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Evaluation of Betaine and Methionine Replacement for Improving Performance and Meat Quality for Broilers reared under Higher Temperature Conditions

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ABSTRACT

4,096 broiler chicks were randomly allocated to 128 floor pens (32 birds/pen). 2,048 day-old male broilers were placed in the east end of a barn, and the following week 2,048 day-old male broilers were placed in the west end. At each placement day, half of the chicks were Cobb 500 and half were Ross 708, and each pen contained only one breed source. East end birds received coccidiostat in the feed, west end received coccidial vaccine, and each end was under separate environmental control. Eight diets contained two levels of coccidiostat (0, 1 lb./ton), methionine (deficient, adequate), and betaine (0, 2 lb./ton). Live weights were measured at days 0, 15, 31, 42 and 56. Cocci scoring was performed on day 22, ammonia flux was measured on day 36, and paw scoring was performed on days 42 and 56. Processing occurred on days 43 (5 birds/pen) and 57 (5 birds/pen). There were no significant differences between treatments in live weights for days 15, 31, 42, or 56. Day 42 paw scores for birds fed betaine + deficient methionine were significantly lower than other treatments, for Cobb Treatments 3 and 7, and for Ross Treatment 3. Birds in the west end had no cocci lesions, while the east did. Ross birds receiving coccidiosis-vaccine, fed betaine + reduced methionine had lower ammonia flux than Ross birds receiving either coccidiostat, fed no betaine + reduced methionine or Ross birds receiving coccidiosis-vaccine, fed betaine + adequate methionine. At day 57, Cobb birds fed betaine + reduced methionine had significantly higher breast and tender weights than all other Cobb birds, while Ross birds fed reduced methionine + no betaine had higher wing weights than any Ross birds receiving betaine. These findings indicate betaine supplementation can act to partially spare methionine. Betaine supplementation was also shown to decrease single-day heat-related mortality and also affect processing performance in broilers reared to heavy weights.

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DEDICATION

This dissertation is dedicated to

my husband, Kyle Frank

my mother, Kate Graham

my father, Lonnie Graham

my son, Graham Xavier Frank

and my daughter, Sophia Joy Frank.

Graham and Sophia, go explore the world and rise to meet any challenge ahead of you.

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INTRODUCTION

Methionine and betaine are two major methyl group donors in broiler diets. Methyl groups are involved in many reactions including DNA and RNA methylation and protein synthesis. In addition to methyl donor functions, methionine and betaine each provide unique contributions in poultry nutrition. Betaine has been firmly established as an osmolyte and methyl donor in poultry, but some effects associated with these functions are unclear and are still under investigation. Methionine supplementation is required for optimum growth and development of broiler chickens. Methionine is an essential amino acid, and also the first limiting amino acid in chickens, and for this reason, is frequently incorporated into commercial broiler diets. Betaine and methionine are poultry ration ingredients that share some functions, while also offering exclusive benefits to poultry. The effects of betaine and methionine, supplemented either in combination or separately, are still not fully understood in broiler nutrition.

The current research was performed to investigate the effects of methionine and betaine on growth performance and processing performance of male broilers grown to heavy weights. A second goal was to evaluate the benefits of betaine for heavy broilers exposed to heat stress as well as measure the effects of methionine and betaine fed at various inclusion levels. This dissertation contains two chapters. Chapter 1 is the literature review and Chapter 2 describes the effects of betaine on big broilers reared under heat stress conditions, when provided with a methyl donor at various inclusion rates.

Chapter 1: Literature Review.

Chapter 2: Evaluation of Betaine and Methionine Replacement for improving performance and meat quality for broilers reared under higher temperature conditions.

CHAPTER I

REVIEW OF LITERATURE

BETAINE AND METHIONINE

Introduction

Betaine and methionine each have important roles in broiler nutrition. Much research exists that indicates betaine can act as both a methyl donor and an osmolyte. Betaine contains methyl groups, suggesting it can partially spare methionine as a methyl group donor. Betaine is also a zwitterion, which provides osmolytic properties, which can be beneficial to an animal during periods of osmotic stress. Past research suggest various effects of betaine, though these studies do not always produce the same findings. Many parameters have been investigated in the research of betaine. Live performance, processing performance, and body composition are frequently evaluated in respect to betaine. Some parameters that are often described in the literature as being affected by betaine are breast meat yield, flock livability, and tolerance to high temperatures. The possibility of betaine sparing some methionine is also a common topic in betaine research. Betaine research does not always reach the same conclusions, but there are some findings that occur with relative consistency. The following chapter serves to describe the the chemistry and effects of methionine and betaine, and the results of research on betaine in poultry nutrition.

METHIONINE

Methionine is the first limiting amino acid for commercial broilers fed corn-soybean based diets. Broilers are unable to synthesize the carbon skeleton of methionine, thus methionine is an essential amino acid and must be supplemented in the diet to optimize poultry growth and feed efficiency (Matterson et al., 1953; Wallis, 1999). Research has established that methionine can affect broiler performance, including protein deposition, feathering, and immune function (Almquist and Grau, 1944; Matterson et al., 1953; Kidd, 2004; Sauer et al., 2008; Jankowski et al., 2014). Methionine has an important role as a sulfur donor. Methionine is a key metabolite in the trans-sulfuration pathway where it serves as a precursor in the synthesis of cysteine, and also as an intermediate in the transmethylation pathway (Wallis, 1999). Since methionine is an integral constituent of protein synthesis, and broilers cannot synthesize the carbon skeleton portion, supplementation is essential in broiler diets.

Methionine is typically supplemented in commercial broiler diets in either of two forms: DL-methionine or Methionine Hydroxy Analogue-free acid. DL-methionine, or DLM, is 99% active and supplemented in dry form. An analogue to DLM is Methionine Hydroxy Analogue-free acid, often referred to as MHA. MHA is reported to be 88% active, compared with DLM that is 99% active (Vazquez- M et al., 2006b; Sauer et al., 2008; Vedenov and Pesti, 2010). The relative bioefficacy of DLM and MHA is a source of continued discussion within the poultry industry. Results from studies investigating the bioefficacy of both methionine forms are not always in agreement. Some researchers have stated that the statistical analysis models used are often inappropriate for the type of data obtained (Kratzer and Littell, 2006; Vedenov and Pesti, 2010). Different study results show that environmental temperature and dietary factors such as amino acid ratios, sodium levels, or dietary protein levels can influence poultry production parameters (Balnave and Brake, 2004; Nukreaw et al., 2011; Swennen et al., 2011; Conde-Aguilera et al., 2013). Regardless of the form of methionine, be it DL-methionine or an analogue, it is well established that supplemental methionine plays a significant role in poultry nutrition. However, the effects of methionine on performance parameters are not clear. Therefore, research quantifying some effects of dietary methionine is an important endeavor.

Methionine - Metabolism and Effects

DL-methionine contains a D-isomer and an L-isomer in a 1:1 ratio (Dilger and Baker, 2007). The most active, readily-usable methionine isomer is the L-isomer. Dietary DL-methionine has been shown to have the same effectiveness as dietary L-methionine, in nutrition studies (Martin-Venegas et al., 2006; Dilger and Baker, 2007). For DL-methionine, broilers are able to convert 70-100% of the D-isomer to the L-isomer (Baker and Boebel, 1980; Dilger and Baker, 2007).

Absorption of dietary methionine occurs in the small intestine. Methionine must first be absorbed across the intestinal epithelium, and then can be metabolized to L-methionine (Martin-Venegas et al., 2006). The liver is the predominant location for methionine metabolism, but the kidney and small intestine can also metabolize methionine (Martin-Venegas et al., 2006; Martinov et al., 2010; Martín-Venegas et al., 2013). DL-methionine is able to be absorbed by a Na+-dependent mechanism (Dibner and Knight, 1984), or a Na+-independent mechanism (Soriano-Garcia et al., 1999). Transport of DL-methionine is mediated by systems that transport neutral amino acids and also by systems that transport cationic amino acids (Soriano-Garcia et al., 1999). DL-methionine is converted to L-methionine, once inside the liver. The activity of L-methionine is the same, regardless of the original methionine source. L-methionine is then able to enter the transmethylation cycle for methyl donor activity, or the trans-sulfuration pathway as a metabolite in protein synthesis. Sequential descriptions of the transmethylation cycle and trans-sulfuration pathway are included previously in this chapter, in the betaine discussion. Entering the trans-sulfuration pathway or the transmethylation cycle is the route for methionine to perform its unique functions.

Understanding of methionine activity in poultry has been vastly improved through research for many decades. Methionine is an essential amino acid, and the first limiting amino acid, in broiler diets. For proper feathering, protein synthesis, and sufficient methylation, methionine must be supplemented in commercial broiler diets that are based on corn-soy ingredients. Many studies have addressed the effects of methionine on broilers, in various experiments, with varying results.

Effects

One study evaluated the effects of methionine levels on 448 male Ross 308 broilers (Ribeiro et al., 2005). Experimental vegetable diets were fed from day 22-42, and the broilers were subjected to cyclic heat stress from days 24-42. Broilers were processed at day 42. DL-methionine was supplemented at 0, 0.73, or 0.93% of the diet. Feed conversion ratio was the parameter most sensitive to varying methionine levels during the experimental period: day 22-42. Broilers fed rations containing 0.93% dietary methionine had significantly lower, thus better, feed conversion than birds fed rations containing 0.73% dietary methionine, with the respective levels of 1.821 and 1.926. Supplementing methionine at either 0.73 or 0.93% resulted in feed conversion ratios that were significantly lower and better than control fed birds. However, there were no significant differences between any of the supplemental methionine levels, 0, 0.73, or 0.93% for the parameters feed intake, weight gain, or body weight. Processing results at day 42 did not show any significant differences between the three treatments for carcass yield, breast yield, leg yield, or thigh yield. Therefore, increased supplemental methionine levels were able to improve feed conversion, but did not improve feed intake, weight gain, or any processing parameters at day 42 of age.

Another investigation utilized chronic high temperature conditions when rearing broilers and supplementing various methionine forms and inclusion levels (Willemsen et al., 2011). A total of 320 Ross male broilers were reared to 6 weeks of age, and methionine was supplemented at 1.0 or 1.2 g/kg of feed. Birds were divided into two different groups at 2 weeks of age. One group lived in a constant temperature of 32° C until week 6, and another group's temperature gradually decreased until it reached a final temperature of 18° C by week 6. Results indicate that chronic heat stress caused a decrease in performance parameters, including body weight and feed intake. Processing parameters not affected by heat stress in this study include breast meat and liver yield. Chronic heat stress treatment, under the conditions of this study, caused a decrease in heart yield and an increase in abdominal fat yield.

The effect of supplemental methionine in broilers was investigated in regards to feed conversion, weight gain, and breast meat yield (Hoehler et al., 2005). Five different experiments were conducted at various facilities, in which male broilers were supplemented different methionine sources and inclusion levels. Breed source, concluding at approximately day 40. Treatments were graded levels of various methionine sources, supplemented to create multiple levels of methionine supplied by different sources. The results of these multiple studies showed that when supplementing with increasing methionine levels, significant performance improvements were observed. Though this study focused on the differences between methionine sources, the general trends showed significant improvements as methionine levels increased for parameters including body weight gain, feed conversion, and breast meat yield. These trials provide further evidence that methionine does affect live performance and processing parameters.

