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I am submitting herewith a thesis written by Candy Ranee Lint-Kessler entitled "Effects of betaine supplementation on growth performance, carcass characteristics and blood parameters of broilers reared under thermoneutral or heat stress conditions." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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Effects of betaine supplementation on growth performance, carcass characteristics, and blood parameters of broilers reared under thermoneutral or heat stress conditions

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

Candy Ranee Lint-Kessler
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Dedicated to my parents Rick and Vicky Lint,
my sister Spring and my brother Rickey whose
encouragement has always given me strength
and to my husband, friend, and fellow adventurer Matt

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Abstract

Betaine has been studied as an osmolyte and methyl group donor for many species. Recent studies in pigs have found that betaine is able to act as an energy nutrient in the diet. The purpose of this study was to observe its effects in broiler diets, on growth performance, carcass characteristics, blood parameters, pH, hematocrit, plasma Na and K levels of heat-stressed and non-heat stressed broilers, and investigate the possibility that betaine may improve intestinal strength. In the first experiment, thirteen hundred and eighty one day old male broilers (Cobb x Cobb) were obtained. Six replicate pens were assigned to each of five treatments with 46 birds per pen. Diets were supplemented with 0 grams betaine/metric ton, 879 g/ton, 1,209 g/ton, and 1,539 g/ton. Birds and feed were weighed on days 1, 21, 42, and 49. On day 49, eight birds from each pen were selected and slaughtered. At slaughter, carcass weight without giblets (WOG), breast weight, and abdominal fat was recorded. Gut strength was determined using an instron test stand/load cell machine.

In Experiment two, 21 day old broilers (Cobb x Cobb) were moved from floor pens to each of two environmental chambers. Diet treatments were the same as Experiment 1, in addition to a sixth treatment in which the basal diet was supplemented with 1,868g betaine/metric ton. There were 20 birds per chamber for each of the six dietary treatments. One chamber was maintained at 23.9°C (thermoneutral), and the other chamber was cycled as follows: 10 hours at 23.9°C, 3 hours to 35°C, 8 hours at 35°C, and 3 hours to 23.9°C (heat stress). Weights of birds and feed were measured on days 21, 28, 35, 42, and 49 and water consumption was recorded daily. Blood samples

were taken on day 28, 35, and 45, and blood pH, hemotocrit, and plasma Na and K were measured. On day 49, all birds from both chambers were slaughtered. Carcass weight WOG, breast weight, abdominal fat, and gut strength were measure.

In Experiment 3, high density and low density diets were compared with and without the addition of betaine. Fourteen hundred and seventy-two straight-run broilers (Ross x Cobb) were obtained at one day of age. Birds were divided into groups of 46 and placed into randomly assigned floor pens. Eight replicates of four treatments were used. Treatment 1 was a high density diet with no betaine added, treatment 2 was a high density diet with 1209 g betaine/metric ton, treatment 3 was a low density diet, and treatment 4 was a low density diet with the addition of 1209 g betaine/metric ton. Birds and feed were weighed on days 1, 21, 42, and 49 of the experiment. On day 49, eight birds (four males and four females) were selected from each pen and slaughtered. The pre-slaughter weight, carcass weight WOG, breast weight, and abdominal fat were measured and recorded. Intestinal strength was also measured.

The addition of betaine to the diet did not improve growth performance of broilers given reduced energy diets, or low density diets. When birds were exposed to heat stress conditions, there was no significant difference between dietary treatments ($P > 0.05$) on broiler performance, live weight, carcass weight, and breast weight. Adding betaine to the diets of heat stressed broilers did not alleviate the deleterious effects of high ambient temperatures. Heat stressed birds were significantly smaller than their thermoneutral counterparts, and had significantly lower carcass yields. There were no significant differences among dietary treatments for the blood parameters measured, however heat

stress altered pH, hematocrit and plasma Na and K levels. Broilers that were fed low density diets had significantly lower feed consumption, gain, and did not utilize feed efficiently, thus birds given low density diets also had significantly smaller carcass values, and the addition of betaine to the diet did not affect growth performance of these birds.

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Chapter 1

Introduction

Broiler production continues to grow in the southeastern United States with the five top broiler producing states being Georgia, Arkansas, Alabama, North Carolina, and Mississippi respectively (USDA, 1998). Along with milder temperatures in the winter, this area of the U.S. also experiences hot, humid summers which can be detrimental to poultry flocks if not managed properly. Decreased feed intake, decreased gain, decreased feed efficiency, and increased mortality are just a few examples of the adverse effects producers face when temperatures rise, and can result in significant economic losses. House management techniques such as providing proper ventilation, adequate water, and removing feed to reduce metabolic heat have proven effective, however researchers are learning that diet manipulation may also provide relief (Teeter et al., 1985).

Betaine (trimethyl glycine), an extract from sugar beets, has been found to act as an osmolyte and a methyl group donor for methylation reactions (Virtanen, 1995). In poultry diets, betaine may spare methionine, reduce osmotic disorders, and improve nutrient utilization under stress (Virtanen, 1995). Betaine supplementation to broiler diets has resulted in increased gain (Virtanen and Rumsey, 1996) increased breast weight (Virtanen and Rosi, 1995; Lobo, 1999) and a decrease in intestinal lesion scores (Remus et al., 1995). A review of cellular physiology is important to facilitate an understanding of betaine's ability to accomplish these effects.

Osmoregulation is the ability of a cell to maintain its structure and function by regulating movement of water in and out of the cell (Kidd, 1997). During times of

osmotic stress, such as heat stress conditions, the cell becomes hyperosmotic, resulting in dehydration as water is lost. Water moves from high concentration to low concentration, and the cell can not control its movement because it is freely permeable to cell membranes. Cells can however, use ion pumps to regulate electrolyte levels within the cell and help attract water back into the cell. This method of regulation costs 1 ATP for every K^+ pumped into the cell, so can only be used as a temporary solution. Another problem that occurs after stress is prolonged is that the high level of electrolytes within the cell begin to interfere with the enzyme activity. Organelles most sensitive to this are mitochondria. Since it is the mitochondria that supplys the energy for the ion pumps, it has a harder time meeting the demands of the cell as mitochondrial enzymes are being affected (Remus, 1998).

The cell can also controls the movement of water through the use of betaine. The use of betaine during osmoregulation occurs in many forms of life, including bacteria (Chambers and Kunin, 1987) plants (Yancey et al., 1982) and animals (Law and Burg, 1991). Betaine accumulates in the cell either by synthesis or by the uptake of dietary betaine (Remus,1998). Betaine is primarily obtained by active uptake although a carrier has not been identified in farm animals. The carrier concentrates betaine in the cell until the level inside the cell is greater than the level outside of the cell. This will attract water back into the cell. The advantage of using betaine is that it does not interfere with the mitochondrial enzymes. Betaine accumulation does not interfere with energy production of the cell, and it does not cost the cell as much energy as the ion pump (Remus, 1998).

Betaine has also been studied as a methyl group donor. This is important because animals cannot synthesize methyl groups, and it must be supplied in the diet. There are

three major sources of methyl groups found in practical diets; betaine, choline and methionine (Finnsugar Bioproducts, 1996). In early studies using betaine, it was determined that betaine was formed from choline. Betaine will then donate a methyl group to homocysteine to form methionine, and ultimately it spares methionine as a methyl donor by recycling homocysteine (Kidd 1997). This is beneficial when the diet is supplying an inadequate source of methyl groups.

Some preliminary studies have been conducted to observe the effects of betaine when added to diets of coccidia infected birds to improve gut strength. Coccidiosis is a condition that affects the ionic balance in the gut and also affects the intestinal morphology, including shortened gut length and truncated intestinal villi (Finnsugar Bioproducts, 1996). When betaine was added along with an ionophore coccidiostat in the feeds of coccidia-challenged birds, an improvement in nutrient absorption was observed. This improvement resulted in higher weight gains (Finnsugar Bioproducts, 1996). More studies are needed before conclusions can be made about the potential benefits of adding betaine to poultry diets.

The following experiments were conducted to assess the effects of betaine supplementation on growth performance, carcass characteristics, blood parameters, and intestinal strength, of broilers reared under thermoneutral and heat stress conditions.

Chapter 2

Literature Review

Heat Stress

Heat stress occurs in chickens when the ambient temperature and humidity extend above the bird's comfort zone (Smith, 1993). Exposure of broilers to high temperatures results in decreased feed intake, feed efficiency, increased mortality, increased risk of disease, and smaller carcasses. The increased growth rate of commercial broilers causes an increase in metabolic heat production, while the ability to dissipate heat does not; making commercial broilers more susceptible to heat stress (Teeter et al.,1996). Heavier breeds generally have increased difficulty with heat dissipation because they have a smaller surface area per unit of weight (Teeter et al.,1996). In order to lower metabolic heat, birds decrease their feed consumption to reduce overall heat load (Teeter et al., 1996). Ain Baziz et al. (1996), found that the decrease in feed consumption was about 3.6% per degree increase between 22 and 32 °C, and the reduction in growth was greater than that accounted for by reduction of feed intake.

It has been shown that ambient temperatures of 32 °C or greater will result in significant increases in body temperature (Cooper and Washburn, 1998). Birds lose excess heat to the environment by the processes of radiation, conduction, convection and evaporation of moisture (Whittow, 1965). Radiation heat loss occurs only when the temperature of the surface of the animal is greater than the temperature of the surface of the surrounding objects. If the temperature of the bird is greater than the surface of the

cage, for example, the cage will absorb some of the heat from the animal. Conduction accounts for only a very small amount of heat loss, and is the heat that is lost through direct transfer from the bird's body surface, to the air or any solid objects with which the bird may come into contact. Heat is lost through convection when the air that comes in contact with the skin's surface is warmed. This warmer air is less dense and therefore rises, allowing cooler air to replace it. Moving air helps to increase the amount of heat that can be lost by this method of cooling. It should be noted that convection works best when the temperature of the moving air is lower than the temperature of the body surface. The final method of cooling that can be used by broilers is evaporative cooling. Because birds do not have sweat glands, their primary means of evaporative cooling is through panting. In a study performed by Harrison and Biellier (1969), it was found that the chickens begin panting when their body temperature rises to 1.22 °C above their initial body temperature, and birds will begin to pant after 30 minutes to 2 hours of exposure at 35 °C.

All of the methods of heat transfer can be divided into two categories: nonevaporative cooling and evaporative cooling. Nonevaporative cooling is used by almost all poultry, and is the principal means of heat dissipation when birds are exposed to low to moderate ambient temperatures (Teeter et al.,1996). This includes heat lost through radiation, conduction and convection, and involves changes in the circulatory system. Vasodilation of blood vessels, and increased cardiac output allow the bird to dissipate heat by bringing heat from internal organs to the surface of the skin where it can be lost (Yahav et al., 1997). Heat exposure has also been found to increase capillary

blood flow in the comb, wattle, skin of the breast, and tissues that are associated with evaporative heat loss, such as the trachea (Wolfenson et al., 1981).

Evaporative cooling allows broilers to lose heat through the evaporation of water. As ambient temperatures increase,and nonevaporative cooling can no longer alleviate the effects of the external environment, respiratory evaporation becomes a more important means of heat loss (Siegel, 1968). Panting occurs when the body temperature reaches approximately 42 °C, and along with this, respiratory frequency and minute volume increase while amplitude and tidal volume decrease (Whittow, 1965). Siegel preformed studies to determine broilers' response to air temperature and air velocity. Results indicated a significant increase in respiratory rates of birds at higher ambient temperatures, demonstrating the bird's dependence on respiratory evaporation as a means of heat exchange. Birds that were placed in chambers with high ambient temperatures and low air velocities had the highest increase in respiration, while high air velocities at high ambient temperatures helped to decrease respiration rates. When temperatures were held at 40 °C for a prolonged period of time, however, the differences between the air velocity rates were not as significant, indicating that high air velocity can not prevent accumulation of body heat over a prolonged period of time (Siegel, 1968).

