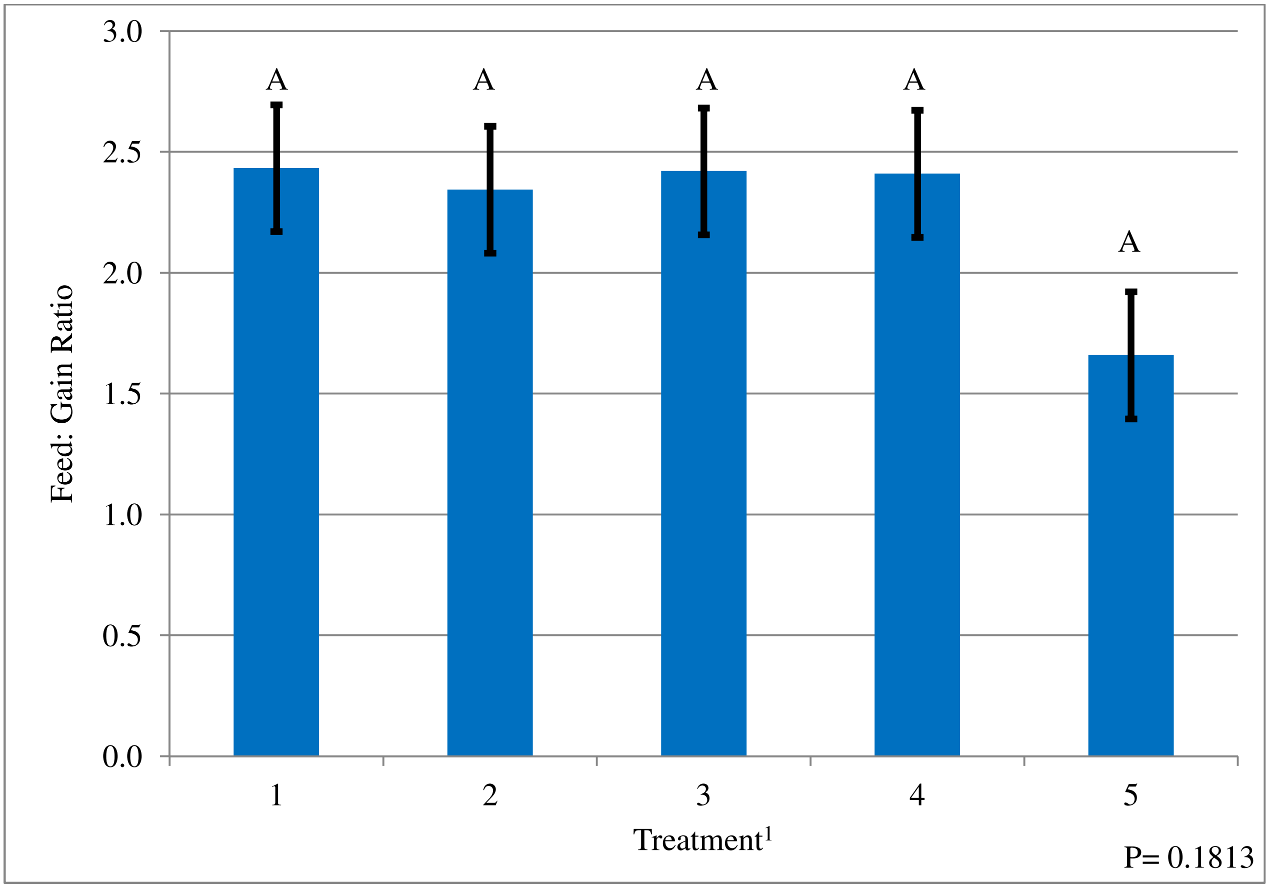

Figure 10. Feed conversion for day 1-21 amongst dietary treatments.

Values are expressed as mean ± SE. Means with different letters differ (P < 0.05).

[1] Treatment 1=control, treatment 2=low choline, treatment 3=high choline, treatment 4=low betaine, and treatment 5=high betaine.