BETAINE

Chemistry and Metabolism

Betaine was first isolated from the sugar beet plant, *Beta vulgaris*, in the 1860's by a German chemist named Scheibler (Lever and Slow, 2010). Research on the effects of betaine in poultry dates to the 1940's (McGinnis et al., 1942; Moyer and Du Vigneaud, 1942; Almquist and Grau, 1943; Almquist and Grau, 1944). The early studies of betaine in poultry focused primarily on the prevention of perosis and the promotion of growth (McGinnis et al., 1942; Almquist and Grau, 1943; Almquist and Grau, 1944). Betaine is a by-product of sugar beet processing. Thus, the pronunciation of betaine is attributed to the nutrient's source. Betaine in animal nutrition is commonly referred to as "betaine," "trimethylglycine" or "glycine betaine." Dietary betaine is most common in two forms: anhydrous betaine and betaine hydrochloride, $C_5H_{11}NO_2$ and $C_5H_{12}ClNO_2$, respectively. Betaine is highly soluble in water, and can be supplemented in feed, in dry powder form or liquid form, or the crystalline powder form dissolved in drinking water.

Many studies have investigated the effects of betaine as an osmolyte. An osmolyte enhances the cell's ability to regulate the movement of water in and out of the cell, which helps maintain cellular function and structure (Kidd et al., 1997). The chemical structure of betaine is an important reason for its osmo-protective properties. Betaine is a zwitterion. Zwitterionic molecules possess a net neutral charge, while having a positive charge on one atom and a negative charge on another atom within the molecule, and the charged atoms must be joined by one or more covalent bonds. This zwitterion is quite soluble in water, enhancing its ability to accumulate in cells and cell organelles that are exposed to osmotic stress (Kidd et al., 1997). Since the molecule is a zwitterion, and possesses no net charge, betaine is able to accumulate in cells without altering intracellular ionic balance, and is undisruptive to the molecular conformation of native intracellular proteins (Lever and Slow, 2010). Additionally, betaine does not bind or attach to intracellular proteins, thus leaving the molecule's mobility unrestricted, and not inducing conformational changes in native proteins (Porter et al., 1992). Accumulation of betaine in cells during times of dehydration allows the cells to maintain osmotic homeostasis by minimizing water loss to hyperosmotic extracellular fluid (Kettunen et al., 2001b).

Exogenous betaine absorption and transport

Dietary betaine enters the digestive tract via feed or water and is absorbed in the intestine via Na^+-dependent and Na^+-independent factors (Kettunen et al., 2001a). The two epithelial betaine transport systems are the betaine γ-aminobutyric acid transporter (BGT-1) and amino acid transport system A (Kettunen et al., 2001a; Craig, 2004; Metzler-Zebeli et al., 2009). The addition of dietary betaine has been shown to increase its own rate of absorption via Na+-dependent transport, most markedly in the duodenum (Kettunen et al., 2001a). After intestinal absorption, betaine enters the portal blood where it is carried to the liver. Upon entering the liver, betaine enters hepatic cells and remains in the cytosol. Once in the cytosol, betaine is then able to either donate a methyl group to homocysteine, or remain structurally intact and confer osmo-protective properties to the cell.

Endogenous betaine synthesis

Chickens are capable of synthesizing betaine from dietary choline. Endogenous betaine is synthesized in the liver of chickens. Following intestinal epithelial absorption, choline enters the portal blood and is transported to the liver. In the liver, choline then enters hepatic cells, and then is predominantly transported via active transport across the mitochondrial membrane (Porter et al., 1992; Porter et al., 1993). Passive diffusion only accounts for a minor percentage of choline entry into hepatic mitochondria (Porter et al., 1992; Porter et al., 1993). Within the hepatic mitochondria, choline undergoes two enzymatic reactions to yield betaine.

Choline is first acted upon by choline oxidase, yielding betaine aldehyde. Betaine aldehyde is then acted upon by the enzyme betaine aldehyde dehydrogenase, resulting in the formation of betaine. Following synthesis, endogenous betaine passively diffuses across the mitochondrial membrane, into the cytosol (Porter et al., 1992). After entering the cytosol, betaine is then able to donate a methyl group, regardless of whether the source of betaine was endogenous or exogenous. As is the case for endogenous betaine, exogenous betaine can either remain intact in the cytosol and confer osmolytic benefits to the cell, or act as a methyl donor to synthesize methionine.

Methyl Donor Functions

The methyl donor function of dietary betaine has also been investigated in many studies. As the name trimethylglycine suggests, the molecule consists of three methyl groups bound to the nitrogen atom of a glycine molecule. Methionine, choline, and betaine are the three dietary sources of methyl donors in broiler diets. The liver is the site of methyl donor activity for nearly all exogenous betaine (Kidd et al., 1997). Betaine is the only methyl donor that does not require activation to enable methyl group donation, once in the hepatic cytosol (Kidd et al., 1997). The ability of dietary methionine, choline, and betaine to spare each other has been the subject of extensive research. Collectively, the results across many years indicate that each of these methyl donors can only spare each other to the extent that the spared compound acts as a methyl group donor (Kidd et al., 1997; Eklund et al., 2005; Ratriyanto et al., 2009). Requirements and properties not related to methyl donation, and exclusive to one methyl donor, cannot be performed by either of the other two methyl donors (Kidd et al., 1997; Eklund et al., 2005; Ratriyanto et al., 2009).

Betaine in transmethylation

Homocysteine is an important molecule in sulfur amino acid metabolism because it conserves the sulfur moiety and is involved in the synthesis of both sulfur amino acids: cysteine and methionine. Two metabolic pathways compete for homocysteine: trans-sulphuration pathway and homocysteine trans-methylation pathway. The presence of homocysteine is a requirement for either pathway to function. In the trans-sulphuration pathway, homocysteine is metabolized to cysteine, which is irreversible. Betaine is not directly involved in trans-sulphuration. Betaine is, however, directly involved in the transmethylation pathway as a methyl donor. The transmethylation pathway is a recycling pathway involving the metabolism of methionine. Previous investigators have studied the metabolic pathway of transmethylation and formed a comprehensive description of the metabolic activity (Saunderson and Mackinlay, 1990; Kidd et al., 1997; Ratriyanto et al., 2009), described herein. The transmethylation pathway has two possible routes of methionine synthesis. These routes are catalyzed by different enzymes: methionine synthase (MS) and betaine homocysteine methyltransferase (BHMT). BHMT is the enzyme that catalyzes methyl transfer from betaine to homocysteine. Betaine serves as the only molecule that can methylate homocysteine. BHMT is responsible for approximately 50% of all methionine synthesis in the transmethylation pathway (Pillai et al., 2006). After betaine donates a methyl group, the remaining product is dimethylglycine, which possesses two methyl groups. Dimethylglycine is further catabolized to sarcosine, and then to glycine, via removal of a methyl group in each step. During this catabolism, these resulting one-carbon fragments enter the carbon pool. MS does not directly act on betaine. The one-carbon fragments resulting from betaine catabolism are able to bind to the tetrahydrofolate precursor molecule (Pillai et al., 2006). MS then catalyzes the methyl group transfer from 5-methyltetrahydrofolate to homocysteine, forming methionine. BHMT can catalyze the transfer of a methyl group from betaine to homocysteine, and MS can catalyze the transfer of methyl groups formed in part from the catabolism of betaine, to methylate homocysteine. Thus, betaine is directly and indirectly involved in homocysteine transmethylation.

Betaine methyl donor activity occurs in liver cytosol (McKeever et al., 1991; Kidd et al., 1997). Betaine donates a methyl group to homocysteine, in the transmethylation cycle, described previously. Methyl group transfer, whether from endogenous or exogenous betaine, follows the same enzymatic steps once betaine enters the cytosol. The enzymes and products of the transmethylation cycle that uses betaine as a methyl donor is as follows: Homocysteine and betaine are acted upon concurrently by betaine homocysteine methyltransferase (BHMT). This reaction transfers a methyl group from betaine to homocysteine, and yields dimethylglycine and methionine. This newly synthesized methionine is the product of homocysteine methylation. Said methionine can then be acted upon by methionine adenosyltransferase and adenosine triphosphate (ATP), to yield S-adenosylmethionine (SAM). S-adenosylmethionine donates a methyl group for methylation reactions, and the new product yielded is S-adenosylhomocysteine. Next, S-adenosylhomocysteine is acted upon by the enzyme S-adenosylhomocysteine hydrolase, which produces homocysteine. This newly-synthesized homocysteine is now available to enter either the transsulphuration pathway or the transmethylation pathway, depending on the metabolic demands of the bird.

Carcass composition and growth performance

Using male broilers fed diets containing increasing levels of methionine and betaine, one study reported that betaine supplementation could increase breast meat mass, but could not decrease abdominal fat pad weight, in broilers grown to 42 days (McDevitt et al., 2000). Betaine was supplemented at 0 or 0.5 grams per kilogram, and methionine was supplemented at 0, 0.6, or 1.2 grams per kilogram, for six total diets. Betaine was ineffective at decreasing abdominal fat pad weights. When fed diets containing 0.6 grams of supplemental methionine per kilogram and 0.5 grams of supplemental betaine per kilogram, breast meat mass was significantly increased compared with diet supplemented with 0.6 grams per kilogram of methionine without added betaine. Further, when betaine was added to a ration devoid of added methionine, there was no significant effect on breast meat mass between said treatment and the control diet, suggesting a limited effect of betaine alone. Interestingly, the combination of betaine with supplemental methionine at 1.2 grams per kilogram was statistically similar to the treatment containing 1.2 grams per kilogram methionine with no added betaine. The only combination that produced a significant difference between treatments of the same methionine level was the 0.5 grams per kilogram of betaine with the marginally deficient 0.6 grams per kilogram of methionine. These results suggest a synergistic response affecting breast meat mass, between betaine and methionine, when methionine is deficient.

Other investigators have also demonstrated that betaine supplementation can improve breast meat yield (Zhan et al., 2006; Waldroup et al., 2006; Rao et al., 2011). Waldroup et al. fed diets at decreasing methionine levels of 0.31, 0.259, 0.209, 0.158, and 0.108 % of ration (2006). These diets were then supplemented with either nothing (control), 1,000 mg/kg choline, 1,000 mg/kg betaine, or 500 mg/kg of betaine and 500 mg/kg of choline. Day old male broilers were raised in pens and processed on days 42, 49, and 56. Birds supplemented with betaine, choline, or both in combination demonstrated significantly higher breast meat yield than control on all processing dates. Further, on day 56 the broilers supplemented with 1000 mg/kg of betaine had significantly higher breast meat yield than the broilers supplemented with 1000 mg/kg of choline. The present study found that decreasing methionine levels negatively impacted breast meat yield for all betaine levels, which conflicts with McDevitt et al.'s findings that show breast meat yield increased for diets supplemented with betaine in combination with deficient methionine (2000).