Respiration plays a key role in regulating the body's acid-base balance. The lungs work to control the levels of extracellular CO_2 concentrations by increasing pCO_2 in extracellular fluid to decrease blood pH when it is elevated, or increasing the pCO_2 to raise blood pH when it is too low (Guyton and Hall, 1996). When overventilation of the lungs occurs, such as panting, the condition of respiratory alkalosis develops (Guyton and

Hall, 1996). Data indicate that broiler chicks that are exposed to chronic heat stress conditions will develop respiratory alkalosis (Teeter et al., 1985). The increase in blood pH causes a decrease in feed consumption, which, ultimately slows growth rate. Although evaporative cooling is necessary for survival during heat stress, it ultimately has a negative effect on productivity or feed efficiency of the animal.

Heat stress can also have a significant impact on carcasses and meat quality. Smith (1993) found that broilers raised under cycling high temperatures gained 21% less weight ($P<.05$) than those raised in a thermoneutral environment (23.9 °C). This study also revealed that whole breast weight and breast yield was significantly greater in the birds raised under thermoneutral conditions. The leg quarter and thigh weights were also lower in the heat distressed birds, while the drumstick, wing, and skeletal frame weights were not significantly different for either of the two environmental treatments. An earlier study conducted by Howlider and Rose (1989) also looked at meat yield of broilers exposed to high ambient temperature. In this study, birds were not slaughtered until they reached predetermined weights, and it was found that the broilers raised at 31 °C took longer to reach the slaughter weights than those birds raised at 21 °C. It was also determined that females ate more food than males in order to reach slaughter weight for both of the temperature groups making them less efficient at feed conversion. Females deposited more fat and had a greater skin weight than males for both temperature groups, but the females did have a greater amount of breast meat for both treatments when compared to males. A reduction of the basal metabolic rate and the physical activity of the birds under heat stress may cause spared energy to be stored as fat (Ain Baziz et al.,

1996). Results of fat measurements have been mixed, however, and some studies have shown a decrease in the amount of fat deposited (Smith and Teeter, 1987b).

Several management practices are available to producers to combat the deleterious effects of heat stress. Management options include: building the poultry house with an east-west orientation, making sure the roof has an overhang to help provide shade from the sun, and providing proper ventilation. When bird survival becomes the main objective, fasting should probably be the management option of choice. Fasting has been shown to decrease heat production by as much as 50%, and has been shown to decrease mortality during heat stress (Teeter, 1996). Water management including providing cool water, or adding electrolytes to the water to increase water intake will also help with production losses during the hot summer months (Teeter et al., 1996). A study performed by Lott (1991), looked at the effect of feed intake on body temperature and water consumption of male broilers exposed to heat stress. It was found that feed in the digestive tract of birds during exposure to heat was detrimental to the ability of the birds to maintain body temperatures below lethal levels. If feed was removed from birds prior to heat exposure, then water consumption initially decreased, which caused an increase in body temperature. In unacclimated birds, increased water consumption appears to be associated with a lower body temperature. It was concluded that water consumption is a major component of the broilers' ability to acclimate to high temperatures, and the time at which feed is removed plays an important role in survival.

Electrolytes

An electrolyte is an ionized salt found in blood, tissue fluids, and cells, with the most common being sodium, potassium and chloride (Thomas, 1997). The most important electrolytes found within the cell are potassium, magnesium, phosphate, sulfate, bicarbonate, and in smaller quantities, sodium, chloride, and calcium. Electrolytes provide inorganic chemicals for a variety of reactions that occur in the cell (Guyton and Hall, 1996), and help to maintain water balance.

The importance of electrolyte balance becomes evident during heat stress conditions. As the consumption of water increases, with increasing ambient temperatures, there is an increase in urinary output. Respiratory alkalosis decreases the competition between hydrogen and potassium for urinary excretion and resulting in an increase in urinary potassium loss (Smith and Teeter, 1987a). Birds that are placed in environments of 35 °C were found to excrete 27.3% more K^+ than birds housed at 24 °C. Results also indicated that the net K^+ loss by heat stressed broilers was 633% greater than the potassium loss of broilers housed in thermoneutral conditions. Previous studies found that turkeys placed in heat stress conditions had a significant decrease in the plasma concentrations of sodium, calcium, and magnesium and inorganic phosphorus, while the concentration of plasma potassium was significantly increased (Kohne and Jones, 1975).

The results of an experiment performed by Smith and Teeter (1987a) determined that the level of dietary potassium that is considered adequate under thermoneutral conditions may not be sufficient for birds exposed to high ambient temperatures because of the increase in potassium loss. It was also found that when KCl was added to the drinking water, the negative effects of heat stress on growth and feed efficiency were

decreased. Because of the electrolytes close association with thirst and water consumption, they are able to decrease mortality and minimize the performance reductions that occur when broilers are under heat stress. Ammonium chloride, and sodium bicarbonate along with potassium chloride added to feed or water have been proven beneficial in heat stress situations (Hooge, 1998).

Betaine, methionine, choline and their interrelationship

Betaine has been described as a methyl group donor, and to understand the significance of adding betaine to poultry diets, it is important to understand how it interacts with other methyl group donors, specifically methionine and choline.

Betaine

Betaine (glycine betaine, trimethylglycine) is the designation used for the compound 1-carboxy-N,N,N-trimethylmethanaminium hydroxide (Finnsugar Bioproducts, 1996). Betaine has a molecular weight of 117.15 and is freely soluble in water. It is a zwitterionic quaternary ammonium compound (Fig. 1) that can also be

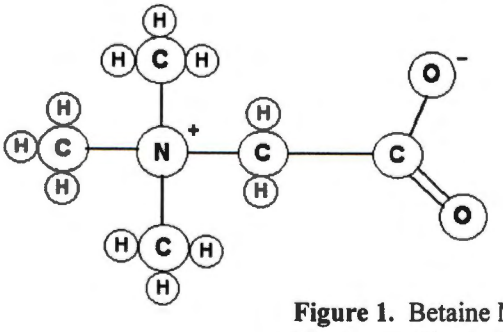


Figure 1. Betaine Molecule
(Finnsugar Bioproducts, 1996)

characterized as a methylamine because it has three reactive methyl groups that are attached to the nitrogen atom of a glycine molecule (Yancey et al., 1982). Betaine is the end product of a two-step oxidation reaction of choline: choline→betaine aldehyde→betaine (Dragolovich, 1994), and it is commercially extracted from sugar beets. The potential for betaine use in the poultry industry is its ability to function as a methyl group donor in the transmethylation cycle, and its ability to function as an organic osmolyte. Betaine may also interact with lipid metabolism by stimulating the oxidative catabolism of fatty acids through its role in the synthesis of carnitine (Schutte et al., 1997). This may potentially result in a decrease in carcass fatness.

Methionine

Methionine is recognized as an essential amino acid for birds and mammals (Ewing, 1963). The availability of methionine is necessary for normal growth and development of mammals (Finkelstein, 1990) and methionine is an essential amino acid for poultry (Schutte et al., 1997). This amino acid provides methyl groups needed in several metabolic reactions, including protein synthesis, synthesis of carnitine and creatine, and the transfer of CH_3 groups to DNA and RNA (Finnsugar Bioproducts, 1996, Schutte et al.,1997). More than one hundred methylation reactions involving methionine are known (Dudley-Cash, 1996). Methionine is formed in the liver by the enzyme betaine-homocysteine methyltransferase, which as its name implies, transfers a methyl group from betaine to homocysteine, forming methionine (Kidd et al., 1997).

Methionine plus adenosine triphosphate (ATP) forms S-adenosyl methionine which itself is a major donor of methyl groups (Kidd et al., 1997). When methionine is

available in excess, the carbons can be used for energy or for gluconeogenesis, while the sulfur is retained for the formation of the sulfhydryl of cysteine (Devlin,1997). The formation of S- adenosylmethionine (AdoMet, or SAM) from methionine and adenosine triphosphate is facilitated by the enzyme methionine adenosyltransferase. The methyl group of SAM is a very good leaving group, and can be transferred and used in a variety of reactions including, methylation of DNA, synthesizing carnitine from lysine, epinephrine from norepinephrine and creatine from guanidine acetate (Simon,1999).

After the methyl group is removed with the aid of the enzyme methyltransferase, the resulting molecule is S-adenosylhomocysteine. The enzyme adenosylhomocysteinase removes the adenosine group forming homocysteine. When homocysteine and serine are combined by the enzyme cystathionine synthase, water is given off, and a new molecule, cystathionine is formed. Cystathionine is then broken down into α-ketobutyrate and cysteine by the enzyme cystathionase. α-Ketobutyrate is decarboxylated through a multienzyme complex, resulting in the formation of propionyl CoA which is the end product of methionine metabolism (Devlin, 1997).

Choline

Choline, along with manganese, folic acid, nicotinic acid, and biotin, is necessary for the prevention of perosis (slipped tendon) in young chicks (Nesheim et al., 1979). It is a vitamin that needs to be present in larger amounts than other vitamins, because diets lacking sufficient choline will result in decreased growth, poor feed utilization, and perosis (Nesheim et al., 1979). Choline plays an important role in fat metabolism and a deficiency in choline is associated with the development of fatty liver due to a lack of

assembly of lipoproteins (Simon, 1999). Chicks may at times synthesize choline, but the amounts are insufficient, and choline must therefore be added to starting rations (North, 1984). The older a bird gets the more efficient it becomes at synthesizing choline (North, 1984), and hens seem to be able to synthesize choline more efficiently than males (Nesheim et al., 1979). Good sources of choline include: fish meal, fish solubles, yeast, liver meal, soybean oil meal, and distillers solubles (North, 1984).

Choline is a constituent of several phospholipids including; phosphatidylcholine, lysophosphatidylcholine, sphyngomyelin, lysosphyngomyelin and the plasmalogens (Simon, 1999). These phospholipids help to maintain the integrity and functions of the organelle and cell membranes (Simon, 1999). When acetyl CoA is present, choline is acetylated by the enzyme choline acetyltransferase to form acetylcholine, a neurotransmitter involved in the parasympathetic nervous system (Simon, 1999). The parasympathetic nervous system controls such organs as the heart, liver, pancreas, gallbladder, and peristalsis of the intestines (Guyton,1996).

Relationship between betaine, methionine, and choline

There are two pathways that allow a methyl donor to provide a methyl group to methionine, indicating how important methionine is (Fig. 2). The first pathway, is the FHMT pathway. This pathway is catalyzed by the enzyme 5-methyltetrahydrofolate-homo-cysteine methyltransferase (FHMT). This enzyme converts homocysteine and 5-methyltetrahydro-folate (the methyl group donor), to methionine and tetrahydrofolate, respectively (Finnsugar Bioproducts, 1996).

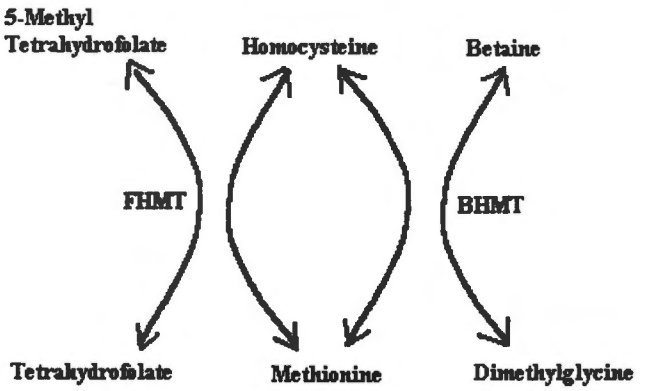


Fig. 2 The formation of methionine by action of 5-methyltetrahydrofolate-homocysteine (FHMT) and betaine-homocysteine (BHMT) methyltransferase.(Finnsugar Bioproducts, 1996)

The second pathway is catalyzed by the enzyme betaine-homocysteine methyltransferase (BHMT). Choline is oxidized to betaine by the enzyme choline oxidase (Kidd et al., 1997). Hydrolysis of betaine generates one free methyl group and the molecule dimethylglycine. The free methyl group is transferred to homocysteine by the action of the enzyme betaine-homocysteine methyltransferase (BH-methyltransferase or BHMT) (Simon,1999). Dimethylglycine is further degraded to sarcosine and glycine (Simon, 1999). When methionine is deficient, there is an increase in BHMT activity and this is especially true when choline and betaine are in excess (Emmert et al., 1996). Saunderson and MacKinlay (1990), found that of these two pathways, the FHMT pathway was less active than the BHMT pathway which utilizes betaine. The FHMT pathway may therefore be a safety measure when betaine is not readily available (Remus, 1998).