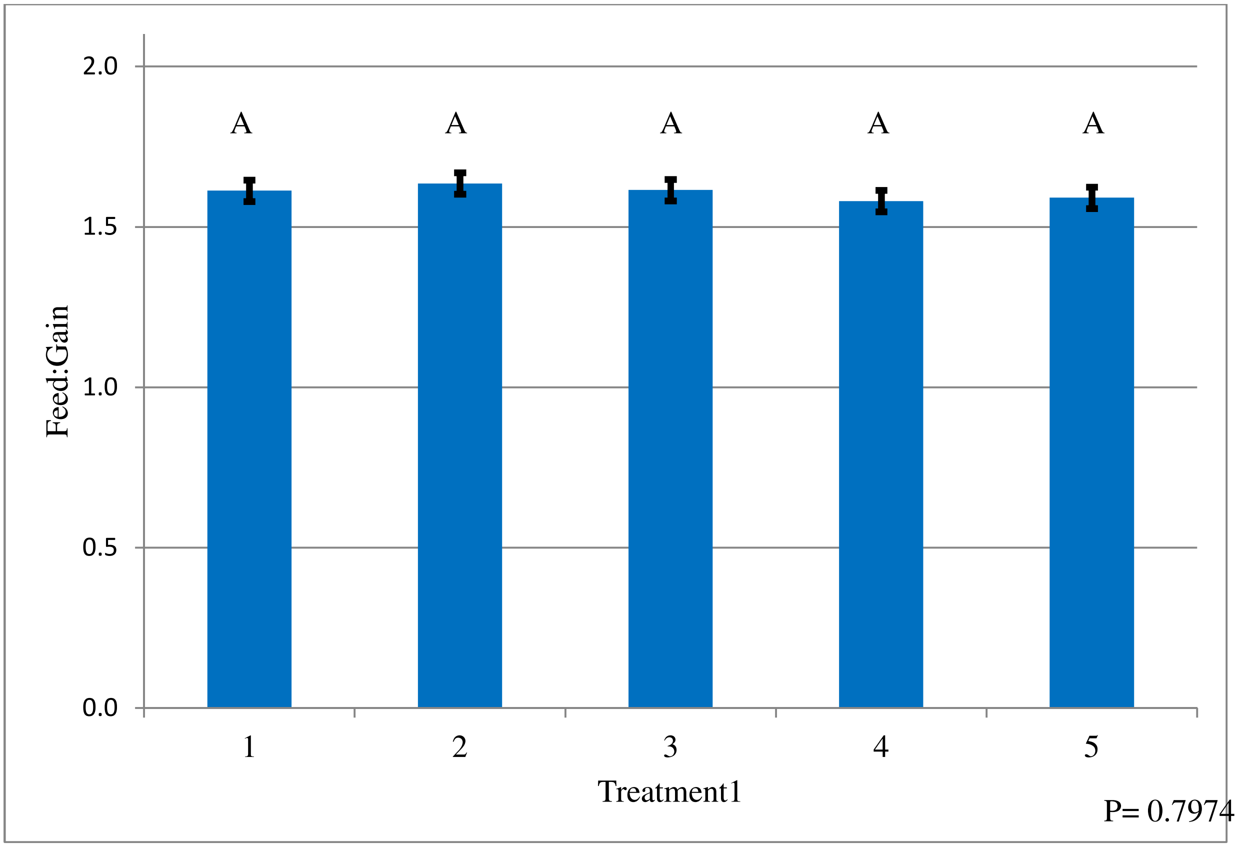

Figure 11. Feed conversion for day 21-42 amongst dietary treatments.

Values are expressed as mean ± SE. Means with different letters differ ($P < 0.05$).

[1] Treatment 1=control, treatment 2=low choline, treatment 3=high choline, treatment 4=low betaine, and treatment 5=high betaine.

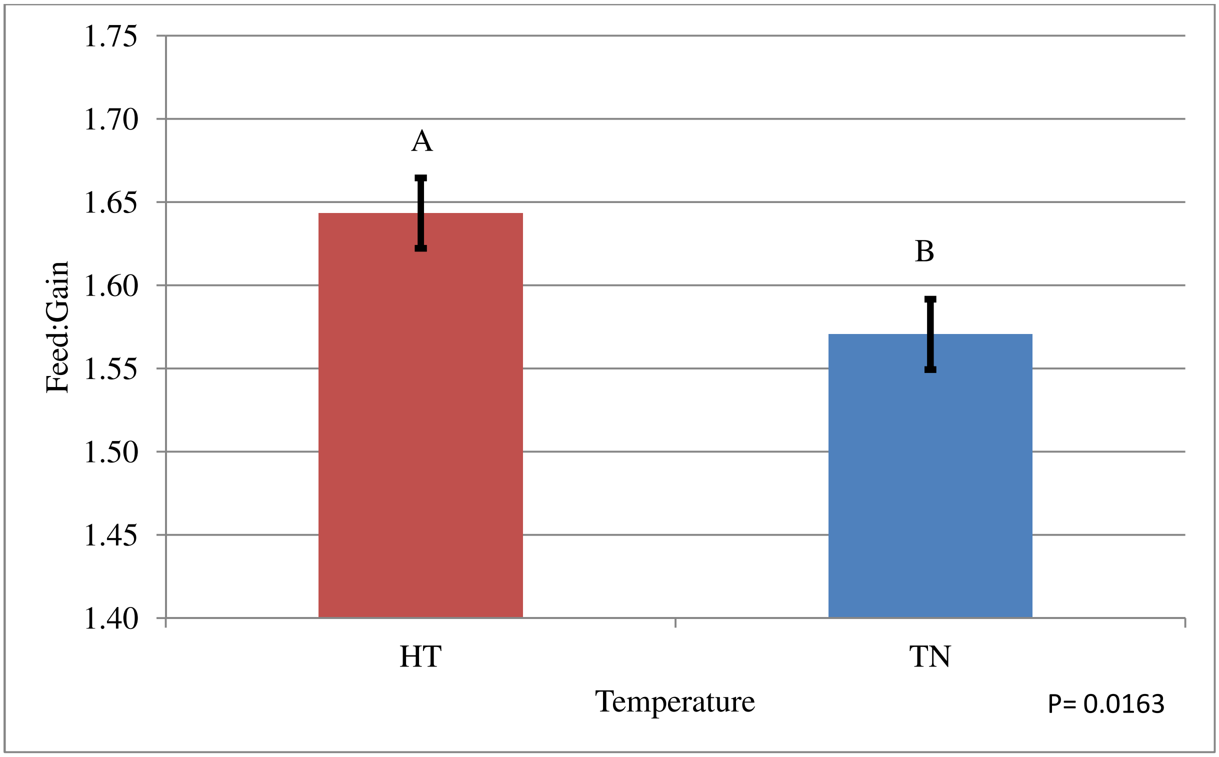

Figure 12. Feed conversion for day 21-42 amongst high temperature and thermoneutral groups.

Values are expressed as mean ± SE. Means with different letters differ ($P < 0.05$).