A different study suggests that betaine can affect carcass composition by increasing breast meat yield and decreasing liver weight, but does not significantly affect abdominal fat pad weight (Rao et al., 2011). Day old male broilers were grown to 42 days, and processed on days 21 and 42. Diets were supplemented with betaine at 0 or 800mg/kg of diet, and with methionine to achieve final methionine levels of 15, 18, 20, 22, and 24 g Met/kg Crude Protein. Abdominal fat pad weight was not significantly influenced by betaine at any age. Breast meat yield on days 21 and 42, however, was significantly improved by the addition of betaine, compared with diets not supplemented with betaine. Additionally, breast meat weight at day 21 was significantly higher for diets supplemented with betaine and deficient in methionine at 15 or 18 g/kg crude protein, or CP, compared with the same methionine-deficient diets that contained no supplemental betaine. There was no interaction between betaine and methionine at any other methionine level in regards to breast meat weight. This synergistic response between betaine and deficient methionine is similar to the response observed by McDevitt et al. (2000), though McDevitt et al. showed a significant day 42 response that the current study could not. Liver weights in the present study were also affected by betaine, but in an inverse fashion. The inclusion of betaine at 800 mg/kg was shown to decrease liver weights at day 42, compared with control. The findings of this study show that betaine can affect the carcass composition of broilers.

Other research suggests that betaine has different impacts on carcass composition, as well as varied performance effects. One such study found that dietary betaine could improve weight gain in broilers fed a diet lacking supplemental methionine, compared with control (Pillai et al., 2006). This study reared broilers from day 8-22 in battery cages. Methionine was supplemented in graded levels at 0. 0.07, 0.11, or 0.24%. Choline was added at either 0 or 0.25% and betaine was supplemented at 0 or 0.28%, for a total of 20 diets. The only growth response significant for betaine was gain at day 22, compared with a diet devoid of added methionine, though the betaine diet also performed statistically similar to the diet supplemented with choline and devoid of supplemental methionine. Feed intake and feed efficiency were no different than control for any combination of betaine and methionine.

Esteve-Garcia and Mack grew female broilers to 41 days and fed diets supplemented with methionine at 0, 0.6, or 1.6 g/kg and supplemented with betaine at either 0 or 0.5 g/kg (2000). Broilers fed betaine-supplemented rations had a significantly higher carcass yield of 81.4%, compared to 80.8% for birds fed diets lacking supplemental betaine. Additional results for other carcass composition parameters, including carcass weight, breast yield, and abdominal fat weight, were not significant for betaine, when broilers were processed at day 41. Betaine's lack of a significant effect on abdominal fat pad agrees with the findings of Rao et al (2011). No significant interactions between methionine and betaine were identified, for any parameters measured. Performance parameters including livability, feed conversion, and live weights were not affected by the addition of dietary betaine, compared with non-betaine-supplemented feed, at days 21 or 41, in the present study.

Methyl donor sparing

One study investigated the effects of adding dietary methionine and betaine to broiler diets and the impact on carcass composition, live performance, and lipid metabolism (Zhan et al., 2006). Methionine and betaine were supplemented at 0 or 1g/kg and 0 or 0.5g/kg, respectively. A methionine-deficient diet served as the control. Betaine supplementation was equal to methionine supplementation for all parameters measured. Compared to control, betaine was statistically similar for carcass yield and feed intake, yet performed significantly better in feed conversion ratio, weight gain, breast yield, and percentage abdominal fat. These results indicate that betaine is capable of affecting carcass composition and growth performance, while producing similar results to added methionine. This also suggests that at the inclusion levels used in this study, betaine can partially spare methionine.

Another study investigated the ability of betaine to spare choline, and included dietary oil level as a factor (Mahmoudnia and Madani, 2012), during hot weather conditions. Betaine replaced choline at 0, 33, 66, or 100% and included dietary oil at either 0 or 2.5% of the diet. Results indicate that betaine can spare choline at all levels of choline replacement, with results that are nearly always equal to the control diet, for both oil inclusion levels. Feed conversion ratio was better from days 0-21 for all diets that included betaine to spare choline, in the diets without oil. However, feed conversion ratio for days 0-21 was not improved for any diets that included 2.5% oil, compared with control. There was also a significant improvement in body weight gain from day 0-21 for both oil levels, compared with the control. The most significant improvements in live performance occur at 33 and 66% sparing of choline by betaine, of which both levels showed similar statistical results. Results indicate that betaine can spare choline at 33, 66, and 100%, in heat stress conditions, for either oil level, with results that meet or exceed those of the control diet.

In a separate trial, researchers evaluated the ability of betaine to replace methionine in diets (Schutte et al., 1997). Two different basal diets were used: a practical diet or a corn-soybean diet. Next, methionine was supplemented at 0. 0.05, or 0.1% of diet, and betaine was supplemented at either 0 or 0.04% of diet. Choline was provided at 220 parts per million in all diets. Birds were reared in floor pens to 39 days and processed. The only significant difference in breast meat yield occurred for a diet with 0.04% betaine, when compared to a control diet with no added methionine. Methionine supplementation alone was able to increase breast meat yield to a greater percent than was betaine supplementation. Similar trends for feed conversion, body weight, and slaughter weight indicate that betaine is not able to replace methionine on a equimolar basis, under the conditions of said study.

Heat Stress

The effects of betaine supplementation during cyclic heat stress were investigated in broilers reared to 45 days (Sakomura et al., 2013). Day old broilers were randomly allocated to 4 dietary treatments and reared to 21 days in similar conditions, and on day 22, allocated to either control or heat stress environment, for a 2x4 factorial arrangement. The dietary treatments were positive control, negative control, negative control + betaine, and negative control + betaine at a higher level. Except for positive control, the treatments had decreased methionine by 11.18% and included no choline. Birds were processed at day 45. Results showed that betaine did not affect live performance or processing parameters, including breast, leg, or carcass yield.

Betaine impact on broilers submitted to heat stress was also investigated in another trial (Sayed and Downing, 2011). Sayed and Downing reared broilers to 32 days of age under thermoneutral conditions and then exposed birds to cyclic heat stress and high relative humidity of 80-100% (2011). Betaine was supplemented in drinking water at 0, 500, or 1000 mg/L. Birds supplemented with 500 mg/L betaine demonstrated an improved body weight gain compared with control, for the period 32-36 days. Interestingly, the birds supplemented with 1000mg/L betaine performed similar to the control and to the treatment using 500 mg/L betaine. Thus, optimum body weight gain for day 32-36 was achieved by supplementing with 500mg/L betaine, but increasing the betaine did not improve body weight gain compared with control. From day 37 to the end of the trial at day 45, there was no difference between any of the treatments.

The activity of betaine as a methyl donor and an osmolyte are well-documented within scientific literature. While the functions of betaine are established, the effects these functions have on broilers is still under some investigation. Researchers have demonstrated the ability of betaine to affect the carcass composition and growth performance of poultry, with varying results. The literature frequently addresses the impact of betaine on breast meat yield, abdominal fat pad weights, and flock livability. Methionine is also an important methyl donor in poultry nutrition, as it is an essential amino acid. The interrelationship between methionine and betaine does exist, but the extent of the effects is not completely established. Some trends have emerged with some consistency, while other results are not clearly explained by the presence of betaine. Other research also addresses the impact of betaine during heat stress conditions. Though much research exists, questions still remain regarding the effects of dietary betaine supplementation in broilers. For this reason, a study was conducted to evaluate the effects of betaine supplementation on broilers grown to heavier weights in heat stress conditions.

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ABSTRACT

4,096 broiler chicks were randomly allocated to 128 floor pens (32 birds/pen). 2,048 day-old male broilers were placed in the east end of a barn, and the following week 2,048 day-old male broilers were placed in the west end. At each placement day, half of the chicks were Cobb 500 and half were Ross 708, and each pen contained only one breed source. East end birds received coccidiostat in the feed, west end received coccidial vaccine, and each end was under separate environmental control. Eight diets contained two levels of coccidiostat (0, 1 lb./ton), methionine (deficient, adequate), and betaine (0, 2 lb./ton). Parameters measured include live weights, cocci scoring, ammonia flux, paw scores, and carcass traits. Live weights were measured at days 0, 15, 31, 42 and 56. Cocci scoring was performed on day 22, ammonia flux was measured on day 36, and paw scoring was performed on days 42 and 56. Processing occurred on days 43 (5 birds/pen) and 57 (5 birds/pen). There were no significant differences between treatments in live weights for days 15, 31, 42, or 56. Day 42 paw scores for birds fed betaine + deficient methionine were significantly lower than other treatments, for Cobb Treatments 3 and 7, and for Ross Treatment 3. Birds in the west end had no cocci lesions, while the east did. Ross birds receiving coccidiosis-vaccine, fed betaine + reduced methionine had lower ammonia flux than Ross birds receiving either coccidiostat, fed no betaine + reduced methionine or Ross birds receiving coccidiosis-vaccine, fed betaine + adequate methionine. At day 57, Cobb birds fed betaine + reduced methionine had significantly higher breast and tender weights than all other Cobb birds, while Ross birds fed reduced methionine + no betaine had higher wing weights than any Ross birds receiving betaine.

Key words: betaine, trimethylglycine, methionine, broiler, heat stress, sparing

Evaluation of Betaine and Methionine Replacement for Improving Performance and Meat Quality for Broilers reared under Higher Temperature Conditions

INTRODUCTION

Betaine and methionine act as methyl donors in poultry nutrition. Much research exists that states betaine can act as both a methyl donor and osmolyte. Betaine contains methyl groups, which suggests that it can partially spare methionine as a methyl group donor. Betaine is also a zwitterion, which provides osmolytic properties, which can be beneficial to an animal during periods of osmotic stress. Past research suggest multiple effects of betaine, but the various trial results are not always in full agreement. The ability of betaine to spare methionine as a methyl donor and also improve tolerance to higher temperature conditions was investigated in the present research. Live performance and processing performance were evaluated. Two different breed strains were used, to increase knowledge of the effects of betaine, across different breed sources. This investigational effort sought to improve the overall understanding of the effects of betaine in broiler nutrition.

MATERIALS AND METHODS

Birds and husbandry

This study had two separate chick starting dates and employed two different breed strains on both of those start dates. The two strains used were Cobb 500 and Ross 708. The start dates were one week, or seven days, apart. The study employed 4,096 male broiler chicks in total. 2,048 day-old chicks were placed on the first start date, of which half, or 1,024, were Cobb 500 broilers and the other half, the remaining 1,024, were Ross 708 broilers. On the second placement date, one week later, an additional 2,048 day-old chicks were placed. This second group of 2,048 chicks were half, or 1,024, Cobb 500 broilers and the other half, the remaining 1,024, were Ross 708 broilers.

A total of 4,096 day-old male broiler chickens were obtained for the study. Cobb 500 by-product [males/female line], and Aviagen Ross 708 were the broiler strains used. One-day-old male broiler chicks were obtained from the Cobb hatchery in Fayetteville, Arkansas (Cobb 500 by-product). The 2,048 chicks were sexed at the Cobb hatchery, and received routine viral vaccinations. An additional 2,048 day-old male chicks were obtained from OK Foods hatchery (Ross 708). The chicks were sexed at the OK Foods hatchery and received routine viral vaccinations. Chicks were randomly allocated to 128 pens starting with 32 chicks per pen (all Aviagen or all Cobb in any one individual pen). All treatments were replicated in 8 blocks per breed source and 8 pens per treatment (64 pens per breed), randomized within blocks for pens. Only healthy appearing chicks were used in the study and chicks were randomly selected. No birds were replaced during the course of the study when mortality, culling or bird sacrifice occurred.