Betaine has been found to methylate homocysteine to methionine approxamately three times more efficiently than choline (Stekol et al., 1953). The conversion of choline to betaine is inefficient because, choline must first be transported from the cytosol into the mitchondria where it is oxidized to betaine (Kidd et al, 1997). Betaine is then transported across the mitochondrial membrane into the cytosol where it can function as a methyl group donor (Mann et al., 1938). Polyether ionophores, commonly used to control coccidiosis in poultry, interfere with mitochondrial membrane transport which further reduces the efficiency of converting choline to betaine (Tyler, 1977). Addition of betaine to the diets of broilers could spare choline and methionine which would be beneficial when diets are low in methionine, or the birds are exposed to stressors, such as coccidiosis or heat stress.

Betaine as an Osmolyte

Osmoregulation is the ability of a cell to maintain its structure and function by regulating movement of water in and out of the cell (Kidd et al., 1997). Because of the high permeability of cell membranes, the net water movements across plasma membranes are primarily driven by the osmotic gradient (Haussinger, 1996). An osmolyte should not be disruptive to the cell, and it should not interfere with protein function even when the osmolyte is at a high intracellular concentration (Haussinger, 1996). Marine invertibrates, which have a high blood osmolarity, use organic compounds to regulate approximately 60-70% of their intracellular fluid osmolarity (Kidd et al., 1997). Organic

osmolytes do not interfere with enzyme function, and differences in their intracellular concentrations will not upset metabolism (Yancey et al., 1982; Draglovich, 1994).

The inorganic intracellular osmolytes include potassium, magnesium and phosphate. The abilities of these osmolytes are limited by the fact that high concentrations of these ions during osmotic stress seriously affect metabolic function, and the maintenance of proper transmembrane potential becomes difficult (Yancey et al., 1982). In response to long-term hyperosmotic stress, cells rely on the accumulation of organic osmolytes (Burg, 1995). The major organic osmolytes found to increase in cells of the renal medulla in water deprived birds were, *myo*-inositol, betaine, and taurine (Lien et al., 1993).

Research has shown that when cells are placed under hyperosmotic conditions there is an inhibition of general cell protein synthesis and the rate of cell proliferation decreases (Petronini et al., 1992). Various solutes were tested to observe their effects on SV-3T3 cells (Simian-virus-40-transformed Balb/c 3T3 cells) placed in a hyperosmotic medium. The cell growth rate was significantly decreased when the cells were placed in the hyperosmotic medium, but the addition of 10 mM-glycerol, -proline, -taurine, or –betaine each produced some increase in the level of proliferation. Betaine, in particular, produced a marked increase in the rate of proliferation of cells placed in hyperosmotic medium. Betaine was also able to protect the cells against the inhibition of protein synthesis that was normally seen in cells placed in hyperosmotic conditions (Petronini et al., 1992).

It is known that *Escherichia coli* and *Staphylococcus typhimurium* will accumulate betaine through increased transport when extracellular osmolality rises (Nakanishi et al., 1990). When Madin-Darby canine kidney (MDCK) cells were placed in culture, they were found to accumulate betaine when extracellular osmolality was increased. It was determined that the accumulation of intracellular betaine requires that betaine be supplied in the medium, and that this accumulation is a result of the uptake of extracellular betaine rather than synthesis by the cells (Nakanishi et al., 1990). It was also discovered that the mode of transport of betaine into the cell is mediated by sodium-dependent betaine transporters.

Betaine may have a stabilizing function in cells that are suffering from osmotic and ionic imbalance (Virtanen, 1996). One of the major intestinal diseases associated with osmotic and ionic disorders is coccidiosis and the ionophoric drugs that are used to treat the disease can cause changes in the ionic and osmotic balance (Virtanen, 1996). Coccidiosis is caused by protozoan parasites of the genus *Eimeria*. The main concern when flocks become infected is the occurance of dehydration due to diarrhea. Many studies have been done to investigate betaine's potential in alleviating the loss of water. Remus et al. (1995), found that the addition of betaine along with the coccidiostat salinomycin to the diets of broilers that were challenged with coccidiosis improved intestinal lesion scores as well as improving the feed conversion ratio of the challenged birds. A similar study investigated the effects of betaine on the growth performance of chicks that were inoculated with mixed cultures of avian *Eimeria* species. Results indicated that betaine in combination with salinomycin in the diets of chicks was able to

protect against a moderate, mixed infection of *E. acervulina*, *E. tenella*, and *E. maxima*. The protection was significantly greater than that of betaine or salinomycin alone. Even when salinomycin alone was able to control the infection, the addition of betaine increased the performance of the chickens. The intestinal lesion scores were also improved, and mortality was significantly decreased in chickens fed betaine and salinomycin (Augustine et al., 1997a). Another study revealed through electron microscopy, that there were ultrastructural changes in the intestinal cells of chickens fed betaine. The cells were less dense indicating that they may have an increased capacity to hold water. This would be beneficial to birds that are infected with coccidiosis by decreasing the risk of dehydration (Augustine et al., 1997b).

Betaine's ability to provide osmotic stability during both coccidiosis challenge, and heat stress was also studied. Mooney et al. (1998), examined betaine as an osmoprotectant for broilers exposed to both high ambient temperatures and coccidiosis. Results from the study showed that betaine was able to improve water retention, especially during heat stress conditions.

Betaine and Animal Production

Several studies have been done investigating betaine as a supplement in the diets of fish, pigs, turkeys and chickens. The goal of producers is to increase feed efficiency and reduce losses when these animals are exposed to various stressors.

For fish producers the potential for betaine to work as an osmolyte is appealing especially to those hatcheries that raise salmon. Salmon are first raised in freshwater

tanks for up to a year before they are placed into saltwater. This transition poses an osmotic stress on the fish and results in production losses. One study investigated the effects of a dietary betaine/amino acid additive on growth and seawater adaptation in yearling chinook salmon (Clarke et al., 1994). Results from this study found that the group of fish that received betaine in the diet had a significantly higher growth rate, also when placed in seawater, the fish receiving the betaine had an improved overall growth performance possibly due to betaine's ability to reduce osmoregulatory stress.

Betaine as a dietary additive for pigs has shown mixed results. Studies done in Australia demonstrated that betaine was able to reduce backfat measurements and increase loin eye area (Zabaras-Krick, 1997). It is not certain how betaine is able to decrease backfat, but it is hypothesized that betaine may improve energy utilization by muscle tissue which in turn, diverts nutrients away from lipid deposition in adipose tissue. Other experiments that evaluated betaine supplementation on carcass characteristics and growth performance found that betaine did not alter growth performance or carcass characteristics (Cera et al., 1995,Webel et al., 1995). Smith et al., (1995) found that although betaine supplementation did not have a significant effect on backfat depths, the pigs fed betaine had a greater loin depth.

In some commercial turkey flocks a diarrhea, or "flushing" syndrome develops for reasons yet unknown. The addition of betaine to drinking water of infected birds was able to alleviate symptoms within 24 to 48 hours, although the mechanism is not known at this time. An investigation of betaine as an osmolyte in turkeys, found that betaine acted as an osmolyte during osmotic stress (Shin et al., 1998). Although birds in the study

did not have diarrhea, they were instead given diets with excess Mg, Na and protein to increase the intestinal lumen osmotic pressure. When betaine was provided in the drinking water, and uptake by the intestinal epithelium was measured using HPLC, it was determined that there was an increased level of betaine in the jejunal epithelial cells. The increase of betaine level within the cells allows it to function as an osmolyte.

Lobo (1999) contends that enhancing the performance of existing physiological systems will be the key that helps to meet the goal of producing the highest quality meat at the lowest possible cost. Studies have been done to investigate the possibility that dietary manipulation may help to enhance growth during heat stress by reducing the effects of alkalosis. When calcium chloride was added to the diet, body weight gains were increased (Teeter et al., 1985), indicating that manipulating the diet can in fact help to alleviate the effects of respiratory alkalosis experienced during heat stress.

Since betaine has been shown to act as an osmolyte, inclusion in the diets of heat stressed poultry may prove beneficial. When betaine was added to the diets of broilers exposed to high ambient temperatures, it had a positive impact on water balance and thermobalance of the birds (Mooney et al., 1998). Some evidence has shown that betaine may also improve feed efficiency during times of heat stress (Lobo, 1999). Although the results were not significant, the trend indicated that there was a numerical increase in the final body weight of betaine-fed birds.

The following experiments were conducted to investigate the effects of betaine addition to various diet formulations, and under both heat stress, and thermoneutral conditions.

Chapter 3

Materials and Methods

Experiment 1

Thirteen hundred and eighty one day old male broilers (Cobb x Cobb) were weighed in groups of 46, and randomly assigned to floor pens. Six replicate pens were assigned to each of five treatments. Dietary treatments (Table 1 and 2) were as follows; treatment 1 normal metabolizable energy diet (3000 kcal/kg 1-21 days, 3150 kcal/kg 21-42 days, 3200 kcal/kg 42-49 days) treatment 2 a reduced energy level diet (2% drop, 2938 kcal/kg 1-21 days, 3087 kcal/kg 21-42 days, 3136 kcal/kg 42-49 days) and treatments 3,4, and 5 were the reduced energy diet with 879, 1209, and 1539 grams of betaine per metric ton added respectively. Feed and water were provided for ad libitum throughout the experiment. Chicks were grown in a 23 hour light: 1 hour dark cycle with incadescent light supplementation. Birds were weighed as a group on days 21, 42, and 49 of the experiment. Any birds that died during the experiment were weighed and the date of death, and the weight of the bird were recorded. On day 49, eight birds from each pen were weighed and then slaughtered after a 12 hour period in which feed was removed while water continued to be available. Birds were slaughtered using the following procedures. Each bird was hung on a rail, stunned with an electrical knife, and then killed by severing the jugular vein and allowing a 4 minute bleeding time. The carcasses were placed in a vat of scalding water (60 °C) for 2 minutes to loosen the feathers. The feathers were removed from the carcass by a rotary drum picker and then were placed

Table 1. Composition of basal diets (Experiment 1 and 2)

Ingredient	Starter		Grower		Finisher	
	Normal	Reduced	Normal	Reduced	Normal	Reduced
	(%)					
Corn	56.03	57.99	53.70	54.57	57.22	58.80
Soybean Meal	27.39	26.47	27.85	27.72	28.07	27.99
Fish Meal	6.09	6.53	3.00	3.00	0.50	0.50
Rice Bran	3.00	3.00	4.54	5.00	2.35	2.26
Fat[1]	1.84	0.45	5.00	3.94	4.91	3.64
Di-Calcium Phosphate	1.57	1.57	1.44	1.43	1.33	1.33
Corn Gluten Meal[2]	1.49	1.45	1.85	1.72	2.88	2.74
Vitamin Mix[3]	1.00	1.00	1.00	1.00	1.00	1.00
Limestone	0.79	0.74	0.88	0.88	1.02	1.02
Salt (NaCl)	0.38	0.38	0.30	0.30	0.30	0.30
Sand[4]	0.20	0.20	0.20	0.20	0.20	0.20
DL-Methionine	0.12	0.12	0.13	0.13	0.10	0.10
Sacox[5]	0.10	0.10	0.10	0.10	0.10	0.10
Choline	---	---	0.01	0.01	0.02	0.02
Total	100	100	100	100	100	100
Nutrient Composition						
ME kcal/kg	3000	2938	3150	3087	3200	3136
CP[6]	21.9	21.9	20.5	20.5	19.7	19.7