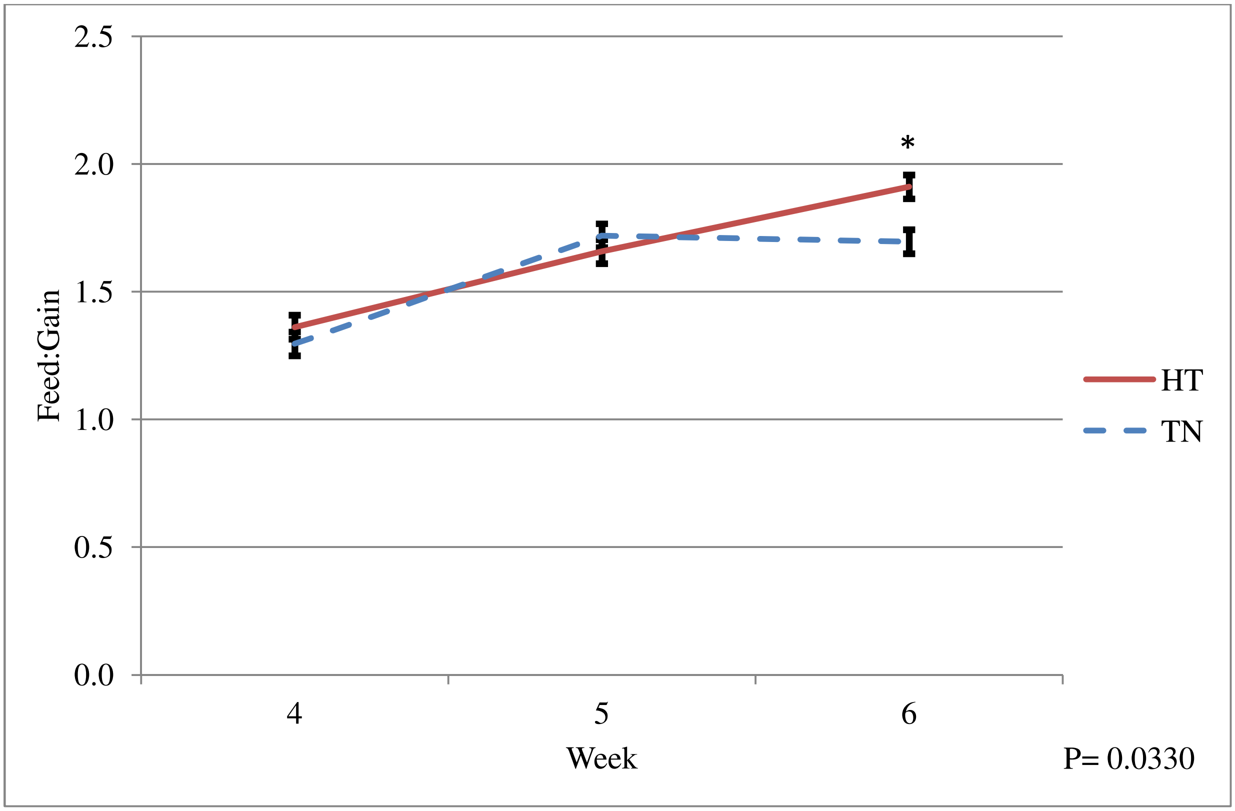

Figure 13. Feed conversion for temperature by week interaction during weeks four five and six.

Values are expressed as mean ± SE. Means that differ between temperature groups for a week ($P < 0.05$) are indicated by (*).

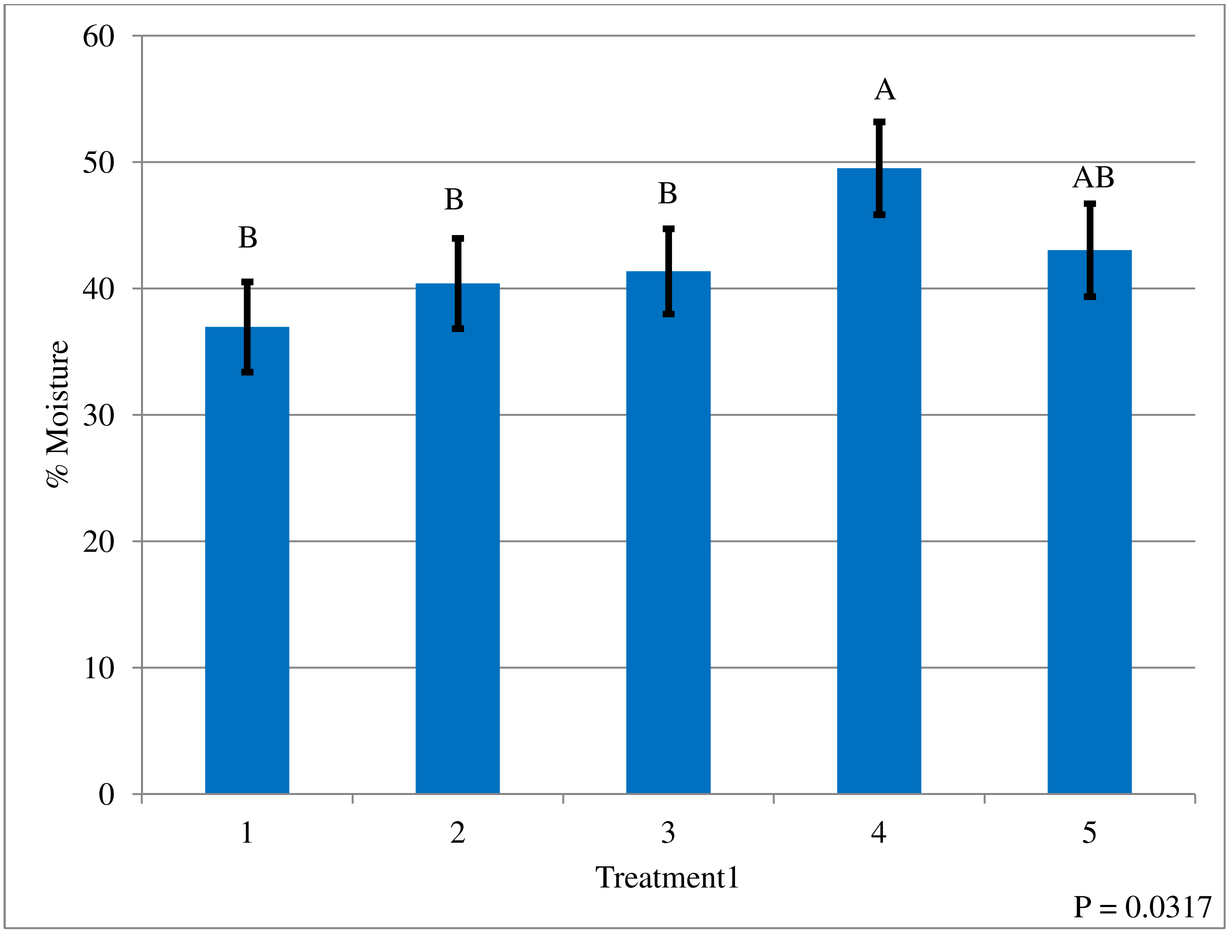

Figure 14. Litter moisture on day 41 amongst dietary treatments.

Values are expressed as mean ± SE. Means with different letters differ ($P < 0.05$).

[1] Treatment 1=control, treatment 2=low choline, treatment 3=high choline, treatment 4=low betaine, and treatment 5=high betaine.

(P = 0.4385). Temperature treatments did not affect litter moisture on day 41 (Figure 15). Foot Scores did not differ amongst dietary treatments or temperature treatments (Figure 16 and 17). Litter moisture has been linked to foot pad dermatitis but in our study there was no correlation between litter moisture and foot scores (P = 0.2514).