Thirty-two birds were initially placed in each of the 3.5 feet (1.1 m) by 6 feet (1.8 m) pens (.061 square meters or .68 SF/bird). Each pen included one hanging feeder equipped with a Chore-Time® feed pan and one Chore-Time® standard flow nipple drinker line complete with flow regulator and four nipple drinkers. Water flow was adjusted weekly to provide optimum flow as per the Chore-Time® drinker specifications. Drinker flows were recorded weekly and all drinkers were within 2-6 mL/minute flow of each other, which is acceptable standard practice.

Birds were provided 23 hours light: 1 hour darkness during the first week, followed by 20 hours light: 4 hours darkness for the remainder of the trial. Dark period started at midnight (0000 Hours). Times were programmed into the Choretronix® controller system.

The experimental design is shown in Table 1.

The environment was maintained via a Choretronix® computer controller and six thermocouple sensors throughout the barn which adjusted the run time of exhaust fans, European inlets, radiant tube heaters and inlet foggers so that temperature and humidity were elevated to a humidity-temperature combined value of "160" (temperature in Fahrenheit + relative humidity % = 160) primarily after the birds were fourteen days old. Hallway floors were saturated with water as needed to maintain increased relative humidity levels.

After the day 42 weigh, temperature was increased in the barn by 5º F and remained 5°F above original temperature set point for the duration of the trial. This was performed on both sides of barn at appropriate dates to achieve this day 42 temperature increase.

Observations were recorded for general flock condition, temperature, lighting, water and feed availability, litter conditions, and unanticipated events for the house at least twice daily. Unexpected events or abnormal reactions were documented in the trial log book but none were recorded. Mortality was removed by checking pens a minimum of twice daily. A bird was culled only to relieve suffering or when it could no longer reach food or water. When a bird was culled or found dead, the date, pen number, and removal weight (kg) was recorded in the log book as well as on the pen record sheet.

All pens contained used litter placed at a depth of approximately 4 inches. Prior to trial start, used litter was removed from research barn 232, mixed to create a uniform blend, and replaced into pens. This was done to assure that any immune challenge stress associated with used bedding would be as uniform as possible to maximize metabolic demand on methionine and maximize benefits of betaine supplementation.

Experimental design

Commercial broiler diets were fed (Corn/SBM /DDGS/ProPlus 54). Diets were formulated to meet the nutrient requirements as recommended by the Cobb-Vantress production guide. All diets contained Axtra®PHY 10000 TPT (DAN phytase at 500 FTU) and Axtra®XAP (DAN NSP enzyme). Ionophore was used in Treatments 1-4 in the starter, grower, and finisher feeds at 60 g/ton inclusion rate (1.0 pound Biocox® per ton finished feed). Commercial coccidial vaccine was used on Treatment 5-8, and none of these feeds (Treatment 5-8) contained coccidiostats. Diets 1, 4, 5, and 8 were all adequate in methionine and were also the only four diets supplemented with choline chloride. This addition of choline chloride to diets 1, 4, 5, and 8 was included to achieve an adequate level of methyl donors. During the feed milling process, feeds with ionophore were mixed after all other feeds to minimize cross contamination.

The eight dietary treatments were Treatment 1 and 5 (Control), Treatment 2 and 6 (Reduced methionine), Treatment 3 and 7 (Betaine/reduced methionine), and Treatment 4 and 8 (Betaine/adequate methionine). Starter diets were fed from 0-15 days, grower diet from 15-31days, finisher diet from 31-42 days, and withdrawal diet from 42-57 days. All diets were pelleted (65 to 95° C), and the starter diet was also crumbled. Finely ground corn was used.

Samples were collected for ingredient analysis, feed analysis and betaine level determination. Diets were representative of industry formulations and calculated to provide the appropriate nutrition. Calculated ingredient content is shown in Tables 2-5. Analyzed nutrient composition is shown in Tables 6-13.

Two sets of one pound samples of major feed ingredients (corn, SBM, and DDGS) were collected. One set was sent to Eurofins for betaine analysis (TMG by HPLC) and the other set was sent to University of Missouri (UMO) for total amino acid analysis. Samples were sent directly from University of Arkansas. One pound of completed feed of each phase as described in Table 2 was sent to Eurofins for betaine activity and to UMO for CML+9 (cysteine, methionine, lysine plus 9 other amino acids) analysis directly from University of Arkansas. One pound of additional feed samples from each phase was retained at 45° F, by the University of Arkansas, for further analysis, if needed.

Two chicks per pen were marked with indelible ink and wing banded upon placement. These marked birds were utilized for cocci lesion scoring and gut histology analysis. The pre-selection of birds at placement minimized sampling bias and at twenty-two days, these birds were individually removed from the pens, weighed, killed via exposure to carbon dioxide gas prior to intestinal evaluation for cocci lesions by a trained veterinarian as well as samples one centimeter sections of the duodenal loop and ileal junction removed and placed in formalin for fixation prior to fixation on slides. Samples were collected and analyzed without any association other than pen of origin.

Initial stocking density was < 0.68 sq. ft./bird for approximately the first three weeks. At day 22, two birds were removed for cocci lesion scoring and gut histology analysis, which adjusted the density to 0.75 sq. ft./bird. On day 43, sampling of birds for processing reduced density to 25 birds/pen to provide approximate stocking density of 0.9 sq. ft./bird until study termination at day 56. Bird weights by pen were recorded at days 0, 15, 31, 42, and 56.

The pen served as the experimental unit. Pen security was sufficient to prevent bird migration and cross-contamination of treatments. Data was recorded in ink. Entries were legible, and each sheet of source data was signed or initialed, and dated by the person making the entry. Any mistake or change in the source data was initialed and dated on the form and when necessary, a brief statement provided as to why the change was made.

Regarding bird and feed disposition, all birds were euthanized by appropriate methods and all remaining feed, including mixer flushes, was disposed of via composting with litter. All mortality was incinerated which is an approved method for dead bird disposal.

Coccidiosis control

Coccidial control was accomplished through use of ionophore (60g/pound) in the starter, grower, and finisher feeds at 60g/ton for Treatments 5-8. Coccidial vaccine (Coccivac-D2 Intervet Schering-Plough Animal Health, Serial #: 94440015, Image 1) was used in Treatments 1-4. Said vaccination was mixed and sprayed, with the use of vaccine dye, at the University of Arkansas hatchery. The only form of coccidiosis control for Treatments 5-8 was dietary coccidiostat, and the only form of coccidiosis control for Treatments 1-4 was coccidial vaccine. No therapeutic drug therapy was used during the study.

Paw Scoring

Paw quality was assessed at time of harvest, days 42 and 56, on two pens of birds for each treatment (n>32 pens). Procedure performed was based on a published procedure and metric for this purpose. The system used was the National Chicken Council Animal Welfare Guidelines and Audit Checklist for Broilers American Association of Avian Pathologists Paw Scoring System. Birds with no blemish or burns which were less than the size of a pencil eraser on the bottom of the foot or pad area received a score of 0. Paw burns larger than a pencil eraser or smaller than quarter with minimum depth were scored a 1. Any paw burns larger than a quarter and deeper than the surface were scored a 2. Each bird was given a single score for paw quality.

Ammonia flux

Ammonia flux was measured at litter surface in the middle of the pen and recorded for each pen at day 36. Floor pen configuration allowed middle of pen to be used for the ammonia flux measurement which was done using a calibrated ammonia analyzer. Ammonia flux was measured using an inverted flux bucket with a cardboard manual paddle fan. After sealing the flux chamber over the litter to be evaluated, the paddle was turned 10 times then the initial pre-ammonia reading was taken. The process was repeated five minutes later. The difference between the two readings was determined to be the flux.

Processing

Broilers were processed on day 43 and day 57, for carcass analysis. Five (5) birds per pen were selected for processing at 42 days and again at 56 days of age. Selected birds were within 2 standard deviations of the treatment mean, when possible, as determined from the 42 day and 56 day pen weights. Selected birds were then double wing tagged for identification and individually weighed. All birds were taken off feed 10 hours prior to processing.

Birds selected for processing were transported in clean coops to the University of Arkansas poultry processing plant at approximately 8:00 a.m. Birds selected for processing were individually weighed on the processing dock prior to shackling, then stunned, and killed via exsanguination. Next, birds were scalded in 130° F water, after which the feathers, feet, head and viscera were removed, and a hot carcass weight was obtained. Carcasses were then chilled in an ice bath for 2 hours. Post chilling, the whole carcass was re-weighed and then pectoralis major, pectoralis minor, wings, and leg quarters were removed and individually weighed.

Data analysis

Raw data were analyzed using SAS 9.3, Cary, North Carolina, United States. Live performance data were analyzed using the pen as the experimental unit. Processing performance data were analyzed using the bird as the experimental unit. Analyses were performed using GLM, the General Linear Model, of SAS, for live performance and also for processing performance.

RESULTS

Performance results

Due to the difference between Cobb and Ross chick performance, results for treatments are described as Treatment 1-8. Treatments 1-8 consist of the 8 diets, described in the materials and methods section of this chapter. Diets 1-4 each contained BioCox60, and diets 5-8 did not contain BioCox60 or any other dietary coccidiostat. Diet 1 and diet 5 are formulated the same, with the only difference between the two being that diet 1 contains BioCox 60. Diet 2 and diet 6 are also formulated the same, with the only difference between the two being that diet 2 contains BioCox 60. Diet 3 and diet 7 are formulated the same, with the only difference between the two being that diet 3 contains BioCox 60. Diet 4 and diet 8 are also formulated the same, with the only difference between the two being that diet 4 contains BioCox 60. Broilers fed treatments 5-8 did receive a coccivaccine, while birds fed treatments 1-4 did not receive any coccivaccine. Results for the strains Cobb and Ross are listed separately, within the tables.

Live production results are shown in Tables 15-22. Ross chicks began the trial numerically smaller than Cobb chicks at day of placement, but attained heavier final live weights by the time they reached Day 57. There were no significant differences between treatments in live weights of the broilers for days 15, 31, 42, or 56.

Bird Selection for Processing

Selecting birds (5/pen) that were within 2 standard deviations of the treatment mean was not always possible. Multiple times every bird in a pen was weighed, and not enough birds (5) were within the acceptable range. This occurred on the west side also, so it is not simply the result of a large amount of mortality from day 43 on the east side. During the weigh, when there were not enough birds within the treatment range within a pen, birds as close to the range as possible were selected. When multiple birds had to be chosen outside of this range, the difference was balanced by selecting one bird slightly larger, and another slightly smaller than the 2 standard deviation range. Any time a bird for processing was selected that was outside of the 2 standard deviation range, all other options had already been exhausted by weighing every bird within the pen.

Processing Results

Processing results are shown in Tables 24-36. For day 43 processing results, for each strain, there were no significant differences among the treatments for pre-slaughter live weight, pre-chill and post- chill carcass weights without giblets (WOG) (Tables 24-25). These day 43 processing results were the same for the birds treated with a coccidiostat or with a coccidiosis-vaccine.

For the day 57 processing, the Cobb control birds given Biocox had the heaviest pre-slaughter live weights followed by the Betaine/Adequate Methionine treatment and these were both significantly heavier than the Betaine/Reduced Methionine treatment (Table 26). This was the same for the post-slaughter hot WOG and the post-chill cold WOG.

For the group that received coccidiosis-vaccine at the hatchery, for the Cobb strain, the birds with the Betaine/Reduced methionine had the significantly heaviest pre-slaughter weight compared to all the other treatments (Table 27). This trend held for the Cobb birds that received coccidiosis-vaccine, with the post-slaughter hot WOG and the post-chill cold WOG.