[1]A mixture of soybean oil and corn oil; Tennessee Farmers Cooperative, La Vergne, TN 37066
[2]Provided by AE Staley; Loudon, TN
[3]Supplied per kilogram of diet: Copper, 8mg; Iodine, 0.4mg; Iron, 100mg; Manganese, 100mg; Selenium, 0.3mg; Zinc, 75mg; Vitamin A, 8800 IU; Vitamin D_3 2992 ICU; Vitamin E, 29.9 IU; Vitamin B_{12}, 0.02mg; Menadione, 1.65mg; Biotin, 0.2mg; Folic Acid, 1.0mg; Niacin, 66mg; d-Pantothenic Acid, 11mg; Vitamin B_6, 4.4mg; Riboflavin, 6.6mg; Thiamine, 1.1mg
[4]Beatine added at the expense of equivalent amount of sand
[5]Sacox (Salinomycin) Hoechst Roussel Vet, Warren NJ 07059
[6]CP = crude protein

Table 2. Betaine and Methionine Composition of Experimental Diets (Experiment 1 and 2)

	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5	Trt 6
Starter						
Betaine (mg/g)	**bdl***	**bdl**	**0.95**	**1.29**	**1.64**	**1.91**
Methionine (mg/g)	**1.15**	**1.08**	**0.98**	**0.96**	**0.97**	**1.03**
Grower						
Betaine	**bdl**	**bdl**	**0.99**	**1.25**	**1.64**	**1.95**
Methionine	**1.16**	**1.14**	**1.05**	**1.03**	**1.09**	**1.04**
Finisher						
Betaine	**bdl**	**bdl**	**0.89**	**1.31**	**1.97**	**2.21**
Methionine	**1.41**	**1.11**	**1.15**	**1.05**	**1.25**	**1.31**

*bdl = below detectable limits

in a chilled ice bath for approximately one hour. The carcasses were then manually eviscerated, and the weight of the carcass without giblets, breast weight, and the weight of the abdominal fat were measured and recorded. The abdominal fat comprised of the fat that surrounded the bursa of Fabricius, cloaca, and abdominal muscles. The intestinal strength was also determined on the ileum using an instron test stand/load cell (Chatillon TCM-201/Chatillon DFIS 2)[1] machine. Two areas of the small intestine were measured, 25 cm above the diverticulum and 25 cm below the diverticulum.

Experiment 2

Cobb x Cobb broiler chicks were raised in floor pens and were provided diets as described in Experiment 1 plus a sixth dietary treatment (1868 g of betaine/metric ton added to the reduced energy diet). On day 21 of the experiment, the birds were assigned to individual cages within each of two environmental chambers. There were 20 birds per chamber for each of the six dietary treatments. One chamber was maintained at a temperature of 23.9°C, indicative of thermoneutral conditions, and the second chamber was allowed to cycle as follows: 10 hours at 23.9°C, 3 hours to 35°C, 8 hours at 35°C and 3 hours to 23.9°C. This cycling of temperatures exposed the birds to heat stress conditions. The photoperiod was held at 23 hours of light/1 hour of dark. Feed and birds were weighed on days 21, 28, 35, 42, and 49 of the experiment. Water consumption was measured and recorded daily, and any bird that died during the experiment was weighed and the date of death and weight of the bird were recorded. Blood samples were taken

[1] Chatillon 7609 Business Park Drive Greensboro, NC 27409

from the wing vein on days 27, 35, and 49. Blood pH, taken immediately after collection was determined using a portable pH meter[2]. The blood samples were then centrifuged for 10 minutes in a microhematocrit centrifuge, and hematocrit values were read and recorded. The plasma was separated, chilled and stored at - 20°C for later analysis. The plasma samples were analyzed for Na and K using a Solaar 969 Atomic Absorption/Atomic Emission spectrophotometer[3]. Samples were prepared as follows: the plasma that was analyzed for sodium was brought to a 1: 10,000 dilution by adding deionized water. The plasma that was analyzed for potassium was diluted to a 1:500 solution by adding a 1% cesium chloride reagent.

On day 49 of the experiment, all birds from both chambers were slaughtered and processed as in Experiment 1. Pre-slaughter weight, the weight of the carcass without giblets, breast weight, and the abdominal fat weight was measured and recorded. Gut strength was also measured using the equipment described in Experiment 1.

Experiment 3

Fourteen hundred and seventy-two one-day-old male and female Ross x Cobb broilers were weighed in groups of 46 and randomly assigned to floor pens. There were four treatments with eight replicates per treatment. Dietary treatments (Table 3 and 4) were as follows: treatment 1 high density diet (low calorie:protein; starter = 134, grower = 151, finisher = 164); treatment 2 high density diet with 1209g betaine per metric ton

[2] Accumet Portable Laboratory AP61 pH meter, Fisher Scientific Co., Fair Lawn, NJ 07410
[3] Jarrell Ash Corp., Franklin MA 02038

Table 3. Composition of Basal Diets (Experiment 3)

Ingredient	Starter		Grower		Finisher	
	High	Low	High	Low	High	Low
			(%)			
Corn	56.88	65.98	60.86	69.66	64.33	75.99
Soybean Meal	33.15	26.47	30.67	22.48	27.75	16.94
Fat[1]	2.90	1.78	3.39	1.96	3.61	1.71
Meat & Bone Meal	2.32	---	---	---	---	---
Limestone	1.14	1.55	1.37	1.39	1.27	1.29
Vitamin Mix[2]	1.00	1.00	1.00	1.00	1.00	1.00
Di-calcium Phosphate	0.99	1.60	1.44	1.47	1.13	1.18
Feather Meal	0.66	0.56	0.43	1.08	0.13	0.91
Salt (NaCl)	0.28	0.35	0.28	0.28	0.28	0.28
DL-Methionine	0.18	0.14	0.16	0.11	0.12	0.10
Sodium Bicarbonate	0.17	0.13	0.09	0.09	0.09	0.09
Sand[3]	0.12	0.12	0.12	0.12	0.12	0.12
Sacox[4]	0.10	0.10	0.10	0.10	0.10	0.10
Lysine	0.06	0.10	0.04	0.17	---	0.16
Threonine	0.05	0.08	0.03	0.04	0.04	0.05
Choline	---	0.04	0.02	0.05	0.03	0.08
Total	100	100	100	100	100	100
Nutrient Composition						
ME kcal/kg	3038	3038	3090	3090	3140	3140
CP[5]	22.75	19	20.5	17.8	19.1	15.5

[1]Choice white grease; Griffin Industries, Inc. Cold Spring, KY 41076

[2] Supplied per kilogram of diet: Copper, 8mg; Iodine, 0.4mg; Iron, 100mg; Manganese, 100mg; Selenium, 0.3mg; Zinc, 75mg; Vitamin A, 8800 IU; Vitamin D_3 2992 ICU; Vitamin E, 29.9 IU; Vitamin B_{12}, 0.02mg; Menadione, 1.65mg; Biotin, 0.2mg; Folic Acid, 1.0mg; Niacin, 66mg; d-Pantothenic Acid, 11mg; Vitamin B_6, 4.4mg; Riboflavin, 6.6mg; Thiamine, 1.1mg

[3]Betaine added at the expense of equivalent amount of sand

[4]Sacox (Salinomycin) Hoechst Roussel Vet, Warren NJ 07059

[5]CP = crude protein

Table 4. Betaine and Amino Acids Composition of Experimental Diets (Experiment 3)

Diet	Betaine (mg/g)	Methionine W/W%*	Threonine W/W%	Lysine W/W%
Starter				
Trt 1	bdl**	0.53	0.77	1.31
Trt 2	1.51	0.56	0.75	1.34
Trt 3	bdl	0.44	0.63	1.09
Trt 4	1.42	0.47	0.68	1.07
Grower				
Trt 1	bdl	0.46	0.67	1.14
Trt 2	1.52	0.44	0.65	1.06
Trt 3	bdl	0.38	0.59	0.97
Trt 4	1.58	0.39	0.58	1.00
Finisher				
Trt 1	bdl	0.47	0.64	1.01
Trt 2	1.60	0.48	0.72	1.05
Trt 3	bdl	0.41	0.57	0.90
Trt 4	1.51	0.38	0.54	0.83

*W/W% = grams per 100 grams of sample
**bdl = below detectable limit

added; treatment 3 low-density diet (high calorie:protein; starter =160, grower = 174, finisher = 203); treatment 4 low density with 1209g betaine per metric ton added. Feed and water were provided for ad libitum consumption. The lighting program for the house was 23 hours of light, and 1 hour of dark, with incandenscent lighting provided. Birds were weighed as a group on day 1, 21, 42, and 49 of the experiment. Feed weight was also recorded on these days in order to calculate feed efficiency. On day 49 of the experiment, eight birds from each pen (4 males and 4 females) were selected and slaughtered using the method described in Experiment 1. The pre-slaughter weight, carcass weight without giblets, breast weight, and abdominal fat as well as intestinal strength were measured as described in Experiment 1 and recorded.

Statistical Analysis

The data from each experiment were analyzed using the General Linear Model Procedure of SAS (SAS Institute, 1987), with the error term being birds within treatments. A completely randomized design with repeated measures was used. Least square means were compared using Tukey analysis and significance was detetmined at the 5 % level of probability. In Experiment 2, the effect of temperature, diet, and the diet X temperature interaction were evaluated, while in Experiment 3, effect of diet, sex, and diet X sex interaction were determined.

Chapter 4

Results

The amount of feed consumed during the seven weeks of Experiment 1 is presented in Table 5. There was no significant difference in feed consumption among treatments on days 1-21 ($P>0.11$), however during days 43-49 ($P>0.06$), birds tended to consume more feed. On days 22-42 ($P<0.02$), the treatment 1 birds consumed significantly larger amounts of feed than treatments 3 and 4.

The average weight gain of the broilers was measured and results are displayed on Table 6. There was no significant difference among treatments on days 22-42 ($P>0.09$), or days 43-49 ($P>0.37$). There was a difference among treatments during the 1-21 day time period ($P<0.0001$), with birds on treatments 1 and 2 gaining more weight than treatments 3, 4, and 5. The total gain also showed significant differences, ($P<0.004$) with treatment 1 having a 10 % larger gain than treatment 3 and an 8% larger gain than treatment 4.

Adjusted feed efficiency values are given in Table 7. There were differences in feed efficiency during the 1-21 day time period ($P<0.01$), with treatment 1 having a 9% higher feed efficiency value than treatment 3. There tended to be differences among treatments for the 22-42 day time period ($P>0.07$), but on the 43-49 day time period there were no significant differences ($P>0.79$). Total feed efficiency values for Experiment 1 also showed that treatments differed ($P<0.01$). Feed efficiency for the birds on treatment 1 was 6% higher than for treatment 2, and 8% higher than treatment 3.

Table 5. Feed Consumption (kg) for Experiment 1[1]

Treatment	Feed Day 1-21	Feed Day 22-42	Feed Day 43-49
1	39.5	97.5[a]	29.4
2	38.0	94.6[ab]	29.8
3	37.5	92.8[b]	27.0
4	36.9	93.0[b]	27.8
5	36.2	95.7[ab]	29.7
Pooled SEM	0.84	1.04	0.78

[1]Data are least square means
[a,b]Values within same column not sharing letters are different (P<0.05).

Table 6. Average weight gain (kg) for Experiment 1[1]

Treatment	Gain Day 1-21	Gain Day 22-42	Gain Day 43-49	Total Gain
1	0.58[a]	1.35	0.26	2.19[a]
2	0.56[a]	1.29	0.24	2.08[ab]
3	0.50[b]	1.22	0.25	1.97[b]
4	0.50[b]	1.27	0.24	2.01[b]
5	0.48[b]	1.30	0.28	2.06[ab]
Pooled SEM	0.01	0.03	0.01	0.04

[1]Data are least square means

[a,b] Values within same column not sharing letters are different ($P<0.05$).