Intestinal Tensile Strength and Mortality

Intestinal tensile strength did not differ across dietary treatments (Figure 18). Mortality did not differ amongst dietary treatments or between the first three weeks and the second three weeks (Table 1). Mortality did not differ across temperature treatments during weeks four through six either (Figure 19).

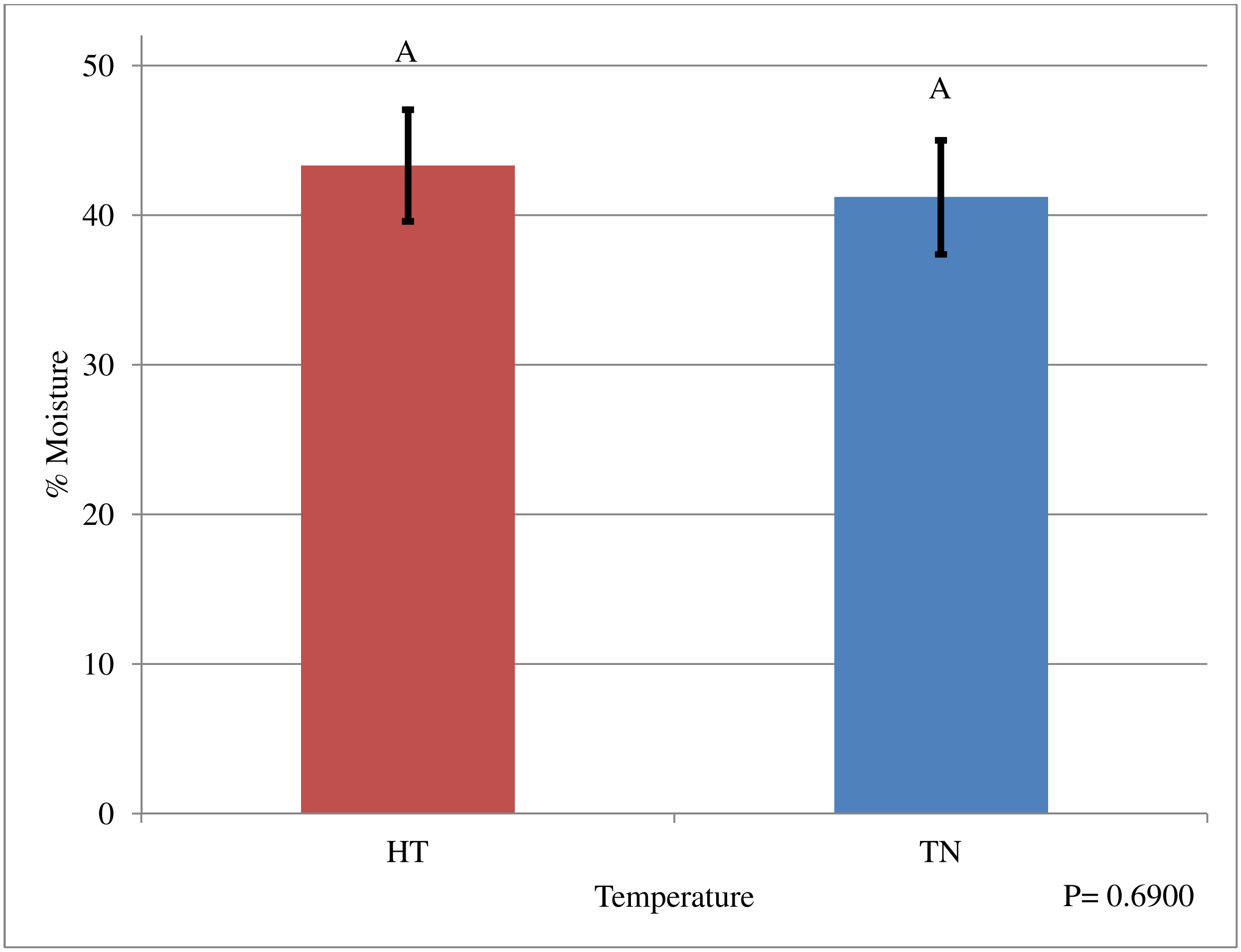

Figure 15. Litter moisture on day 41 amongst high temperature and thermoneutral groups.

Values are expressed as mean ± SE. Means with different letters differ ($P < 0.05$).