For parts yield at day 43, there was a treatment effect for the Ross birds given the coccidiostat for percent breast yield (Table 28). The highest breast meat yield was seen for the

Ross birds receiving the Betaine/Reduced methionine diets but this was significantly similar to the Control diets and the Betaine/adequate methionine diets. Only the reduced methionine diets supported a significantly lower breast yield. Percent wing yield was significantly less for the Ross birds receiving the control diet as compared to the Ross birds receiving the reduced methionine diets.

For the day 43 yield results, for the birds that received coccidiosis-vaccine at day of age, the breast meat percent yield was highest for the Cobb and Ross birds receiving the Betaine/Reduced methionine diets (Table 29). For the Cobb birds, this breast meat percent yield was significantly higher than the control fed, reduced methionine diets. For the Ross birds, this breast meat percent yield was significantly similar to the Betaine/Adequate methionine fed birds and the control fed birds. Percent leg yield for the Ross birds was highest for the birds fed the reduced methionine diets.

For the processing results for day 57, for the birds receiving the coccidiostat, the Cobb birds fed the Betaine/Adequate methionine had a significantly lower carcass yield than the Betaine/reduced methionine diets and the control fed diets (Table 30). The only other significant day 57 yield was seen for the Ross birds for percent leg quarter yield. Birds receiving the Betaine/adequate methionine diet had the lowest yield followed by the Betaine/reduced methionine. For the day 57 yield for Cobb birds which had coccidiosis-vaccine, the lowest percent yield were the birds receiving the reduced methionine diets. Yield for all the other treatments were significantly similar (Table 31). For percent breast meat yield, for both Cobb and Ross, the significantly lowest yield was for the birds fed the reduced methionine diets. Highest yield for both strains was for the birds fed the Betaine/reduced methionine diets but these were significantly similar to the control and Betaine/adequate methionine diets fed birds.

The only other yield that had significant differences was for the wing yield for the Ross birds. The Ross reduced methionine birds had the highest wing yield as compared to all the other treatments.

Regarding day 43 parts absolute weight, the only significant difference for the birds receiving the coccidiostat was for the Ross birds for their wing weight (Table 33). The highest wing weight was for the birds receiving the reduced methionine diets and this was significantly greater than the other treatments. This was the same for the Ross leg quarter weight. The reduced methionine fed birds had significantly greater leg quarter weights than all the other treatments.

For day 43 absolute weight of parts for birds which were coccidiosis-vaccine treated, the only significant difference was for the Ross birds receiving the reduced methionine diets (Table 34). Their leg quarter weight was similar to the Betaine/reduced methionine fed birds but was significantly higher than the birds receiving the control diets or the Betaine/adequate methionine diets.

For the day 57 absolute weight of parts, the birds receiving the diets with coccidiostat had several significant differences (Table 35). For the Cobb birds, the tender weight was highest for the control birds followed by the Betaine/adequate methionine fed birds and these were significantly greater than the birds receiving Betaine/reduced methionine diets.

For both breed strains, the wing weight was highest for the control fed birds and this was significantly greater than the birds receiving the Betaine/reduced methionine diets for Cobb birds and Betaine/adequate methionine diets for Ross birds. For the leg quarter weights, for both strains, the control fed birds had the highest weights and these were significantly greater than the

Betaine/reduced methionine fed birds for both strains and also for the Betaine/adequate methionine fed birds for the Ross strain.

For day 57 absolute weight of parts for the birds that received coccivac at day of age, there were several significant differences (Table 36). For the Cobb birds, the breast and the tenders weight for the Betaine/reduced methionine fed birds was significantly higher than all the other treatments. For the Ross birds, the wing weight for the reduced methionine fed birds was significantly higher than the Betaine/reduced methionine and Betaine/adequate methionine fed birds.

Litter Temperature, Ammonia, and Flux

Litter temperatures for coccidiosis control were significantly different, as shown in Table 37. However, it should be noted that the Biocox method of coccidiosis control was on the east side of the barn, while the coccidiosis-vaccine method was on the west side. The different sides of the barn were placed on different dates, thus litter temperatures were also taken on different dates. There may have been an effect on ammonia levels and flux caused by this temperature difference, yet the cause in temperature increase cannot solely be attributed to the form of coccidiosis control.

Coccidiosis Scoring

One bird per pen was randomly selected and wing banded on day 1 of life. These birds were then sacrificed on day 22. Each bird was scored in three locations of the gastrointestinal tract, including the duodenum, mid-gut, and ceca. Scoring was performed by Dr. H. David Chapman. Table 39 shows coccidiosis scores for each bird strain, and for both strains combined. The coccidiosis-vaccine form of coccidiosis control (232 West) did not have a single coccidiosis lesion above a “0”. The Biocox method of coccidiosis control (232 East) did have coccidiosis scores above “0” for some birds. For statistical analysis, the three scores for each bird were averaged. This averaged number was then used to analyze by treatment.

The Cobb birds tended to have higher cocci scores than the Ross birds. One exception to said trend was Treatment 3 (Betaine/Reduced Methionine) Cobb birds, which had lower scores than the Treatment 3 (Betaine/Reduced Methionine) Ross birds.

Paw Scores

Paw scores were taken on day 42 and day 56 (Table 41). Day 42 paw scores show an interesting trend. Cobb birds in Treatment 3 (Betaine w/reduced methionine) and Treatment 7 (Betaine/reduced methionine) both had paw scores significantly better than all other treatments. Further, Treatment 3 was significantly better than Treatment 7 for Cobb. Similar trends also existed for Ross birds in Day 42. Treatment 3 for Ross Day 42 birds had the lowest incidence of paw score lesions. Treatment 7 for Ross Day 42 was also lower than nearly all other treatments. This suggests that Betaine with reduced methionine can produce birds with improved paw scores at 42 days of age.

Day 56 paw score data for Cobb do not follow the same trends that they did at day 42. Day 56 Cobb birds performed similarly to each other, with Treatment 1 and Treatment 2 having statistically lower paw scores than all other treatments. For Ross birds at day 56, the trend of

Treatment 3 (Betaine/Reduced methionine) having the lowest and best paw scores is present, similar to day 42 results. Overall, for both breed strains at day 42 and day 56, a trend is present that shows Betaine with reduced methionine can produce paw scores that are lower, and thus better, than other treatments.

DISCUSSION

Live Performance

In the present research, there was no significant difference in live weights between treatments for Ross strain broilers for days 15, 31, 42, or 56. Similar results were also observed for Cobb strain broilers, as there was no significant difference between treatments in live weights for days 15, 31, 42, or 56. These results differ with the findings of another study that did show a significant increase in broiler live weights at day 22 for diets supplemented with betaine and deficient methionine, compared with diets not supplemented with betaine (Pillai et al., 2006). Though the live weights in the present research were not measured on day 21, the lack of significant differences on all days, both before and after day of age 21, make it unlikely that the birds would have had significant differences in live weights at day 21, thus this research is conflicting with the findings of Pillai et al. (2006).

However, the findings of a separate study do support the results of the present research for live weights. Female broilers were grown to day 41, and exhibited no significant difference in live weights for days 21 or 41 (Esteve-Garcia and Mack, 2000). Further, the betaine supplemental levels were either 0 or 0.5 g/kg, which is the same percent inclusion rate as the current study, which demonstrates similar response to similar betaine inclusion levels.

Other investigators also supplemented betaine at the inclusion rates of 0 or 0.5 g/kg, which is the same inclusion level as the present investigational effort and further is the same as those of Esteve-Garcia and Mack (2000), and yet those investigators were able to demonstrate a significant difference in weight gain between treatments containing betaine and treatments that did not contain betaine (Zhan et al., 2006b). There are several factors that may affect the variability in response, including age of study, changes in genetic lines over time, sex of broilers, and other dietary factors.

Feed intake was only affected early in the current trial, for both genetic strains, but did not show statistical difference on days 31, 42, or 56. In one study, betaine was statistically similar to the control diet (Zhan et al., 2006), which coincides with the findings of our present research in that the final live weight stages there was not a significant difference between the treatments. These findings are supported by the investigational efforts of another researcher. Said study exposed the birds to cyclic heat stress at day 22, and birds were grown until day 45 (Sakomura et al., 2013). The heat stress, even in combination with varying levels of betaine and methionine, did not induce a significant response to affect feed intake. The present study employed heat stress from the beginning of the trial, and much like the Sakomura study, had little or no differences in feed intake. By the end of the Sakomura trial, there was no measurable difference between any of the treatments.

In one heat stress study, feed intake was significantly decreased during conditions of high heat and varying methionine inclusion levels. Though this does not align with the results of the current study, it does introduce the challenge of raising birds in heat stress conditions. The current study saw gross feed intake decrease as a response to heat stress, which was incongruent with our research findings.

Considering these factors, there was enough inconsistency in the results of multiple trials to warrant a more comprehensive study to investigate multiple variables.

Cocci Scores

The cocci lesion scores were significantly different between coccidiosis prevention methods. Coccivac broilers did not have any observed cocci lesions. The broilers receiving coccidiostat in the feed did, however, have observable cocci lesions.

Ross broilers did not have statistically significant differences between any treatments for coccivacine birds or for coccidiostat-treated birds. Cobb did not have any differences between treatments for the birds that received coccivaccine. Cobb, however, did have significant differences between the treatments for the coccidiostat method of coccidiosis prevention. Cobb broilers fed betaine with reduced methionine had significantly lower cocci scores than broilers fed either betaine with adequate methionine or broilers fed no betaine with reduced methionine. These results suggest that cocci lesions may be decreased significantly when broilers are supplemented with betaine and reduced methionine. This is one parameter that demonstrates potential benefit from supplementing with betaine and reduced methionine.

Paw Scores

Day 42 paw scores for Cobb strain birds fed betaine with reduced methionine had paw scores significantly lower, thus better, than all other Cobb treatments. The birds on Biocox had the lowest paw scores, followed by the same treatment of betaine with reduced methionine, receiving coccivac having the second-best paw scores at day 42. Ross birds displayed the same trends. Therefore, for both forms of cocci control and both bird strains, the treatment of betaine with reduced methionine produced paw scores that were significantly better than any of the other treatments.

Day 56 paw scores demonstrated similar trends to the day 42 paw score data. The separation between treatments was less pronounced, but still existed. One notable difference between day 42 and day 56 is the improvement in scores made by the Cobb control and the Cobb reduced methionine diets on the Biocox cocci control plan. While the results from day 56 are not as differentiated as the results from day 42, the trends observed with paw scores are still present.

Overall, for both breed strains at day 42 and day 56, a trend is present that shows Betaine with reduced methionine can produce paw scores that are lower, and thus better, than other treatments.

Litter Temperature, Ammonia Emission, Ammonia Flux

Litter temperatures for broilers that received BioCox trended higher than for broilers that received coccivac, with some significant differences between threatments. This trend was consistent for both the Cobb and Ross strains. However, it should be noted that the Biocox method of coccidiosis control was used exclusively on the east side of the barn, while the coccidiosis-vaccine method was used exclusively on the west side. The different sides of the barn were placed on different dates, one week apart, thus day 36 litter temperatures were also taken on different dates. There may have been an effect on ammonia levels and flux caused by this temperature difference, yet the cause in temperature increase cannot solely be attributed to the form of coccidiosis control.