Table 7. Adjusted Feed Efficiency[1] for Experiment 1[2]

Treatment	AdEff Day 1-21	AdEff Day 22-42	AdEff Day 43-49	Total AdEff
1	0.66[a]	0.54	0.34	0.53[a]
2	0.65[ab]	0.51	0.31	0.50[b]
3	0.60[b]	0.50	0.34	0.49[b]
4	0.62[ab]	0.53	0.33	0.51[ab]
5	0.61[ab]	0.53	0.35	0.51[ab]
Pooled SEM	0.01	0.01	0.02	0.01

[1](Live weight + mortality weight)/ Feed intake
[2]Data are least square means
[a,b] Values within same column not sharing letters are different (P<0.05).

Carcass measurements evaluated post-slaughter were carcass weight, dressing percent, breast weight, percent breast, abdominal fat weight and percent abdominal fat (Table 8). The birds' live weight prior to slaughter was measured, and showed differences between treatment groups ($P<0.005$). The average live weights of birds in treatment 1 were 7% heavier than birds in treatment 3, and 6% heavier than birds in group 4. The carcass weights were also significantly different among treatments ($P<0.05$), with treatment 1 having a 6% higher average carcass weight than treatment 3. There were no differences between treatments in dressing percent, breast weight, breast percent or percent abdominal fat ($P>0.05$). The abdominal fat weights did differ among treatments ($P<0.02$), with treatment 1 birds having 19% more abdominal fat than birds in treatment 4.

The results for Experiment 2 include the addition of environmental temperature as a variable. Feed consumption during Experiment 2 for both the thermoneutral and heat stress chambers is presented in Tables 9 and 10. The feed consumed during the 29-35 day time period in the thermoneutral chamber was the only group that had significant differences ($P<0.0001$) among diet treatments. Birds in treatment 2 consumed 16% more feed than the birds in treatment group 4. Looking at the main effect means shows that there were differences between diet treatments during the 21-28 day time period ($P<0.04$), and during the 43-49 day time period ($P<0.03$). Treatment 2 birds consumed 11% more feed than birds in treatment 5 during the 21-28 day period. During the 43-49 day time period there were significant differences between birds in treatment group 6 and

Table 8. Carcass weight, dressing percent, breast weight, percent breast, abdominal fat weight, and percent abdominal fat for experiment 1[1]

Treatment	Live Wt	Carcass Wt	Dressing	Breast Wt.	Breast	Ab Fat Wt.	Ab Fat
	---- (kg) -----		%	(g)	%	(g)	%
1	2.43[a]	1.83[a]	75.24	602.56	32.91	48.88[a]	2.70
2	2.34[ab]	1.76[ab]	75.62	587.13	33.19	44.11[ab]	2.52
3	2.27[b]	1.72[b]	75.72	565.66	32.88	43.11[ab]	2.51
4	2.28[b]	1.73[ab]	75.87	572.44	33.22	39.61[b]	2.33
5	2.33[ab]	1.75[ab]	75.00	582.17	33.34	44.22[ab]	2.55
Pooled SEM	0.03	0.03	0.34	0.01	0.28	0.002	0.11

[1]Data are least square means from 48 birds per treatment

[a,b]Values within same column not sharing letters are different ($P<0.05$).

Table 9. Feed consumption (kg) for Experiment 2[1]

Treatment	Temp[2]	Feed Day 21-28	Feed Day 29-35	Feed Day 36-42	Feed Day 43-49
1	TN	0.623	0.870[ab]	1.156	0.911
	HS	0.568	0.688	0.841	0.675
2	TN	0.666	0.992[a]	1.250	0.942
	HS	0.583	0.703	0.881	0.736
3	TN	0.604	0.914[ab]	1.196	0.865
	HS	0.566	0.698	0.904	0.677
4	TN	0.588	0.831[bc]	1.189	0.943
	HS	0.543	0.682	0.882	0.779
5	TN	0.606	0.868[ab]	1.223	0.937
	HS	0.512	0.689	0.815	0.754
6	TN	0.643	0.883[ab]	1.281	1.004
	HS	0.567	0.671	0.865	0.753
Pooled SEM		0.02	0.03	0.05	0.04

[1]Data are least square means
[2]TN = thermoneutral environment, HS = heat stress environment
[a,b]Values within same column, within same temperature environment, not sharing letters are different ($P<0.05$)

Table 10. Main effect means of feed consumption for Experiment 2[1]

Treatment	Feed Day 21-28	Feed Day 29-35	Feed Day 36-42	Feed Day 43-49
Treatment				
1	0.598[ab]	0.786	1.007	0.803[ab]
2	0.625[a]	0.851	1.070	0.844[ab]
3	0.585[ab]	0.809	1.053	0.774[b]
4	0.567[ab]	0.761	1.043	0.865[ab]
5	0.558[b]	0.776	1.002	0.843[ab]
6	0.605[ab]	0.777	1.073	0.882[a]
Temperature[2]				
TN	0.621[a]	0.893[a]	1.215[a]	0.933[a]
HS	0.556[b]	0.689[b]	0.864[b]	0.729[b]
Source of Variation	**Probabilities**			
Diet	0.042	0.077	0.703	0.029
Temp	0.0001	0.0001	0.0001	0.0001
Diet x Temp	0.783	0.342	0.727	0.836

[1]Data are least square means
[2]TN = thermoneutral environment, HS = heat stress environment
[a,b] Values within same column not sharing letters are different

treatment group 3, with birds in receiving diet treatment 6 consuming 12% more feed than birds receiving diet treatment 3. The main effect of environmental temperature showed that the birds in the thermoneutral chambers consumed significantly more feed than the birds that were housed in the heat stress chambers ($P<0.05$).

The average weight gain of birds from experiment 2 are given in Tables 11 and 12. Results showed no significant differences except among those birds housed in the thermoneutral house during the 43-49 day time period ($P<0.0001$). During this time period there were treatment differences with treatment 5 having a 27% higher gain than the birds that were given diet treatment 3. The main effect means also show that birds receiving diet treatment 5 had a 21% higher gain than treatment 2 birds and a 30% higher gain than treatment 3 birds during the 43-49 day time period. Temperature had a significant effect on gain for birds throughout the experiment ($P<0.05$), with birds in the thermoneutral environment having a higher gain than those birds housed in the chambers receiving the cycling high ambient temperatures. The total gain values showed that birds housed in the thermoneutral environment had a 23% higher gain than those birds housed in the heat stress chambers ($P<0.05$).

Feed efficiency for Experiment 2 is listed in Tables 13 and 14. Results indicate there was no significant difference between diet treatments or environmental treatments on feed efficiency throughout the experiment. The main effect means for diet treatments show birds given diet treatment 5 having a 22% higher feed efficiency value than diet treatments 2,3, and 4 ($P<0.05$) during the 43-49 day period. Temperature affected feed efficiency during the 21-28 day time frame, and during the 43-49 day time frame

Table 11. Average weight gain (kg) for Experiment 2[1]

Treatment	Temp[2]	Gain Day 21-28	Gain Day 29-35	Gain Day 36-42	Gain Day 43-49	Total Gain
1	TN	0.398	0.494	0.565	0.343[ab]	1.799
	HS	0.371	0.402	0.465	0.196	1.433
2	TN	0.438	0.544	0.613	0.278[abc]	1.872
	HS	0.348	0.400	0.464	0.204	1.415
3	TN	0.389	0.469	0.592	0.267[bc]	1.717
	HS	0.364	0.398	0.495	0.165	1.421
4	TN	0.392	0.516	0.648	0.312[ab]	1.868
	HS	0.322	0.398	0.454	0.196	1.369
5	TN	0.393	0.512	0.580	0.367[a]	1.853
	HS	0.321	0.417	0.439	0.252	1.428
6	TN	0.412	0.495	0.657	0.346[ab]	1.911
	HS	0.370	0.392	0.449	0.213	1.424
Pooled SEM		0.02	0.02	0.02	0.02	0.05

[1]Data are least square means
[2]TN = thermoneutral environment, HS = heat stress environment
[abc]Values within same column, within same temperature environment, not sharing letters are different ($P<0.05$)

Table 12. Main effect means of average weight gain for Experiment 2[1]

Treatment	Gain Day 21-28	Gain Day 29-35	Gain Day 36-42	Gain Day 43-49	Total Gain
Treatment					
1	0.385	0.451	0.519	0.276[abc]	1.630
2	0.394	0.474	0.540	0.242[bc]	1.650
3	0.376	0.434	0.545	0.217[c]	1.573
4	0.359	0.460	0.556	0.257[abc]	1.632
5	0.356	0.463	0.508	0.308[a]	1.635
6	0.391	0.444	0.553	0.279[ab]	1.667
Temperature[2]					
TN	0.404[a]	0.505[a]	0.609[a]	0.318[a]	1.836[a]
HS	0.349[b]	0.401[b]	0.461[b]	0.205[b]	1.415[b]
Source of Variation			**Probabilities**		
Diet	0.194	0.387	0.310	0.0004	0.491
Temp	0.0001	0.0001	0.0001	0.0001	0.0001
Diet xTemp	0.396	0.542	0.107	0.611	0.316

[1]Data are least square means
[2]TN = thermoneutral environment, HS = heat stress environment
[abc] Values within same column not sharing letters are different

Table 13. Feed efficiency[1] for Experiment 2[2]

Treatment	Temp[3]	FdEff Day 21-28	FdEff Day 29-35	FdEff Day 36-42	FdEff Day 43-49	Total FdEff
1	TN	0.634	0.571	0.491	0.374	0.507
	HS	0.647	0.584	0.557	0.287	0.516
2	TN	0.659	0.552	0.502	0.292	0.488
	HS	0.598	0.568	0.539	0.266	0.488
3	TN	0.641	0.531	0.500	0.308	0.482
	HS	0.636	0.574	0.548	0.243	0.500
4	TN	0.661	0.620	0.566	0.330	0.530
	HS	0.584	0.593	0.528	0.221	0.478
5	TN	0.650	0.600	0.489	0.389	0.507
	HS	0.622	0.605	0.546	0.338	0.518
6	TN	0.643	0.569	0.527	0.344	0.506
	HS	0.650	0.585	0.524	0.290	0.502
Pooled SEM		0.02	0.02	0.02	0.03	0.01

[1]Feed efficiency = gain:feed
[2]Data are least square means
[3]TN = thermoneutral environment, HS = heat stress environment
[a,b]Values within same column not sharing letters are different (P<0.05)

Table 14. Main effect means of feed efficiency[1] for Experiment 2[2]

Treatment	FdEff Day 21-28	FdEff Day 29-35	FdEff Day 36-42	FdEff Day 43-49	Total FdEff
Treatment					
1	0.64	0.58	0.52	0.33[ab]	0.51
2	0.63	0.56	0.52	0.28[b]	0.49
3	0.64	0.55	0.52	0.28[b]	0.49
4	0.62	0.61	0.55	0.28[b]	0.51
5	0.64	0.60	0.52	0.36[a]	0.51
6	0.65	0.58	0.53	0.32[ab]	0.50
Temperature[3]					
TN	0.65[a]	0.57	0.51	0.34[a]	0.50
HS	0.62[b]	0.58	0.54	0.27[b]	0.50
Source of Variation			**Probabilities**		
Diet	0.839	0.036	0.879	0.004	0.178
Temp	0.024	0.338	0.052	0.0001	0.640
Diet x Temp	0.107	0.674	0.263	0.710	0.044

[1]Feed efficiency = gain:feed
[2]Data are least square means
[3]TN = thermoneutral environment, HS = heat stress environment
[a,b]Values within same column not sharing letters are different (P<0.05)

($P<0.05$). The birds in the heat stress chambers had significantly lower feed efficiency values than those birds in the thermoneutral environment. There were no significant differences between dietary treatments or temperature treatments on total feed efficiency ($P>0.05$).