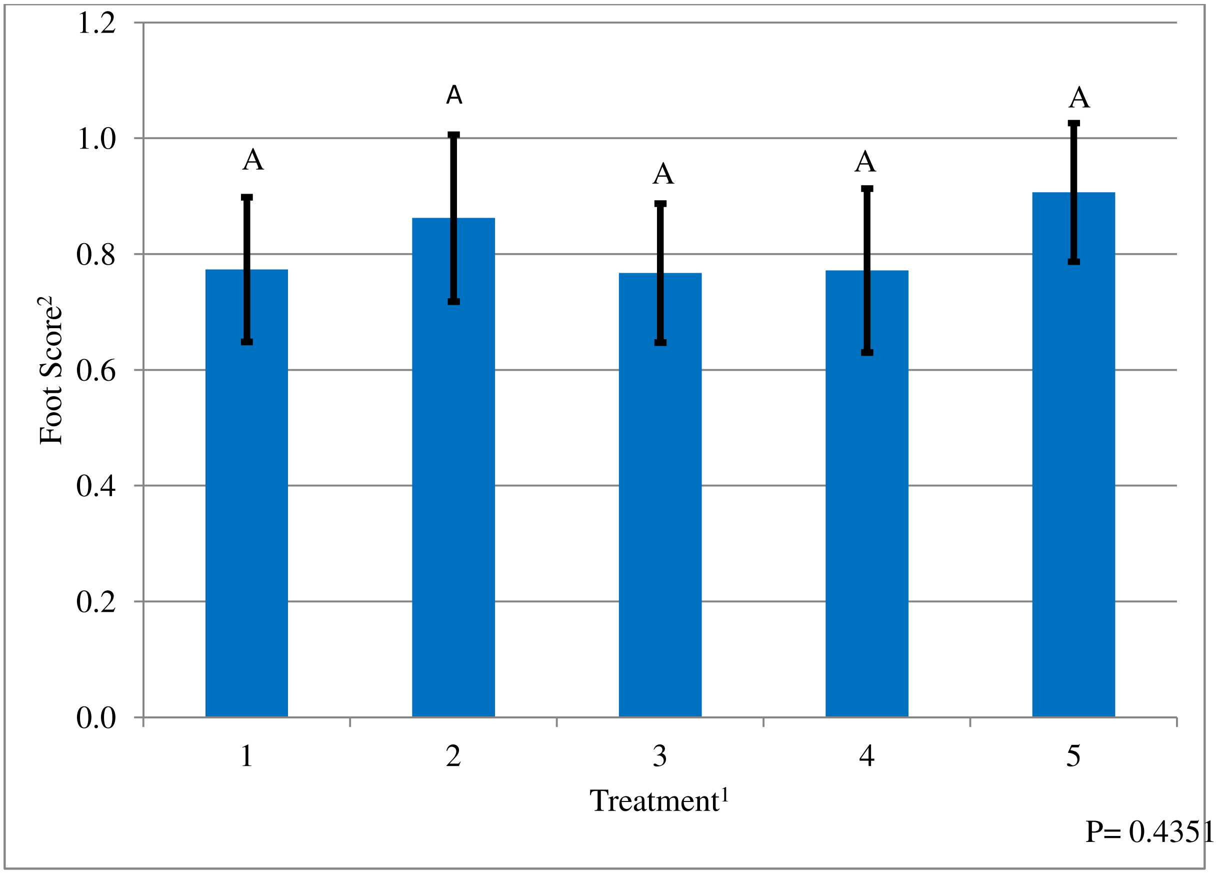

Figure 16. Foot scores amongst dietary treatments.

Values are expressed as mean ± SE. Means with different letters differ ($P < 0.05$).

[1] Treatment 1=control, treatment 2=low choline, treatment 3=high choline, treatment 4=low betaine, and treatment 5=high betaine.

[2] Foot scores were measured on a zero to three scoring system.

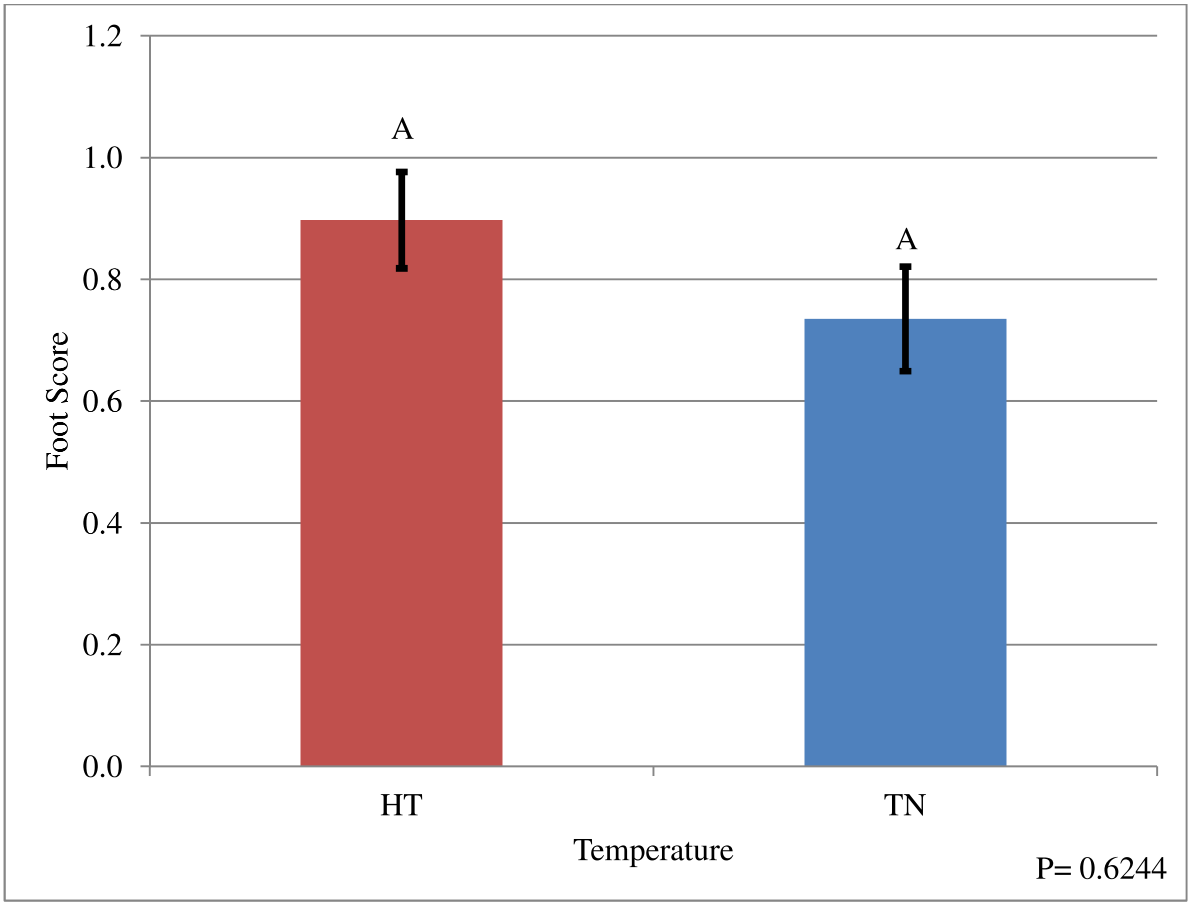

Figure 17. Foot scores amongst high temperature and thermoneutral groups.

Values are expressed as mean ± SE. Means with different letters differ ($P < 0.05$).