When considering the Table 37 data collectively, a trend of lower day 36 litter temperatures is associated with treatments that are either lacking in methionine or do not include betaine. A decrease in dietary nitrogen would likely be associated with a decrease in nitrogenous waste, ammonia, and thus a potential decrease in litter temperature. This may explain part of the trend associated with lower litter temperatures observed in pens fed diets lower in nitrogenous ingredients. Again, consideration should be given to the fact that litter temperatures were taken on different days.

Ammonia emissions showed a consistent trend between cocci prevention methods. Broilers that received coccivac had the lowest ammonia emissions, while the broilers receiving dietary BioCox had higher ammonia emissions. Another trend emerged, showing that the Ross strain always had numerically lower ammonia emissions than the Cobb strain, within the same coccivac control method and treatment.

Ammonia flux data did not show any significance between any of the treatments. This held true for the Cobb and Ross strains. Further, the form of cocci control did not have a significant effect on ammonia flux.

Processing Performance

At day 43 processing, there were significant differences between the dietary treatments. For birds receiving dietary coccidiostat, Cobb did not have significant differences, but Ross did have differences for breast meat yield and for wing yield. Ross birds fed reduced methionine with no betaine had significantly less breast meat yield than all other dietary treatments. Ross birds fed control rations had significantly less wing yield than birds fed reduced methionine with no betaine.

At day 43 processing, for birds that received coccivac at day of age, there were also significant differences between the dietary treatments. Ross strain birds fed reduced methionine with no betaine had higher leg yield than all other dietary treatments. Broilers fed reduced methionine with no betaine had lower breast meat yield than all other dietary treatments, and this was true for both the Cobb strain and the Ross strain birds. Cobb strain birds receiving betaine with reduced methionine ration had breast meat yield similar to the birds receiving betaine and adequate methionine, but significantly larger than the birds receiving the control ration. These day 43 processing results indicate that betaine supplementation can significantly increase breast meat yield, and are in agreement with other study results obtained at similar ages (McDevitt et al., 2000; Waldroup et al., 2006). McDevitt et al. reared broilers to 42 days of age, and when supplementing betaine in the ration, with methionine at 0.6 grams/kilogram, showed they could increase breast meat yield, compared to rations without betaine. However, when McDevitt supplemented betaine with a higher inclusion rate of 1.2 grams/kilogram of methionine, that treatment produced breast meat yield results that were statistically similar to control diet. These results are in partial agreement to the results we obtained in our research, as the lower level of methionine with betaine was able to produce significantly larger breast meat yield for the Cobb strain receiving coccivaccine compared with control, but was not able to produce the same results for any other strain or coccidiosis prevention at day 43.

The findings of Waldroup et al. are also in partial agreement with the results of our research (2006). Our research at 43 days showed some increase in breast meat yield with betaine supplementation, which agrees with Waldroup et al. (2006). A different study is also in partial agreement with our research, as they also found that breast meat yield was significantly higher in rations containing betaine, compared with no betaine, at 42 days of age (Rao et al., 2011). Another study found no significant difference in breast meat yield at day 41, for any level of betaine or methionine supplementation (Esteve-Garcia and Mack, 2000), which conflicts with the findings of our research. However, the same study did find a significant improvement in carcass yield with betaine supplementation at day 41 (Esteve-Garcia and Mack, 2000), while our study showed an increase in carcass yield for rations supplemented with betaine at only day 57. Waldroup et al., also showed that betaine supplementation caused an increase in breast meat yield at day 56, compared with control (2006), which our research did not show. Our broilers supplemented with betaine showed breast meat yield statistically similar to the control treatments at day 57 for both breed strains and also for both coccidiosis prevention methods. These collective results show that betaine supplementation can affect the processing yield performance of broilers, yet the results across the research can vary.

Day 43 East Mortality

On day 42, following the weigh, the environmental temperature was increased by 5º F to exacerbate heat stress in the flock. The East side of 232 barn sustained unexpectedly high mortality one day following the temperature increase (Day 43), which is shown in Table 23. Unlike the east side, the west side only had minor mortality one day following that temperature increase (Day 43). It is believed that the difference between the two sides' mortality levels was due to the weather conditions on those respective days. The morning of east side day 43 (10-1-13) there was elevated humidity, fog in the areas adjacent to the 232 barn, and it rained the previous night and early that morning. Alternately, the morning of the west side day 43 was cooler, dry, and the region had low relative humidity. Though the mortality on the east side was unexpectedly high, the information gained from analyzing mortality across treatments does appear to be valuable.

Results indicate that treatment 4 with betaine and adequate methionine had significantly lower mortality than the control group (2.28% and 4.41%, respectively). This suggests that betaine with adequate methionine may have improved the ability of betaine to alter bird performance under such conditions. The mean mortality on the east side of the barn on day 43 was 4.20 birds per pen. Treatments 1 and 2, neither of which contained betaine, had the highest numeric mean mortality, 4.81 and 5.50 birds per pen, respectively. Interestingly, Treatment 4, which contained betaine and adequate methionine, had the lowest numeric east single day 43 mortality, with 2.25 birds per pen. Treatment 4 had more than 2 birds per pen survive during the heat challenge on day 43 than any other treatment. Treatment 2, the treatment with no betaine and reduced methionine, and Treatment 4, which contained betaine and adequate methionine, are significantly different from each other for the mean mortality on day 43. The results from the day 43 east mortality suggest that supplementing betaine can significantly improve flock livability in high humidity and high temperature conditions.

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CHAPTER III

CONCLUSION

The findings of our investigational efforts show that betaine is able to partially spare the other two methyl donors, choline and methionine, in corn-soy based broiler diets. Additionally, our findings indicate that dietary betaine supplementation has an effect across different strains, both Cobb 500 and Ross 708, and can induce some positive responses in each of these breed strains. The effects in live performance were minor, and did not show major, significant differences in weights as a result of differing betaine supplementation levels. One area of live performance that did show positive results associated with betaine supplementation was single-day mortality that was significantly lower than birds fed rations not supplemented with dietary betaine. At day 43, an acute, unanticipated heat event caused elevated mortality, and the birds that received betaine had significantly lower mortality.

Additionally, there were results from processing performance parameters that were significant, in regard to both parts weight and parts yield. The research herein shows that betaine supplementation was able to increase breast meat yield at day 43. Day 57 processing weights for Cobb birds receiving coccivac showed that diets supplemented with betaine and deficient in methionine produced the heaviest breast weight and heaviest tender weight, compared with all other treatments. These results suggest that betaine supplementation can affect body composition and processing performance of broilers grown to heavy weights. In addition to improving some processing performance parameters, it was demonstrated that betaine supplementation can also significantly improve single-day mortality in acute higher temperature environments. These results collectively show that betaine is effective at altering body composition and improving the response to heat stress in broilers.

Table 1. Experimental design

Location	Strain	Trt	Cocci Control	Betaine	Methionine
232 East	Ross 708	1	Biocox	None	Adequate
232 East	Cobb 500	1	Biocox	None	Adequate
232 East	Ross 708	2	Biocox	None	Reduced as met as per Betachek
232 East	Cobb 500	2	Biocox	None	Reduced as met as per Betachek
232 East	Ross 708	3	Biocox	1 kg/Metric tonBetafin	Reduced as met as per Betachek
232 East	Cobb 500	3	Biocox	1 kg/Metric tonBetafin	Reduced as met as per Betachek
232 East	Ross 708	4	Biocox	1 kg/Metric tonBetafin	Adequate
232 East	Cobb 500	4	Biocox	1 kg/Metric tonBetafin	Adequate
232 West	Ross 708	5	CocciVac	None	Adequate
232 West	Cobb 500	5	CocciVac	None	Adequate
232 West	Ross 708	6	CocciVac	None	Reduced as met as per Betachek
232 West	Cobb 500	6	CocciVac	None	Reduced as met as per Betachek
232 West	Ross 708	7	CocciVac	1 kg/Metric tonBetafin	Reduced as met as per Betachek
232 West	Cobb 500	7	CocciVac	1 kg/Metric tonBetafin	Reduced as met as per Betachek
232 West	Ross 708	8	CocciVac	1 kg/Metric tonBetafin	Adequate
232 West	Cobb 500	8	CocciVac	1 kg/Metric tonBetafin	Adequate

Table 1A. Treatments

Treatments by Betaine Dosage	Diet	Betaine	Methionine	Rep 8/8	Axtra® PHY 10K TPT	Axtra XAP 102
T1	Basal & *Salinomycin*	None	Adequate	16	500 FTU	Xylanase
T2	Basal *&* *Salinomycin*	None	Reduced in met as per Betacheck	16	500 FTU	Xylanase
T3	Basal *&* *Salinomycin*	1 kg/MT	reduced in met as per Betacheck (trt 2 + Betaine)	16	500 FTU	Xylanase
T4	Basal *&* *Salinomycin*	1 kg/MT	Adequate	16	500 FTU	Xylanase
T5	Basal/ *Vaccine*	None	Adequate	16	500 FTU	Xylanase
T6	Basal/ *Vaccine*	None	Reduced in met as per Betacheck	16	500 FTU	Xylanase
T7	Basal/ *Vaccine*	1 kg/MT	reduced in met as per Betacheck (trt 2 + Betaine)	16	500 FTU	Xylanase
T8	Basal/ *Vaccine*	1 kg/MT	Adequate	16	500 FTU	Xylanase

Table 2. Ingredient composition of the starter diets (Fed days 0-15)

Ingredient	Diets							
	T1	T2	T3	T4	T5	T6	T7	T8
	(%)							
Corn - AR	56.658	56.852	56.752	56.558	56.708	56.902	56.802	56.608
Soybean Meal	32.591	32.591	32.591	32.591	Same as T1	Same as T2	Same as T3	Same as T4
Corn DDGS[1]	5.000	5.000	5.000	5.000				
Pro-Plus 55 H.J. Baker[2]-AR	3.000	3.000	3.000	3.000				
Poultry Fat	0.500	0.500	0.500	0.500				
L-Lysine HCl	0.140	0.140	0.140	0.140				
DL-Methionine	0.327	0.233	0.233	0.327				
L-Threonine	0.044	0.044	0.044	0.044				
Choline Chloride 60%	0.100	0.000	0.000	0.100				
Limestone	0.805	0.805	0.805	0.805				
Dicalcium Phosphate	0.275	0.275	0.275	0.275				
Salt	0.356	0.356	0.356	0.356				
Vitamin Premix[3]	0.025	0.025	0.025	0.025				
Trace Mineral Premix	0.075	0.075	0.075	0.075				
XAP 102 (40)	0.050	0.050	0.050	0.050				
AxtraPhy TPT[4] 10000 FTU/g	0.005	0.005	0.005	0.005				
Betafin S4	0.000	0.000	0.100	0.100				
Biocox®	0.050	0.050	0.050	0.050	0.000	0.000	0.000	0.000
Total	100	100	100	100	100	100	100	100

[1]Distillers dried grains with soluble; [2]H. J. Baker & Bro., 595 Summer Street, Stamford, CT 06901-1407. [3]Provides per kg of diet: vitamin A (from vitamin A acetate) 7715 IU; cholecalciferol 5511 IU; vitamin E (from dl-alpha-tocopheryl acetate) 16.53 IU; vitamin B_{12} 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione (from menadionedimethylpyrimidinol) 1.5 mg; folic acid 0.9 mg; choline 1000 mg; thiamin (from thiamin mononitrate) 1.54 mg; pyridoxine (from pyridoxine HCl) 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg. [4]Phytase enzyme.