Various blood parameters were measured during Experiment 2 including hematocrit, pH (Table 15 and 16), and plasma Na and K (Table 17 and 18). The hematocrit results show that there were no treatment differences on day 28 or on day 45 ($P>0.05$), however there were treatment differences on day 35 ($P<0.007$). The hematocrit was 10% higher in birds given diet treatment 1 than birds receiving diet treatment 4, and 12% higher than birds in treatment group 5. Birds in the thermoneutral chambers had a significantly higher hematocrit value ($P<0.05$) than birds housed in the heat stress chambers. There were no significant treatment differences for pH ($P>0.05$). The temperature of the chamber had an effect on pH on day 35 of the experiment. Birds in the thermoneutral environment had a lower pH than birds in the heat stress chambers ($P<0.05$). The plasma sodium and potassium values are listed on Table 13. Plasma sodium values only differed among diet treatments on day 28 of the experiment ($P>0.05$). There were no diet treatment differences on plasma potassium levels for any of the days tested ($P>0.05$). Temperature had some effect on plasma sodium and potassium levels. On day 28, the heat stressed birds had a higher plasma sodium value than the birds in the thermoneutral chambers ($P<0.05$). Plasma sodium levels were also significantly lower in the thermoneutral birds for day 35, and day 45 ($P<0.05$). Temperature had an effect on

Table 15. Hematocrit and pH values for Experiment 2[1]

Treatment	Temp[2]	Hematocrit Day 28	Hematocrit Day 35	Hematocrit Day 45	pH Day 35	pH Day 45
1	TN	28.40	35.82	32.78	7.30	7.54
	HS	28.53	33.03	29.51	7.35	7.51
2	TN	27.94	35.58	34.80	7.28	7.51
	HS	26.98	29.75	28.64	7.37	7.54
3	TN	27.74	33.39	33.01	7.28	7.53
	HS	26.84	29.68	26.86	7.35	7.54
4	TN	27.33	30.84	34.00	7.28	7.49
	HS	27.82	30.86	27.13	7.35	7.54
5	TN	27.90	31.51	30.97	7.26	7.50
	HS	26.56	28.98	29.21	7.37	7.56
6	TN	28.28	33.65	33.58	7.27	7.48
	HS	25.82	29.75	26.80	7.35	7.52
Pooled SEM		0.76	1.16	0.98	0.02	0.03

[1]Data are least square means
[2]TN = thermoneutral environment, HS = heat stress environment
[a,b]Values within same column not sharing letters are different (P<0.05)

Table 16. Main effect means of hematocrit and pH values for Experiment 2[1]

Treatment	Hematocrit Day 28	Hematocrit Day 35	Hematocrit Day 45	pH Day 35	pH Day 45
Treatment					
1	28.46	34.39[a]	31.23	7.32	7.52
2	27.43	32.82[ab]	31.88	7.32	7.52
3	27.30	31.54[ab]	29.93	7.31	7.54
4	27.55	30.85[b]	30.65	7.32	7.51
5	27.20	30.21[b]	30.07	7.32	7.53
6	27.05	31.70[ab]	30.19	7.31	7.50
Temperature[2]					
TN	27.92[a]	33.48[a]	33.21[a]	7.28[b]	7.51
HS	27.05[b]	30.35[b]	28.02[b]	7.36[a]	7.54
Source of Variation			**Probabilities**		
Diet	0.510	0.007	0.409	0.975	0.718
Temp	0.055	0.0001	0.0001	0.0001	0.083
Diet x Temp	0.422	0.247	0.037	0.514	0.605

[1]Data are least square means

[2]TN = thermoneutral environment, HS = heat stress environment

[a,b]Values within same column not sharing letters are different ($P<0.05$)

Table 17. Plasma sodium and potassium values for Experiment 2[1]

Treatment	Temp	Na Day 28	K Day 28	Na Day 35	K Day 35	Na Day 45	K Day 45
		----------	----------	---(mg/g)---	----------	----------	----------
1	TN	328.94	25.60	328.31	22.46	334.57	22.23
	HS	358.02	27.20	349.00	24.07	341.85	22.06
2	TN	325.99	24.69	316.76	22.47	335.66	21.80
	HS	350.93	25.33	348.22	23.91	340.19	21.63
3	TN	331.15	25.24	330.62	22.17	334.65	21.46
	HS	351.45	25.95	344.13	23.98	338.66	21.20
4	TN	327.11	25.10	330.97	21.51	335.90	21.92
	HS	352.77	25.81	347.38	24.86	340.56	22.10
5	TN	326.14	25.37	328.99	22.08	335.28	21.48
	HS	347.22	25.61	344.03	23.63	337.12	22.45
6	TN	328.72	25.61	333.19	23.11	337.93	22.72
	HS	349.88	25.63	346.33	24.83	339.40	21.43
Pooled SEM		2.56	0.66	5.01	0.51	2.10	0.45

[1]Data are least square means
[2]TN = thermoneutral environment, HS = heat stress environment

Table 18. Main effect means of plasma sodium and potassium values for Experiment 2[1]

Treatment	Na Day 28	K Day 28	Na Day 35	K Day 35	Na Day 45	K Day 45
			---(mg/g)---			
Treatment						
1	343.48[a]	26.40	338.39	23.25	338.02	22.15
2	338.46[ab]	25.01	332.49	23.19	337.86	21.72
3	341.30[ab]	25.59	337.37	23.08	336.65	21.33
4	339.26[ab]	25.43	338.72	23.09	338.17	22.01
5	335.83[b]	25.48	336.70	22.87	336.22	21.97
6	339.01[ab]	25.62	339.76	23.97	338.67	22.08
Temperature[2]						
TN[3]	328.00[b]	25.27	328.20[b]	22.31[b]	335.65[b]	21.93
HS	351.84[a]	25.94	346.45[a]	24.20[a]	339.58[a]	21.81
Source of Variation					**Probabilities**	
Diet	0.150	0.458	0.730	0.360	0.819	0.463
Temp	0.0001	0.093	0.0001	0.0001	0.001	0.635
Diet x Temp	0.490	0.894	0.434	0.477	0.772	0.265

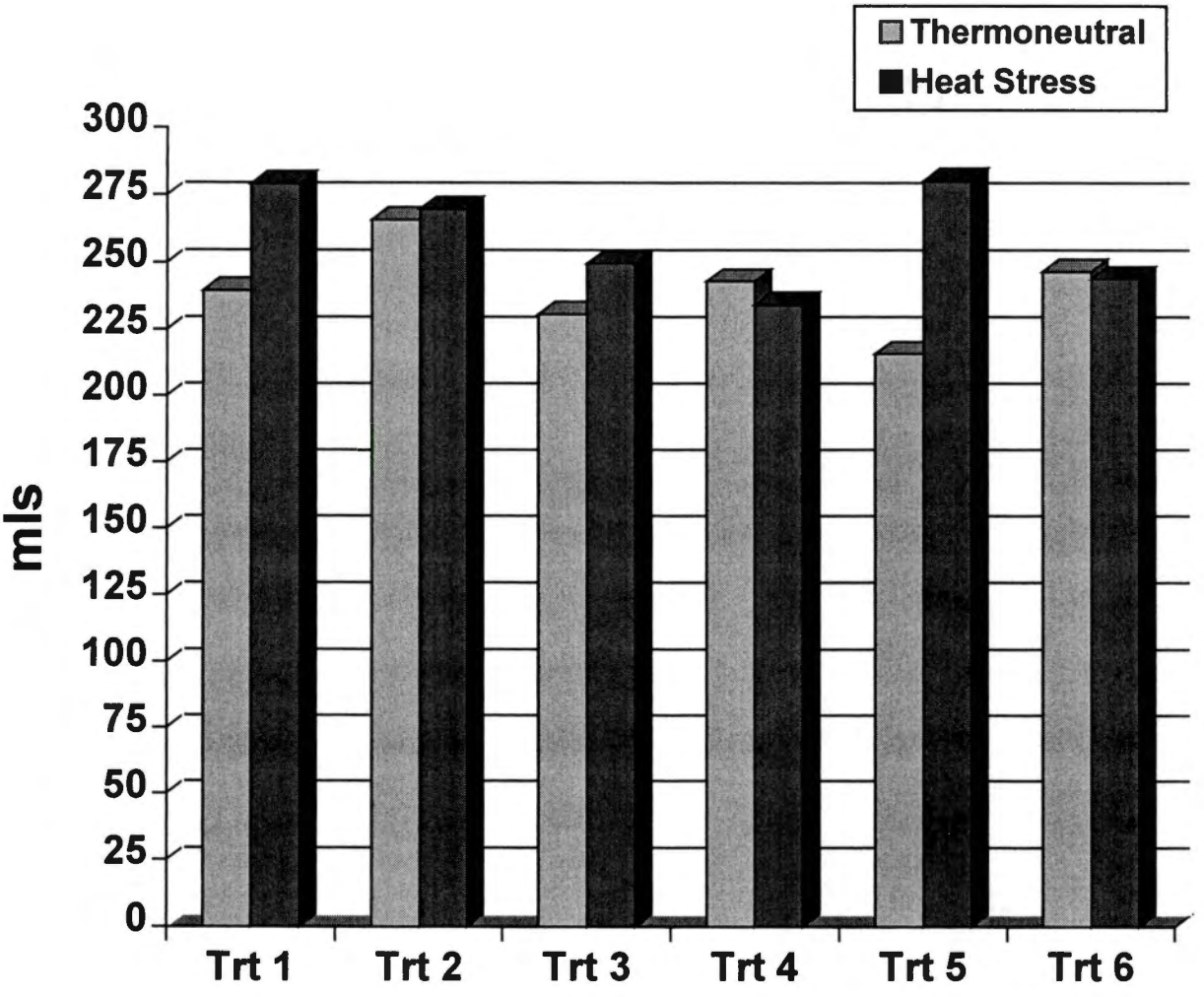
[1]Data are least square means
[2]TN = thermoneutral environment, HS = heat stress environment

potassium levels on day 35 of the experiment. Potassium levels were significantly higher in the heat stressed birds than in the thermoneutral birds ($P<0.05$).

Water intake was measured daily from day 22 to day 49 of the experiment. There were no significant differences ($P>0.05$) in water intake between diet treatments, or environmental temperatures. Figure 3 shows that although there were no significant differences ($P>0.34$), there was an increased water intake for some of the birds in the heat stress chambers.

The carcass values that were recorded are given on Tables 19, 20, 21 and 22. Diet treatments had no effect on the average live weight or the carcass weight of birds in this experiment ($P>0.05$). There were no significant differences in the dressing percent, breast weight, breast percent, abdominal fat weight, and percent abdominal fat for any of the dietary treatments ($P>0.05$). Temperature did have an effect on live weight and carcass measurements. The live weight of birds housed in the thermoneutral temperatures was 18% higher than birds exposed to cycling high ambient temperatures. Carcass weights of thermoneutral birds were 15% larger than heat stressed birds, however the dressing percent for heat stressed birds was significantly higher ($P<0.05$). Breast weight was severely affected by heat stress, with the thermoneutral birds having a 20% larger breast weight, and the percent breast weight was 6% higher in the thermoneutral birds. The thermoneutral birds had a larger abdominal fat weight ($P<0.05$) than the heat stressed birds, however there was no significant difference ($P>0.05$) in the percent of abdominal fat between the two temperature environments.