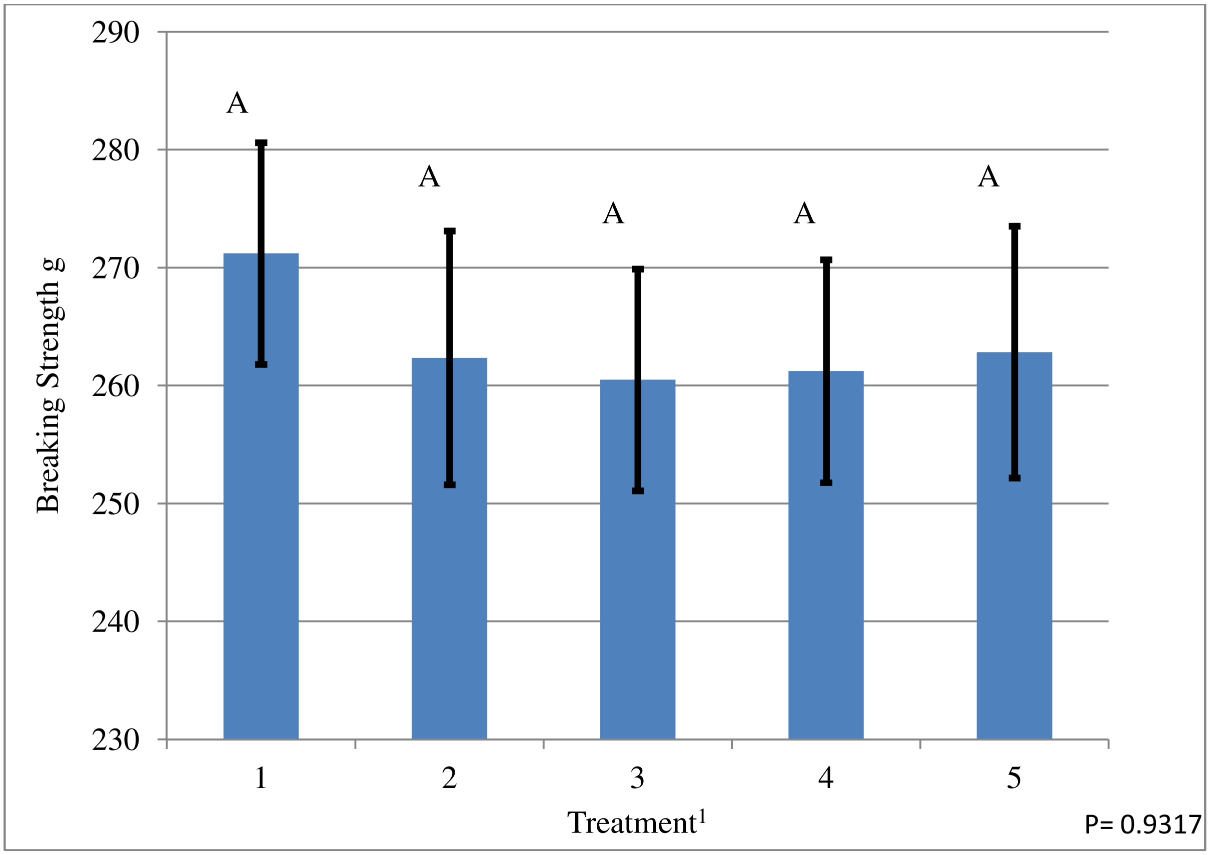

Figure 18. Intestinal tensile strength amongst dietary treatments.

Values are expressed as mean ± SE. Means with different letters differ ($P < 0.05$).

[1] Treatment 1=control, treatment 2=low choline, treatment 3=high choline, treatment 4=low betaine, and treatment 5=high betaine.

Table 4. Least squares means for mortality rate for days 1-21, days 21-42, and days 1-42.[1]

Treatment	Day 1-21	Day 21-42	Day 1-42
Control	0.625^{A}	0.00^{A}	0.625^{A}
Choline 500	1.875^{A}	3.125^{A}	5.000^{A}
Choline 1000	0.625^{A}	1.25^{A}	1.875^{A}
Betaine 500	1.25^{A}	0.625^{A}	1.875^{A}
Betaine 1000	1.25^{A}	1.875^{A}	3.125^{A}

[1]Means within a column with different letters differ ($P < 0.05$).

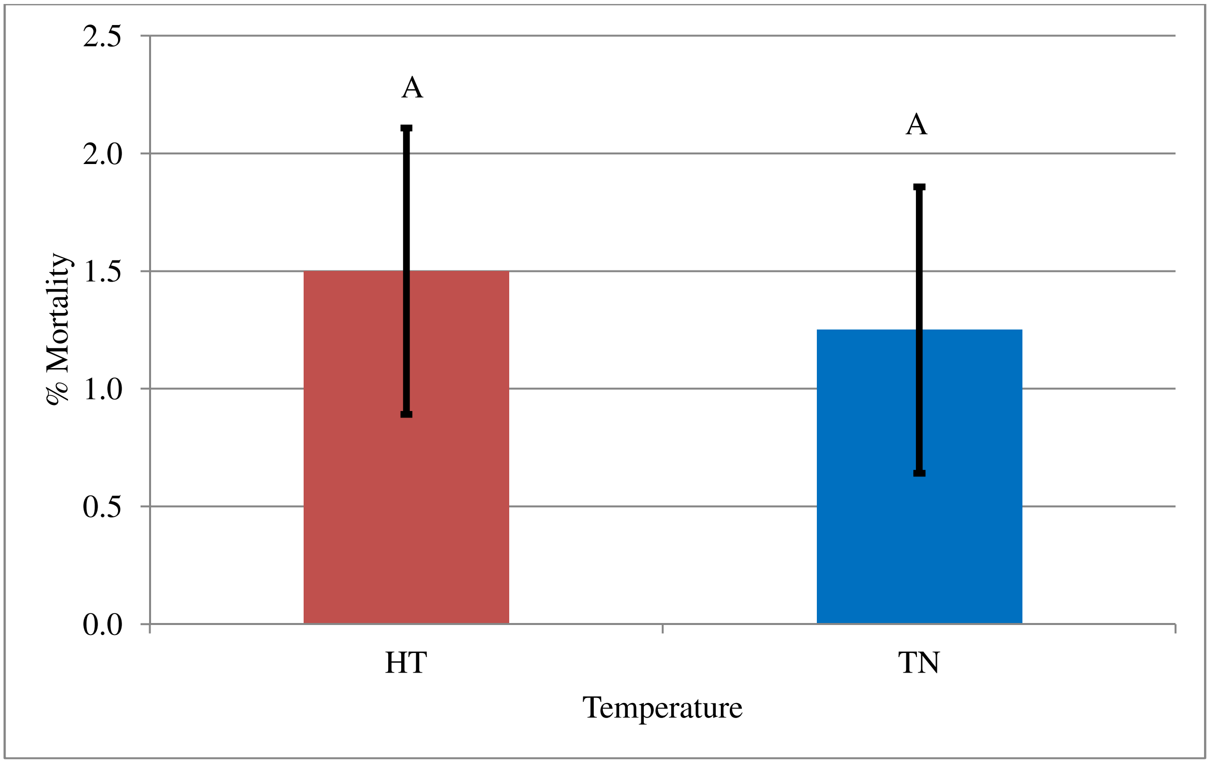

Figure 19. Percent mortality for day 21-42 amongst high temperature and thermoneutral groups.