Table 3. Ingredient composition of the grower diets (Fed 15-31 days)

Ingredient	Diets							
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**
	(%)							
Corn	62.350	62.519	62.419	62.250	62.400	62.569	62.469	62.300
Soybean Meal	25.371	25.371	25.371	25.371	Same as T1	Same as T2	Same as T3	Same as T4
Corn DDGS[1]	7.000	7.000	7.000	7.000				
Pro-Plus 55 H.J. Baker[2]-AR	3.000	3.000	3.000	3.000				
Poultry Fat	0.500	0.500	0.500	0.500				
L-Lysine HCl	0.185	0.185	0.185	0.185				
DL-Methionine	0.263	0.169	0.169	0.263				
L-Threonine	0.070	0.070	0.070	0.070				
Choline Chloride 60%	0.075	0.000	0.000	0.075				
Limestone	0.631	0.631	0.631	0.631				
Dicalcium Phosphate	0.042	0.042	0.042	0.042				
Salt	0.308	0.308	0.308	0.308				
Vitamin Premix[3]	0.025	0.025	0.025	0.025				
Trace Mineral Premix	0.075	0.075	0.075	0.075				
XAP 102 (40)	0.050	0.050	0.050	0.050				
AxtraPhy TPT[4] 10000 FTU/g	0.005	0.005	0.005	0.005				
Betafin S4	0.000	0.000	0.100	0.100				
Biocox®	0.050	0.050	0.050	0.050	0.000	0.000	0.000	0.000
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1]Distillers dried grains with soluble; [2]H. J. Baker & Bro., 595 Summer Street, Stamford, CT 06901-1407. [3]Provides per kg of diet: vitamin A (from vitamin A acetate) 7715 IU; cholecalciferol 5511 IU; vitamin E (from dl-alpha-tocopheryl acetate) 16.53 IU; vitamin B_{12} 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione (from menadionedimethylpyrimidinol) 1.5 mg; folic acid 0.9 mg; choline 1000 mg; thiamin (from thiamin mononitrate) 1.54 mg; pyridoxine (from pyridoxine HCl) 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg. [4]Phytase enzyme.

Table 4. Ingredient composition of the finisher diets (Fed 31-42 days)

Ingredient	Diets							
	T1	T2	T3	T4	T5	T6	T7	T8
	(%)							
Corn - AR	67.221	67.391	67.291	67.122	67.271	67.441	67.341	67.172
Soybean Meal	18.001	18.001	18.001	18.001	Same as T1	Same as T2	Same as T3	Same as T4
Corn DDGS[1]	10.000	10.000	10.000	10.000				
Pro-Plus 55 H.J. Baker[2]-AR	2.500	2.500	2.500	2.500				
Poultry Fat	0.500	0.500	0.500	0.500				
L-Lysine HCl	0.217	0.217	0.217	0.217				
DL-Methionine	0.207	0.113	0.113	0.207				
L-Threonine	0.072	0.072	0.072	0.072				
Choline Chloride 60%	0.075	0.000	0.000	0.075				
Limestone	0.756	0.756	0.756	0.756				
Dicalcium Phosphate	0.000	0.000	0.000	0.000				
Salt	0.282	0.282	0.282	0.282				
Vitamin Premix[3]	0.015	0.015	0.015	0.015				
Trace Mineral Premix	0.050	0.050	0.050	0.050				
XAP 102 (40)	0.050	0.050	0.050	0.050				
AxtraPhy TPT[4] 10000 FTU/g	0.005	0.005	0.005	0.005				
Betafin S4	0.000	0.000	0.100	0.100				
Biocox®	0.050	0.050	0.050	0.050	0.000	0.000	0.000	0.000
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

[1]Distillers dried grains with soluble; [2]H. J. Baker & Bro., 595 Summer Street, Stamford, CT 06901-1407. [3]Provides per kg of diet: vitamin A (from vitamin A acetate) 7715 IU; cholecalciferol 5511 IU; vitamin E (from dl-alpha-tocopheryl acetate) 16.53 IU; vitamin B_{12} 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione (from menadionedimethylpyrimidinol) 1.5 mg; folic acid 0.9 mg; choline 1000 mg; thiamin (from thiamin mononitrate) 1.54 mg; pyridoxine (from pyridoxine HCl) 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg. [4]Phytase enzyme.

Table 5. Ingredient composition of the withdrawal diets (Fed 42-56 days)

Ingredient	Diets							
	T1	T2	T3	T4	T5	T6	T7	T8
	(%)							
Corn - AR	67.921	68.065	67.965	67.821	67.921	68.065	67.965	67.821
Soybean Meal	15.446	15.446	15.446	15.446	Same as T1	Same as T2	Same as T3	Same as T4
Corn DDGS[1]	12.000	12.000	12.000	12.000				
Pro-Plus 55 H.J. Baker[2]-AR	2.500	2.500	2.500	2.500				
Poultry Fat	0.500	0.500	0.500	0.500				
L-Lysine HCl	0.202	0.202	0.202	0.202				
DL-Methionine	0.167	0.073	0.073	0.167				
L-Threonine	0.053	0.053	0.053	0.053				
Choline Chloride 60%	0.050	0.000	0.000	0.050				
Limestone	0.784	0.784	0.784	0.784				
Dicalcium Phosphate	0.000	0.000	0.000	0.000				
Salt	0.258	0.258	0.258	0.258				
Vitamin Premix[3]	0.015	0.015	0.015	0.015				
Trace Mineral Premix	0.050	0.050	0.050	0.050				
XAP 102 (40)	0.050	0.050	0.050	0.050				
AxtraPhy TPT[4] 10000 FTU/g	0.005	0.005	0.005	0.005				
Betafin S4	0.000	0.000	0.100	0.100				
Biocox®	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

[1]Distillers dried grains with soluble; [2]H. J. Baker & Bro., 595 Summer Street, Stamford, CT 06901-1407. [3]Provides per kg of diet: vitamin A (from vitamin A acetate) 7715 IU; cholecalciferol 5511 IU; vitamin E (from dl-alpha-tocopheryl acetate) 16.53 IU; vitamin B_{12} 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione (from menadionedimethylpyrimidinol) 1.5 mg; folic acid 0.9 mg; choline 1000 mg; thiamin (from thiamin mononitrate) 1.54 mg; pyridoxine (from pyridoxine HCl) 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg. [4]Phytase enzyme.

Table 6. Analyzed proximate analysis of starter diets

Ingredient	Diets							
	T1	T2	T3	T4	T5	T6	T7	T8
	% of diet							
Dry Matter	89.0	89.1	89.2	89.2	88.5	88.6	89.0	89.1
Protein	25.9	24.3	24.6	26.0	27.5	25.0	26.5	25.6
Ash	6.75	6.47	6.44	6.39	6.37	6.60	6.53	6.43
Fat	3.86	3.40	4.02	3.88	3.82	3.72	3.72	3.82

Analyzed by University of Arkansas, Center of Excellence for Poultry Science Central Analytical Laboratory.

Table 7. Analyzed proximate analysis of grower diets

Ingredient	Diets							
	T1	T2	T3	T4	T5	T6	T7	T8
	% of diet							
Dry Matter	87.8	87.8	87.7	87.5	87.1	87.7	87.8	87.6
Protein	24.1	24.7	23.1	23.6	24.2	24.0	23.7	23.4
Ash	4.74	4.68	4.71	4.63	4.65	4.81	4.71	4.74
Fat	3.77	3.90	3.83	3.89	4.01	3.94	3.79	2.92

Analyzed by University of Arkansas, Center of Excellence for Poultry Science Central Analytical Laboratory.

Table 8. Analyzed proximate analysis of finisher diets

Ingredient	Diets							
	T1	T2	T3	T4	T5	T6	T7	T8
	% of diet							
Dry Matter	90.6	90.5	90.4	90.3	90.4	90.3	90.4	90.8
Protein	20.5	19.0	20.6	20.7	19.9	19.1	20.0	20.1
Ash	4.50	4.35	4.56	4.35	4.22	4.48	4.40	4.52
Fat	4.05	3.92	4.12	3.91	3.45	3.80	4.09	4.16

Analyzed by University of Arkansas, Center of Excellence for Poultry Science Central Analytical Laboratory.

Table 9. Analyzed proximate analysis of withdrawal diets

Ingredient	Diets							
	T1	T2	T3	T4	T5	T6	T7	T8
	% of diet							
Dry Matter	90.3	90.3	90.6	90.4	90.2	90.3	90.2	90.4
Protein	21.7	19.0	20.9	22.3	21.2	21.7	21.5	18.6
Ash	4.37	4.14	4.24	4.09	4.09	4.20	3.90	4.18
Fat	3.73	4.39	3.51	3.86	3.67	3.61	4.22	3.87

Analyzed by University of Arkansas, Center of Excellence for Poultry Science Central Analytical Laboratory.

Table 10. Analyzed amino acid composition of the starter diets

Amino Acid	Diets							
	T1	T2	T3	T4	T5	T6	T7	T8
	W/W %							
Aspartic Acid	2.54	2.62	2.65	2.59	2.56	2.46	2.53	2.49
Threonine	0.88	0.88	0.87	0.87	0.85	0.82	0.86	0.84
Glutamic Acid	4.45	4.58	4.57	4.48	4.46	4.28	4.37	4.33
Proline	1.33	1.51	1.45	1.40	1.43	1.41	1.33	1.43
Glycine	1.19	1.31	1.21	1.33	1.19	1.20	1.19	1.20
Alanine	1.32	1.37	1.31	1.36	1.29	1.29	1.29	1.30
Cysteine	0.42	0.43	0.45	0.45	0.42	0.41	0.41	0.41
Valine	1.34	1.38	1.38	1.39	1.35	1.31	1.33	1.31
Methionine	0.66	0.59	0.61	0.64	0.65	0.59	0.66	0.67
Isoleucine	1.13	1.15	1.17	1.15	1.14	1.09	1.12	1.10
Leucine	2.20	2.22	2.21	2.22	2.17	2.12	2.14	2.13
Lysine	1.52	1.56	1.57	1.55	1.55	1.47	1.53	1.48
TOTAL:	18.98	19.60	19.45	19.43	19.06	18.45	18.76	18.69
Crude Protein*	26.01	26.45	25.59	26.04	26.08	26.54	25.74	25.60

Analyzed by University of Missouri-Columbia, Agricultural Experimentation Station Chemical Laboratories.