Figure 3. Average daily water intake for birds housed in thermoneutral and heat stressed chambers[1]



[1]Data are least square means. There were no significant differences among diet treatments or between temperature environments (P>0.05)

Table 19. Live weight, carcass weight, and dressing percent for Experiment 2[1]

Treatment	Temp[2]	Live Wt (g)	Carcass Wt (g)	Dressing %
1	TN	2476.50	1766.00	71.33
	HS	2054.21	1515.69	73.17
2	TN	2525.50	1810.46	71.66
	HS	2030.00	1493.09	73.54
3	TN	2295.50	1645.27	71.19
	HS	2022.50	1483.51	73.30
4	TN	2460.50	1775.17	72.21
	HS	1961.05	1444.80	72.94
5	TN	2428.42	1745.01	71.80
	HS	2009.50	1484.62	73.86
6	TN	2493.68	1784.65	71.52
	HS	2014.44	1478.32	73.27
Pooled SEM		58.20	44.50	0.31

[1]Data are least square means
[2]TN = thermoneutral environment, HS = heat stress environment

Table 20. Main effect means of live weight, carcass weight, and dressing percent for Experiment 2[1]

Treatment	Live Wt (g)	Carcass Wt (g)	Dressing %
Treatment			
1	2.270	1.647	72.20
2	2.284	1.656	72.58
3	2.159	1.562	72.28
4	2.217	1.614	72.57
5	2.213	1.611	72.85
6	2.260	1.636	72.37
Temperature[3]			
TN	2.446[a]	1.755[a]	71.62[b]
HS	2.015[b]	1.483[b]	73.36[a]
Source of Variation	**Probabilities**		
Diet	0.349	0.433	0.393
Temp	0.0001	0.0001	0.0001
Diet x Temp	0.381	0.431	0.278

[1]Data are least square means
[2]TN = thermoneutral environment, HS = heat stress environment
[a,b]Values within same column not sharing letters are different (P<0.05)

Table 21. Breast weight, percent breast, abdominal fat weight, and percent abdominal fat for Experiment 2[1]

Treatment	Temp[2]	Breast Wt (g)	Breast %	Ab Fat Wt (g)	Ab Fat %
1	TN	605.01	34.20	38.00	2.16
	HS	500.48	32.96	29.72	1.97
2	TN	624.57	34.43	36.78	2.07
	HS	497.32	33.25	31.59	2.10
3	TN	583.54	35.47	33.93	2.09
	HS	495.84	33.45	25.46	1.73
4	TN	639.83	35.94	32.26	1.84
	HS	477.58	33.00	27.31	1.94
5	TN	611.86	35.05	38.81	2.26
	HS	492.40	33.13	28.54	1.95
6	TN	630.77	35.42	37.33	2.11
	HS	487.44	32.85	29.96	2.06
Pooled SEM		17.50	0.41	2.35	0.15

[1]Data are least square means
[2]TN = thermoneutral environment, HS = heat stress environment

Table 22. Main effect means of breast weight, percent breast, abdominal fat weight, and percent abdominal fat for Experiment 2[1]

Treatment	Breast Wt (g)	Breast %	Ab Fat Wt (g)	Ab Fat %
Treatment				
1	555.49	33.61	34.08	2.07
2	562.58	33.86	34.32	2.08
3	538.56	34.43	29.59	1.90
4	562.97	34.51	29.85	1.88
5	550.60	34.07	33.54	2.10
6	561.04	34.17	33.74	2.08
Temperature[2]				
TN[3]	616.12[a]	35.07[a]	36.20[a]	2.09
HS	491.97[b]	33.11[b]	28.70[b]	1.95
Source of Variation	**Probabilities**			
Diet	0.836	0.227	0.147	0.535
Temp	0.0001	0.0001	0.0001	0.137
Diet x Temp	0.311	0.225	0.858	0.581

[1]Data are least square means
[2]TN = thermoneutral environment, HS = heat stress environment
[a,b]Values within same column not sharing letters are different ($P<0.05$)

Feed consumption for Experiment 3 is given on Table 23. In this experiment there were significant differences in feed consumption during the 1-21 day time period ($P<0.005$), and during the 22-42 day time period ($P<0.0001$). Birds in treatment group 2 consumed 9% more feed than birds in group 1, and 12% more feed than birds in treatment group 4. Results of feed consumption data from the 22-42 day time period show that birds in treatments 1 and 2 consumed significant more feed than birds in treatment groups 3 and 4 ($P<0.05$). Birds in treatment 1 ate 21% more feed than birds in treatment group 4. The decrease in feed intake had an effect on gain (Table 24). There were significant differences ($P>0.05$) in gain throughout the experiment. The birds receiving diets 1 and 2 had a 22% greater gain than birds in group 3, and a 25% greater gain than birds in treatment group 4 during the day 1-21 time period. Treatment 1 birds also had a higher gain than diet treatments 3 and 4 during the 22-42 day time period ($P<0.05$). Treatment 1 birds gained 22% more than birds receiving diet 3, and 26% higher than treatment 4 birds. On day 43-49, birds in group 2 had a higher gain ($P<0.05$) than birds in treatment groups 3 and 4. Treatment 2 birds had a 22% higher gain than treatment 3 birds, and a 26% higher gain than birds in treatment 4. Total gain results indicate that birds given diet treatments 1 and 2 had an overall higher gain than birds fed diet treatments 3 and 4 ($P<0.0001$).

Adjusted feed efficiency results show that there were differences ($P<0.05$) between diet treatments (Table 25). The 1-21 day period showed that birds given diet 1 had an 8% higher feed efficiency ratio than treatment 2 birds, and a 22% higher feed

Table 23. Feed consumption[1] for Experiment 3[2]

Treatment	Feed Day 1-21	Feed Day 22-42	Feed Day 43-49
1	40.38[b]	115.25[a]	48.38
2	44.43[a]	111.14[a]	49.29
3	41.00[ab]	98.88[b]	44.75
4	39.25[b]	91.38[b]	42.50
Pooled SEM	0.85	2.67	1.78

[1]Feed was measured in kilograms
[2]Data are least square means
[a,b]Values within same column not sharing letters are different ($P<0.05$)

Table 24. Average weight gain[1] for Experiment 3[2]

Treatment	Gain Day 1-21	Gain Day 22-42	Gain Day 43-49	Total Gain
1	0.67[a]	1.37[a]	0.41[ab]	2.46[a]
2	0.67[a]	1.27[b]	0.46[a]	2.39[a]
3	0.52[b]	1.07[c]	0.36[b]	1.96[b]
4	0.50[b]	1.01[c]	0.34[b]	1.86[b]
Pooled SEM	0.01	0.02	0.02	0.04

[1]Gain was measured in kilograms
[2]Data are least square means
[abc]Values within same column not sharing letters are different ($P<0.05$)

Table 25. Adjusted feed efficiency[1] for Experiment 3[2]

Treatment	AdEff 21	AdEff 42	AdEff 49	Total AdEff
1	0.73[a]	0.51[a]	0.37	0.52[a]
2	0.67[b]	0.50[ab]	0.41	0.52[a]
3	0.57[c]	0.49[b]	0.36	0.47[b]
4	0.57[c]	0.48[ab]	0.35	0.47[b]
Pooled SEM	0.01	0.01	0.01	0.005

[1]Feed efficiency = gain:feed
[2]Data are least square means
[abc]Values within same column not sharing letters are different (P<0.05)

efficiency ratio than birds given diets 3 and 4. The total feed efficiency values show that treatments 1 and 2 had a 10% higher feed efficiency ratio than treatments 3 and 4.

The carcass measurements taken for Experiment 3 are listed in Table 26. Results show that live weights for treatments 1 and 2 were significantly higher than live weights of birds given diet treatments 3 and 4 ($P< 0.0001$). Carcasses were larger as well, with treatment 1 carcasses being 16% larger than treatment 3, and 19% larger than treatment 4 carcasses. Treatments 1 and 2 had a higher dressing percent than treatments 3 and 4 ($P<0.0001$). The breast weights for birds in treatment groups 1 and 2 were significantly higher than the breast weights of birds in treatments 3 and 4 ($P<0.0001$), however, the percent breast was not significantly different for any of the diet treatment groups ($P>0.50$). There were no significant differences in the abdominal fat weights ($P>0.62$), but there was a significant difference in the percent abdominal fat ($P<0.0001$). Treatments 3 and 4 had an 18% higher percent abdominal fat than treatment 1, and a 19% higher percent abdominal fat than treatment 2.

Male and female birds were used in Experiment 3 (Table 26). Male birds had a significantly higher live weight than females ($P<0.0001$), and males had a 21% higher live weight than females. The carcasses of males were also significantly larger than that of females ($P<0.0001$). The dressing percent for males was therefore higher than that of females ($P<0.009$). Males had 21% larger breast weights than females, however females had a higher percent breast weight than males ($P<0.03$). There was no significant treatment difference in the abdominal fat weights ($P>0.05$), but females had a higher percent abdominal fat than males ($P<0.0001$).

Table 26. Live weight and carcass measurements for Experiment 3[1]

Treatment	Live Wt (kg)	Carc Wt (kg)	Dressing %	Breast Wt (g)	Breast %	Ab Fat Wt (g)	Ab Fat %
1	2.73[a]	2.05[a]	74.97[a]	725.44[a]	35.39	55.18	2.77[b]
2	2.68[a]	2.02[a]	75.21[a]	714.39[a]	35.35	53.76	2.74[b]
3	2.34[b]	1.72[b]	73.48[b]	600.62[b]	34.87	57.09	3.37[a]
4	2.25[b]	1.67[b]	73.88[b]	582.23[b]	35.05	54.43	3.37[a]
Pooled SEM	0.04	0.03	0.26	11.66	0.28	1.88	0.11
Sex[2]							
Male	2.80[a]	2.09[a]	74.73[a]	731.91[a]	34.86[b]	54.30	2.64[b]
Female	2.20[b]	1.64[b]	74.04[b]	579.43[b]	35.47[a]	55.93	3.49[a]
Source of Variation				**Probabilities**			
Diet	0.0001	0.0001	0.0001	0.0001	0.503	0.623	0.0001
Sex	0.0001	0.0001	0.009	0.0001	0.029	0.398	0.0001
Diet x Sex	0.529	0.485	0.432	0.338	0.004	0.245	0.229

[1]Data are least square means from 64 birds per treatment
[2]Effect of sex ($P<0.05$)

The tensile strength data is included in Table 27. Two areas of the small intestine were measured and an average of the two measurements was used. There were no significant differences in tensile strength of the small intestine among diet treatments in Experiment 1($P>0.23$), or in Experiment 2 ($P>0.56$), and there was no significant difference between the environmental chambers in Experiment 2 ($P>0.92$). Tensile strength results from Experiment 3 show no significant differences between dietary treatments ($P>0.05$), however there was a significant difference between male and female birds with males having a significantly higher tensile strength ($P<0.0001$) than females.

Table 27. Tensile strength[1] of small intestine (grams)[2]

Treatment	1	2	3	4	5	6
Exp. 1	321.52	297.79	305.71	292.86	299.70	
Exp. 2						
TN	259.33	269.20	263.32	272.18	271.34	259.33
HS	279.63	253.18	268.30	248.32	250.42	263.22
Exp. 3						
Male	327.11[a]	327.81[a]	327.90[a]	325.71[a]		
Female	296.51[b]	300.93[b]	285.29[b]	264.72[b]		

[1]Data are least square means
[2]Strength was determined using an instron test load stand/load cell (Chatillon TCM-201/Chatillon DFIS 2)
[ab]Values within same column not sharing letters are different ($P<0.05$)

Chapter 5

Discussion

The compound trimethylglycine, or betaine has recently become the focus of research investigating its potential as an additive in poultry diets. It has been known to function as a methyl group donor, and as an osmolyte. Results from studies using pigs suggest that betaine may increase the energy/nutrient value of the diet (Zabaras-Krick, 1997). The main benefit of using betaine in swine diets is the "energy boosting" effect, which can result in improvements in feed efficiency in the range of 5 to 8% (Remus, 1998). The ability to adjust the energy level without compromising performance and carcass quality could be economically useful for large producers as energy can be an expensive component in broiler diets (Remus, personal contact). In order to test whether betaine could serve as an energy/nutrient component in broiler diets, a "typical" diet, along with a "typical" diet minus 2% metabolizable energy was generated. The effect of betaine addition was then evaluated in both a floor pen study (Experiment 1), and a chamber study (Experiment 2).

There was no significant difference in feed consumption, gain or feed efficiency due to diet treatments in Experiment 1. Although birds in treatment 1 tended to have higher feed consumption, gain and feed efficiency, it was not significant. Live weight and carcass weight also tended to be higher among treatment 1 birds than in birds given the low energy diet treatments supplemented with betaine. There was no difference among treatments for dressing percent, breast weight, breast percent, and percent abdominal fat. These results suggest that betaine may not have been beneficial in the low

energy diets as an energy nutrient because treatment 1 birds receiving normal metabolizable energy diet without betaine tended to perform better. Results from pig studies have been mixed. Some researchers have been able to demonstrate betaine as an energy nutrient (Zabaras-Krick, 1997), while others have found that betaine was not able to improve energy utilization in pigs (Webel, 1995), thus supporting the finding from this study.