Values are expressed as mean ± SE. Means with different letters differ ($P < 0.05$).

CHAPTER FOUR

Discussion

This study tested the effects of choline or betaine supplementation, above recommended amounts, on broilers exposed to varied temperature treatments. We hypothesized that choline or betaine, when supplemented above recommended amounts, may have a variety of benefits, both performance and economic, which could play a key role in future broiler production. Heat stress causes physiological changes in chickens that present challenges from both a performance side as well as animal welfare consideration. Dietary additives have been shown to aid in heat stress abatement. This study investigated the effect of choline and betaine as dietary additives on broilers during a typical production life of 42 days, while broilers were exposed to different temperature regimens.

Our study did not support the hypothesis that the amount of added choline or betaine along with the amount of methionine present in the diet would have demonstrable effects on performance parameters. While Waldroup et al. (2006) showed improved feed conversion in broilers fed additional choline or betaine, this study had results similar to those of Anderson and Dobson (1982), who demonstrated that additional choline did not improve performance in broilers. Our results were also consistent with those of Esteve-Garcia and Mack (2000), Pillai et al. (2006a), Rafeeq et al. (2011a) and Waldroup and Fritts (2005), which all indicated no positive effects of betaine on broiler performance. Dietary treatments did have an effect on intake during the last three weeks of the study, however, gain and therefore feed conversion did not respond to dietary treatments. The effect of dietary treatments was most likely masked by the actual content of the diets (Table 5). The choline content of treatments two and three were approximately

Table 5. The actual amounts of added choline and betaine in the dietary treatments, and the percent that these values vary from what was expected.

Treatment	Additional Choline	Additional Betaine	Expected Additional Choline	Expected Additional Betaine	% of Expected Additonal Choline	% of Expected Additonal Betaine
1	0.0	0.0	0.0	0.0	0.0	0.0
2	336.5	0.0	500	0.0	67.3	0.0
3	785.7	0.0	1000	0.0	78.6	0.0
4	0.0	190.7	0.0	500	0.0	38.1
5	0.0	374.7	0.0	1000	0.0	37.5

Choline and betaine amounts are in parts per methyl.

seventy percent of what was anticipated, and the betaine contents of treatments four and five were about thirty-eight as treatment two, and the choline was from a reliable source, this was most likely an error in the percent of the expected amount. Since treatment three had roughly double the amount of choline ration formulation, and since choline is very stable, it was most likely a formulation or mixing error. The methionine values for all rations were much higher than anticipated (Table 6). Methionine has been shown to have a choline sparing effect in broiler performance (Baker et al., 1983; Augspurger et al., 2005). It was also shown, in turkey poults, that choline improved performance at adequate methionine levels, but when methionine was increased that effect disappeared (Harms and Miles, 1984). It is possible that methionine levels were above expected values due to an error in diet formulation or feed preparation. Dietary treatments did not have any discernible influence on performance, and this was most likely due to high methionine levels.

Unlike dietary treatments, in this study, temperature treatments had an effect on broiler performance. Temperature treatments were only applied the last three weeks of the study, since chickens require high temperatures during the first three weeks of life (Teeter and Belay, 1996). We found that the high temperature treatment decreased feed intake as well as elevating the feed conversion ratio. Multiple studies, through various temperature schedules, have shown high temperatures to have a negative effect on broiler performance (Dale and Fuller, 1980; Teeter et al., 1985; Sands and Smith, 1999; Bartlett and Smith, 2003; Smith et al., 2003; Quinteiro-Filho et al., 2010; Willemsen et al., 2011; Quinteiro-Filho et al., 2012; Sohail et al., 2012). This study showed decreased intake and increased feed conversion ratio in the high temperature groups during week six, indicating that these broilers did not acclimate to above optimal temperatures.

Table 6. Actual and recommended methionine levels.

	Treatments					
Stage	1	2	3	4	5	Cobb-Vantress Recommendations
Starter	0.85	0.88	0.84	0.83	0.89	0.5
Grower	0.85	0.85	0.87	0.86	0.86	0.48
Finisher	0.9	0.78	0.75	0.84	0.85	0.43

Some studies have shown broilers to acclimate after as little as four days of exposure to high temperatures (May et al., 1987; Teeter et al., 1992; Sayed and Downing, 2011b), on the contrary, we found that temperature had the biggest effect on feed conversion ratio during week six. Temperature effect on performance was consistent with current literature, except for the fact that it continued to impact performance, in some cases more so in weeks six than during week four.

Unlike production performance, dietary treatment was found to affect litter moisture content. The betaine treatments had the highest litter moisture percentages on day 41. Betaine is a known osmolyte (Simon, 1999; Thorne Research Inc., 2003; Craig, 2004), and is usually added to commercial broiler diets for this purpose (Kidd et al., 1997). Betaine has been shown to decrease litter moisture in turkey production systems (Ferket, 1995 ; Kidd et al., 1997). Since choline levels were significantly higher than the betaine levels, this increase in litter wetness cannot be explained by increased nitrogen content. The quality of the betaine, used in this study, may have been subpar. It is possible that the actual betaine content was lower than what was indicated on the label. The product was not analyzed prior to use, and therefore it is possible that there was something other than betaine in the product used, which may have increased litter moisture. On day 48 this difference, among dietary treatments, was not observed ($P = 0.4385$). The lack of difference on day 48 was most likely due to lower and inconsistent bird numbers, since some birds were harvested between day 41 and 48, and thus, bird density was reduced in most pens. Dietary treatments did have an effect on litter moisture, and since the trend went against literature and logic, this could have been due to the supplemental betaine quality. Litter moisture was also not affected by temperature treatments on either day 41 or 48. There is a lack of information linking high environmental temperatures and litter moisture content. In a production scenario, when the ambient temperature reaches heat stress range vents will be opened and fans will be turned on. This will aid in drying litter.