Experiment 2 investigated the same diet formulations as Experiment 1 with the addition of a sixth diet that was a reduced energy diet plus 1868g of betaine/metric ton. Birds from Experiment 2 were placed in one of two chambers at 21 days of age. The ambient temperatures within the chambers was regulated, with one chamber remaining at a thermoneutral temperature, and the second chamber cycled to high ambient temperatures exposing these birds to heat stress conditions (35 °C). Results from this study showed that birds exposed to high ambient temperatures had decreased feed consumption and gain, however feed efficiency results were mixed. During the 21-28 day time period and the 43-49 day period, the feed efficiency for heat distressed birds was significantly less ($P<0.05$), however there was no significant difference in feed efficiency during the 29-35 day period, the 36-42 day period, or for the calculated total feed efficiency ($P>0.05$). It is interesting to note that in some instances the heat stressed birds had higher feed efficiency values. Betaine was not able to alleviate the negative effects of heat stress on growth performance. Birds receiving little or no betaine had similar results in weight gain, feed efficiency, and carcass values as birds receiving higher amounts of betaine.

Various blood parameters were measured during Experiment 2 in order to observe the effect of betaine on blood pH, hematocrit and plasma Na and K levels. There were treatment differences between hematocrit values on day 35 of the experiment with treatment 1 being significantly higher ($P<0.05$) than treatments 4 and 5. During exposure to high ambient temperatures, hematocrit levels have been found to decrease significantly at temperatuures of 35 °C (Yahav, et al., 1997). The reduction in hematocrit is accompanied by peripheral vasodilation (Wolfenson et al., 1981) both of which would reduce the resistance to flow. Ultimately, a reduced resistance would help facilitate heat dissipation (Yahav et al., 1997). In the present study, hematocrit levels were significantly lower in the heat stressed birds ($P<0.05$). This was consistent with previous studies.

There were no differences in blood pH levels due to any dietary treatment for either day 35, or day 45 of the experiment. Ambient temperature did play an important role in blood pH measurements. At high ambient temperatures, birds begin to pant to regulate their body temperature, but in the process they will often experience respiratory alkalosis, or an increase in blood pH (Teeter et al., 1985). Results from the present study showed that on day 35 of the experiment birds in the heat stress environment had a higher blood pH than birds housed in thermoneutral temperatures ($P<0.05$). However, on day 45 there was no difference in blood pH between birds housed at thermoneutral and high temperatures. Unfortunately, the pH meter malfunctioned on day 45 which may have caused this discrepancy.

Potassium is the most abundant exchangeable cation, and it is found primarily within the cell (Thier, 1986). A gradient exists for the diffusion of potassium from

intracellular to extracellular fluid, and the reverse is true for sodium, which is present in high extracellular concentrations and a low intracellular concentration (Thier, 1986). ATPases play an important role in the control of ion absorption and secretion from the kidneys and the intestines. Na,K-ATPase is a crucial enzyme found in the basolateral membrane of the kidneys and intestine that controls Na absorption from the lumen and K excretion from the blood (Chen et al., 1994). Previous studies have shown that when broilers are exposed to heat stress, there is a reduction in blood content of sodium and a 633% increase in potassium excretion (Smith, 1987). However, in that study dietary sodium levels may have affected plasma sodium, and birds were stressed at higher temperatures which may have caused an increase in water consumption. The present study did not show an effect on plasma Na or K due to diet, however ambient temperature did have an effect. On days 35 and 45, plasma Na levels were significantly higher in heat stressed birds than in birds housed in the thermoneutral chamber. The plasma K levels differed on day 35 where heat stressed birds had higher plasma K values. However on day 45 of the experiment there was no difference in K levels for birds at either temperature. It is uncertain if the heat stressed birds were drinking sufficient amounts of water when the ambient temperature increased. If the birds were not drinking enough water, regulation within the kidneys may have caused an increase in plasma Na levels in order to prevent excess water from being excreted. Sodium is absorbed from the tubular lumen into the tubular cell of the distal convoluted tubule or collecting duct of the kidneys, and then is exchanged for potassium in the peritubular fluid (Breazile, 1983). Both hydrogen and potassium secretion involve exchanges for Na at the luminal border

of the tubular cells. Hydrogen will compete with potassium for exchange of sodium, however during respiratory alkalosis less hydrogen is available allowing for an increase in potassium secretion, and an increase in sodium reabsorption (Swenson and Reece ed., 1993). Lien et al. (1993) found that water deprivation significantly increased plasma Na levels, but had no effect on plasma K levels. If birds in this study were not getting adequate water, this may have resulted in the elevated plasma Na levels observed in the heat stressed birds.

Water measurements were taken daily beginning on day 21 of Experiment 2. An increase in water needs at high ambient temperatures is a result of the utilization of water in the dissipation of heat. Evaporative heat loss accounts for 100% of heat loss by birds exposed to ambient temperatures of 35 °C or higher, illustrating the importance of supplying adequate water to birds under heat stress (Yousef, 1985). In this study, results indicate no significant difference in daily water consumption regardless of diet formulation or environmental chamber. It is difficult to draw a conclusion about water consumption because the containers that were used did leak. Adjustments were made to the data that may have affected these results and unfortunately more water containers from the heat stress chamber were leaking than those in the thermoneutral chambers.

The live weight and carcass weights of birds in Experiment 2 were not significantly different between dietary treatments ($P>0.05$), and dressing percent was also not affected by which diet treatment was given. Temperature had a significant impact on live weight and carcass weight, with birds in the thermoneutral environment being significantly larger than the heat stressed birds ($P<0.05$). This is consistent with reported

observations from several studies (Howlider and Rose, 1989; Smith, 1993; Cooper and Washburn, 1998). The dressing percent for the heat stressed birds was significantly higher than birds from the thermoneutral chambers ($P<0.05$). A study performed by Cooper and Washburn (1998) found that birds exposed to heat stress experience a significant reduction in feather cover when exposed to temperatures of 32 °C. Although feather cover was not measured in the present study, this may have been why heat stressed birds had a higher dressing percent.

Breast weight, percent breast, abdominal fat weight, and percent abdominal fat were not altered by dietary treatment in Experiment 2. Studies suggest that betaine may increase breast weight and decrease fat (Virtanen and Rosi, 1995; Lobo, 1999) however results from experiment 2 did not reflect that. Ambient temperature does affect breast weight and abdominal fat. Results from previous studies have shown that birds reared in high ambient temperatures have lower breast weights than birds reared in thermoneutral temperatures (Smith, 1993). In the present study, breast weights of birds placed in the heat stress chambers were significantly lower than their thermoneutral counterparts ($P<0.05$). Percent breast weights were also higher for the thermoneutral chamber birds, suggesting that the addition of betaine to the diet was not able to overcome the detrimental effects of heat stress.

Abdominal fat pad results during heat stress conditions have been mixed in previous studies, with some researchers finding an increase in abdominal fat (Ain Baziz et al., 1996; Brown, 1999), while others have found a decrease in abdominal fat (Smith and Teeter, 1987; Sands, 1997). Results from the current study show that birds placed in

high ambient temperatures had a numerically lower abdominal fat pad weight, but the percent abdominal fat was not affected by environmental temperature ($P>0.05$). Because basal metabolism and physical activity are reduced under heat stress conditions, more energy is available for growth, but this extra energy can be stored as abdominal or subcutaneous fats in chickens (Ain Baziz et al., 1996).

Birds will adjust their feed intake according to the dietary energy level allowing them to maintain weight gain over a range of diet energy levels (Cupo and Cartwright, 1991). Experiment 3 examined the effects of adding betaine to high density (calorie:protein ratio includes more protein) and low density diets. There were significant differences in feed consumption among the dietary treatments, with the birds receiving the high density diets consuming significantly more feed than birds receiving the low density diets ($P<0.05$). In a previous experiment that compared high density and low density diets, the feed consumed per bird was greater among birds that received the high density diet verses those fed the low density diets (Cupo and Cartwright, 1991). Because energy intakes were equal in Cupo and Cartwright's study (1991), birds may have consumed more feed in the high density group in order to meet energy requirements, and at the same time were consuming more protein than birds in the low density group. The high density diets in Experiment 3, contained more fat than the low density diets which may have made it more palatable which would promote an increased feed consumption (Brue and Latshaw, 1985). As a result of lower feed consumption, birds receiving the low density diets did not gain as much weight as birds that received the high density diet. The total adjusted feed efficiency shows that the high density

treatment groups were more efficient at utilizing feed to gain than birds in the low density treatment groups. This was true whether the birds received betaine in the diet or not, suggesting that the addition of betaine was not beneficial to birds receiving low density diet.

The preslaughter weight, carcass weight, and carcass measurements taken also demonstrated significant differences between treatments. Birds that received the high density diets had a significantly larger live weight and carcass weight than the birds that received the low density diets. The dressing percent for the high density birds was also significantly higher. These results are consistent with previous studies which also found that birds given a high density diet had significantly greater body weights than birds given a low density diet (Cupo and Cartwright, 1991). In this study, the addition of betaine to the diet did not have any effect on live weight or carcass values.

Differences between the high and low density diet treatments were also reflected in the breast weight, with the high density treatment birds having significantly larger breast weights than the low density treatment birds, however there was no significant difference in the percent breast weight. Although the low density birds were smaller, they were able to maintain the same percentage of ther body weight as breast weight. The addition of betaine to the diet did not influence body weight ($P>0.05$). There was no significant difference in the abdominal fat weight, however the low density birds had higher percent abdominal fat than the high density birds. From previous studies, it appears that the percent body fat will decrease as the calorie:protein ratio decreases (Robbins, 1981; Pesti, 1982). Results from the present study agree with these findings.

Male and female broilers were used in Experiment 3. Although sex difference in feed:gain could not be calculated because the birds were raised together in floor pens and ate from the same feeders, the live weight and carcass values of males and females could be compared. Males had a significantly higher live weight and carcass weight than females, and as a result the dressing percent for the male birds was also significantly higher ($P<0.05$). Previous studies have also shown that males grow faster than females (Robbins, 1981), and females need to consume more feed to reach an equivalent weight to males (Howlider and Rose, 1989). In the present study, males had larger breast weights than females, however females had higher percent breast than males. Howlider and Rose (1989) also found female broilers gained more breast meat and had a greater breast:dark meat ratio than males. There was no significant difference in abdominal fat weight between males and females, but females had significantly higher percent abdominal fat than males ($P<0.05$).

Intestinal strength was measured in each of the experiments, and the addition of betaine to broiler diets did not affect tensile strength ($P>0.05$). Previous research has shown that addition of betaine in combination with salinomycin to the diet can improve lesion scores of broilers infected with avain *Eimeria* species (Augustine et al., 1997a). Birds used in this study were not infected in order to determine if betaine could improve intestinal strength in healthy birds. If betaine could improve intestinal strength, it would be especially beneficial during processing of the carcass. When intestines break while being processed, the entire carcass is contaminated and must be disposed of which results in economic losses (Remus, personal contact). The current study did not show

improvements in tensile strength for broilers that were given diets supplemented with betaine. There were significant differences between the male and female birds used in Experiment 3, with male birds having a significantly higher tensile strength value ($P>0.05$) than female broilers given the same diet treatment. Further investigation of male and female differences may provide useful information about betaine and its ability to improve intestinal integrity.

Previous research in pigs has shown that betaine can be added to the diet as an energy nutrient resulting in leaner back fat, and larger loin eye areas. The present study produced mixed results. The addition of betaine to the diets of broilers in one experiment produced leaner birds with less abdominal fat. This however was not demonstrated in the other two experiments. Betaine also works as an osmoprotectant, and it was added to the diets of heat stressed broilers to determine if it could alleviate the deleterious effects heat stress has on growth performance. This was not observed in the current study, however adding betaine to the diets of heat stressed broilers is a new area of research and warrants further investigation.

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Vita

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