All remaining birds were foot scored on day 48, using a zero through three scoring system. Dietary treatments showed no effects on foot scores, indicating that dietary treatments did not promote or prevent footpad dermatitis (FPD). Many studies have linked dietary surplus or deficiency, of certain nutrients, to FPD in broilers (Harms et al., 1977; Hess et al., 2001; Bilgili et al., 2006; Eichner et al., 2007) and turkey poults (Jensen et al., 1970; Chavez and Kratzer, 1972; Chavez and Kratzer, 1974; Harms and Simpson, 1975; Harms and Simpson, 1977; Eichner et al., 2007; Abd El-Wahab et al., 2011), but neither choline or betaine has been associated with FPD in broilers. In our study, temperature treatments did not have an effect on foot score. Litter moisture and quality have been shown multiple times to increase FPD (Harms and Simpson, 1977; Ekstrand et al., 1997; Mayne, 2005; Eichner et al., 2007; Mayne et al., 2007; Bilgili et al., 2009; Youssef et al., 2010a; Youssef et al., 2011), and litter moisture is considered the one sure factor to cause FPD (Harms et al., 1977; Harms and Simpson, 1977; Martland, 1984; Martland, 1985; Eichner et al., 2007; Mayne et al., 2007; Youssef et al., 2010a; Youssef et al., 2010b; Youssef et al., 2011), however our study showed no correlation between litter moisture and foot score ($P = 0.2514$). Our study used a paper litter, which is not typically found in commercial settings, and it has been shown in turkeys that different litter materials can alter the affect moisture has on FPD incidence (Youssef et al., 2010b). Multiple dietary modifications have been shown to increase or decrease FPD incidence in broilers broilers (Harms et al., 1977; Hess et al., 2001; Bilgili et al., 2006; Eichner et al., 2007) and turkeys poults (Jensen et al., 1970; Chavez and Kratzer, 1972; Chavez and Kratzer, 1974; Harms and Simpson, 1975; Harms and

Simpson, 1977; Eichner et al., 2007; Abd El-Wahab et al., 2011), but for our study, neither betaine nor choline had this effect.

One source of broiler condemnations is ruptured gastrointestinal tracts during evisceration (Thayer and Walsh, 1993). It is known that phosphatidylcholine is one of the most common phospholipids in membrane formation (Berg et al., 2007), and because minimal research has been conducted to check if there is a link between dietary choline and intestinal integrity, we measured intestinal tensile strength on day 42. We found no dietary effect on intestinal tensile strength. This is consistent with the results of Waldroup et al. (2006). Choline has multiple paths for metabolism and intestinal membranes are made of many different molecules, therefore this lack of an effect of dietary choline supplementation on intestinal strength is not necessarily surprising.

Many studies have shown a positive relationship between heat stress conditions and mortality rates (Bogin, 1996; Teeter and Belay, 1996; De Basilio et al., 2001). Our study showed no significant temperature effect on mortality, which is similar to results obtained by Cooper and Washburn (1998). It is possible therefore that, despite degraded performance of the birds raised at high temperature, these birds were perhaps not truly heat stressed, or that their strain was more heat tolerant than those used in the aforementioned studies.

Our study showed little effect of dietary treatments on the parameters measured. This was possibly due to discrepancies in the projected and the actual content of the diets or it may be because there is a lack of real benefit to feeding supplemental choline or betaine. Esteve-Garcia and Mack (2000) showed betaine to have no significant effect on gain or feed efficiency. Similarly Rafeeq et al. (2011a) and Pillai et al. (2006b) showed that increased betaine did not significantly increase performance. Waldroup and Fritts (2005) also demonstrated no positive effects for weight gain, feed efficiency, or bird mortality. Anderson and Dobson (1982) indicated that additional choline did not improve performance in broilers, and Waldroup and Fritts (2005) showed similar results while testing choline levels similar to those used in our study. Another factor possibly affecting our results was the amount of betaine, choline, and methionine actually fed. The betaine content of our diets was less than fifty percent of what was anticipated, and methionine levels were close to double the recommended amount for Cobb 500™ broilers (Cobb-Vantress, Incorporated, Siloam Springs, AR, USA). Since supplemental methionine has been shown to minimize choline benefits in turkeys (Harms and Miles, 1984) and to have a choline sparing effect in broilers (Baker et al., 1983; Augspurger et al., 2005), it is possible that high methionine caused the lack of effect that we saw. The lack of significant effects amongst dietary treatments was most likely due to increased methionine levels. Since data is conflicting it is also possible that supplemental choline or betaine, when fed with adequate methionine, has little or no benefit

Unlike dietary treatments, we observed temperature effects on performance. Our HT birds were exposed to increased temperatures from 12:00 until 20:00, and the ambient temperatures in the HT room ranged from 30 C to >33 C during this time. Although heat stress is a caused by a combination of temperature, humidity, age and other factors (Teeter and Belay, 1996) and is hard to define (Leeson, 1986), many studies have seen decreased performance in this range (Howlider and Rose, 1989; Smith, 1993a; Yalcin et al., 1997; Yalcin et al., 2001; Quinteiro-Filho et al., 2010; Quinteiro-Filho et al., 2012). Since our HT group had decreased consumption and poorer feed conversion it may be safe to assume that we elicited some heat stress induced physiological changes. Humidity was not measured in this study therefore it was difficult to ascertain the level of heat stress our broilers experienced. Since our temperature range was similar to other studies and performance was degraded, more than likely the reason we did not see a temperature treatment by dietary treatment interaction was due to a lack of dietary treatment effect.

In conclusion, despite being considered essential, additional choline benefits to heat stressed broilers has not been fully explored. Since methionine was higher than required levels in all diets fed few conclusions about supplemental choline effects on heat stressed broilers can be made. This indicates that more research needs to be conducted, with more care being given to ration formulation and assembly.

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