* Percentage N X 6.25. W/W%= grams per 100 grams of sample

Table 11. Analyzed amino acid composition of the grower diets

Amino Acid	Diets							
	T1	T2	T3	T4	T5	T6	T7	T8
	W/W %							
Aspartic Acid	2.20	2.21	2.12	2.13	2.14	2.20	2.11	2.08
Threonine	0.76	0.81	0.76	0.70	0.74	0.77	0.73	0.77
Glutamic Acid	3.69	3.77	3.66	3.66	3.65	3.76	3.65	3.54
Proline	1.37	1.36	1.34	1.34	1.30	1.44	1.32	1.35
Glycine	1.04	1.10	1.09	1.07	1.03	1.28	1.03	1.02
Alanine	1.20	1.26	1.24	1.23	1.20	1.39	1.21	1.19
Cysteine	0.37	0.37	0.36	0.36	0.37	0.37	0.36	0.35
Valine	1.11	1.11	1.09	1.10	1.09	1.24	1.09	1.04
Methionine	0.59	0.50	0.49	0.56	0.53	0.51	0.51	0.56
Isoleucine	0.94	0.95	0.91	0.93	0.93	0.97	0.93	0.89
Leucine	1.92	2.00	1.94	1.96	1.93	2.02	1.96	1.89
Lysine	1.34	1.34	1.28	1.27	1.28	1.35	1.26	1.26
TOTAL:	16.53	16.78	16.28	16.31	16.19	17.30	16.16	15.94
Crude Protein*	21.98	21.53	22.40	22.38	22.12	21.99	22.94	22.58

Analyzed by University of Missouri-Columbia, Agricultural Experimentation Station Chemical Laboratories.

* Percentage N X 6.25. W/W%= grams per 100 grams of sample

Table 12. Analyzed amino acid composition of the finisher diets

Amino Acid	Diets							
	T1	T2	T3	T4	T5	T6	T7	T8
	W/W %							
Aspartic Acid	1.715	1.745	1.76	1.88	1.74	1.8	1.735	1.78
Threonine	0.72	0.71	0.735	0.75	0.735	0.735	0.725	0.72
Glutamic Acid	3.25	3.295	3.32	3.48	3.27	3.37	3.255	3.40
Proline	1.28	1.27	1.26	1.315	1.27	1.28	1.27	1.31
Glycine	0.895	0.875	0.885	0.945	0.865	0.905	0.905	0.92
Alanine	1.11	1.115	1.105	1.155	1.085	1.115	1.105	1.15
Cysteine	0.32	0.32	0.32	0.31	0.31	0.33	0.35	0.33
Valine	0.92	0.94	0.905	0.995	0.93	0.95	0.925	0.95
Methionine	0.49	0.425	0.455	0.49	0.525	0.43	0.445	0.52
Isoleucine	0.755	0.77	0.765	0.825	0.765	0.79	0.755	0.78
Leucine	1.785	1.805	1.795	1.865	1.76	1.82	1.765	1.86
Lysine	1.095	1.075	1.085	1.13	1.045	1.09	1.08	1.13
TOTAL:	14.27	14.275	14.32	15.08	14.24	14.545	14.23	14.76
Crude Protein*	19.95	20.18	20.33	19.915	19.365	19.86	19.62	20.05

Analyzed by University of Missouri-Columbia, Agricultural Experimentation Station Chemical Laboratories.

* Percentage N X 6.25. W/W%= grams per 100 grams of sample

Table 13. Analyzed amino acid composition of the withdrawal diets

Amino Acid	Diets							
	T1	T2	T3	T4	T5	T6	T7	T8
	W/W %							
Aspartic Acid	1.64	1.64	1.62	1.64	1.61	1.59	1.49	1.59
Threonine	0.74	0.73	0.74	0.73	0.72	0.72	0.69	0.70
Glutamic Acid	3.12	3.12	3.05	3.10	3.09	3.03	2.88	3.01
Proline	1.26	1.27	1.21	1.24	1.23	1.24	1.16	1.18
Glycine	0.91	0.91	0.84	0.86	0.83	0.87	0.79	0.84
Alanine	1.11	1.10	1.06	1.08	1.07	1.07	1.03	1.06
Cysteine	0.32	0.33	0.32	0.34	0.31	0.32	0.30	0.33
Valine	0.85	0.83	0.81	0.83	0.80	0.80	0.76	0.80
Methionine	0.47	0.39	0.39	0.45	0.45	0.39	0.37	0.45
Isoleucine	0.71	0.71	0.69	0.70	0.69	0.68	0.64	0.68
Leucine	1.75	1.75	1.70	1.72	1.74	1.70	1.62	1.69
Lysine	1.03	1.00	0.99	0.99	0.98	0.99	0.94	0.97
TOTAL:	13.87	13.75	13.38	13.66	13.49	13.37	12.64	13.28
Crude Protein*	18.41	18.40	18.23	18.30	18.14	18.41	18.36	18.01

Analyzed by University of Missouri-Columbia, Agricultural Experimentation Station Chemical Laboratories.

* Percentage N X 6.25. W/W%= grams per 100 grams of sample

Table 14. Analyzed betaine (trimethylglycine) content of diets[1]

Trimethylglycine	Diets							
	T1	T2	T3	T4	T5	T6	T7	T8
Starter	0.216	0.150	0.899	1.08	0.165	0.153	0.912	1.11
Grower	0.172	0.117	1.02	1.10	0.129	0.121	0.987	1.08
Finisher	0.386	0.262	1.20	1.31	0.383	0.226	1.03	1.39
Withdrawal	0.113	0.0653	0.911	0.960	0.136	0.0620	0.911	1.04

[1]Analyzed by Eurofins, by HPLC - Internal analysis method

Table 15. Impact of Betaine and methionine levels and a coccidiostat in the diet vs coccivac given at one day of age on the feed intake of male Cobb broilers reared to 56 days of age

Cocci Control	Diet	Day 15 Feed Intake (kg/pen)[1,2]	Day 31 Feed Intake (kg/pen)[1]	Day 42 Feed Intake (kg/pen)[1]	Day 56 Feed Intake (kg/pen)[1]
Biocox	Control	19.66[a]	81.48	137.63	181.86
	Reduced Methionine	19.45[a]	80.46	137.09	178.75
	Betaine/Reduced Methionine	19.56[a]	81.39	138.27	180.82
	Betaine/Adequate Methionine	19.65[a]	81.15	139.09	186.17
Coccivac	Control	18.44[b]	75.41	132.74	185.45
	Reduced Methionine	18.47[b]	77.89	137.47	193.14
	Betaine/Reduced Methionine	18.17[b]	77.86	135.15	188.91
	Betaine/Adequate Methionine	18.20[b]	77.95	137.77	190.18
SEM		0.1065	0.5856	1.0181	1.6704
P Value		<.0001	0.0647	0.8468	0.3325

[1]Average pen Feed Intake per bird is unadjusted for mortality.

[2] Means within columns with different letters are statistically different at the $P<0.05$ level.

Table 16. Impact of Betaine and methionine levels and a coccidiostat in the diet vs coccivac given at one day of age on the feed intake of male Ross broilers reared to 56 days of age

Cocci Control	Diet	Day 15 Feed Intake (kg/pen)[2,3]	Day 31 Feed Intake (kg/pen)[2,3]	Day 42 Feed Intake (kg/pen)[2,3]	Day 56 Feed Intake (kg/pen)[1,2]
Biocox	Control diet	17.87[a]	77.66[a]	139.52	196.84
	Reduced Methionine	17.67[a]	76.74[ab]	139.01	196.05
	Betaine/Reduced Methionine	17.55[a]	77.35[ab]	140.20	200.60
	Betaine/Adequate Methionine	17.52[a]	77.32[ab]	139.08	197.04
Coccivac	Control	15.78[c]	72.60[d]	134.37	198.02
	Reduced Methionine	16.58[b]	75.60[abc]	134.96	197.64
	Betaine/Reduced Methionine	16.04[bc]	73.78[cd]	137.17	201.14
	Betaine/Adequate Methionine	16.31[bc]	74.64[bcd]	138.85	203.29
SEM		0.1329	0.4019	0.7275	1.1082
P Value		<.0001	0.0036	0.3561	0.7272

[1] Feed intake is per pen with no mortality weight adjustment for intake per bird.

[2] 232 East experienced unanticipated mortality on Day 43.

[3]Means within columns with different letters are statistically different at the $P<0.05$ level.

Table 17. Impact of Betaine and methionine levels on the average weights of male broilers provided a dietary coccidiostat program and reared to 57 days of age [1]

Treatment	Initial Average Weights (grams)		Day 15 Average Weights (grams)		Day 31 Average Weights (grams)		Day 42 Average Weights (grams)		Day 56 Average Weights (grams)		Average Daily Gain (grams)	
Strain	**Cobb[1]**	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**
Control diet w/ Biocox	45.5^{ab}	37.5	561.0	494.8	1741.1	1695.9	2613.1	2697.3	3213.0	3577.8	56.6	63.2
Reduced Methionine	45.4^{b}	37.5	556.2	499.5	1718.4	1694.7	2576.1	2709.3	3146.6	3598.2	55.4	63.6
Betaine/ reduced Methionine	46.1^{a}	37.5	558.0	495.3	1715.6	1677.8	2573.6	2710.3	3064.0	3528.3	53.9	62.3
Betaine/ Adequate Methionine	45.4^{ab}	37.4	557.9	484.2	1717.0	1675.5	2602.7	2677.6	3199.2	3503.9	56.3	61.9
SEM	0.0001	0.0001	0.0024	0.0032	0.0124	0.0068	0.0245	0.0161	0.0429	0.0206	0.0008	0.0004
P Value	0.0381	0.9727	0.924	0.3791	0.8819	0.6175	0.9312	0.8920	0.6236	0.3542	0.6207	0.3554

[1] Means with different letters are statistically different at the $P<0.05$ level

Table 18. Impact of Betaine and methionine levels on the average weights of 57 day old male broilers vaccinated at day of age with coccivac[1]

Treatment	Initial Average weights (grams)		Day 15 Average weights (grams)		Day 31 Average weights (grams)		Day 42 Average weights (grams)		Day 56 Average weights (grams)		Average Daily gain (grams)	
Strain	**Cobb**	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**
Control no coccidiostat	46.1	37.8[ab]	501.7	430.7	1555.4	1528.3	2511.0	2552.8[b]	3303.9	3531.1	58.2	62.4
Reduced Methionine	46.4	38.0[a]	482.5	448.3	1562.1	1595.8	2519.1	2665.8[a]	3309.4	3525.6	58.3	62.3
Betaine/ reduced methionine	46.0	37.6[ab]	501.1	432.3	1594.9	1563.1	2568.9	2608.3[ab]	3363.3	3542.0	59.2	62.6
Betaine/ adequate methioine	46.3	37.2[b]	484.3	439.8	1569.7	1561.6	2524.3	2566.1[ab]	3345.7	3526.0	58.9	62.3
SEM	0.0001	0.0001	0.0044	0.0045	0.0209	0.0114	0.0261	0.0150	0.0262	0.0168	0.0005	0.0003
P Value	0.523	0.0432	0.2552	0.5177	0.9242	0.2296	0.8755	0.0254	0.8412	0.9863	0.8398	0.9863

[1]Means within columns with different letters are statistically different at the P<0.05 level.

Table 19. Impact of Betaine and methionine levels on the 0-56 day cumulative feed conversion rates of male broilers vaccinated at day of age with coccivac [1]

Treatment	Day 15 Feed-to-Gain Ratios grams:grams		Day 31 Feed-to-Gain Ratios grams:grams		Day 42 Feed-to-Gain Ratios grams:grams		Day 56 Feed-to-Gain Ratios grams:grams	
Strain	**Cobb**	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**
Control diet w/ Biocox	1.107	1.143	1.506	1.484	1.715	1.697	1.965	1.956
Reduced Methionine	1.108	1.128	1.516	1.482	1.739	1.700	1.988	1.955
Betaine/ reduced Methionine	1.114	1.121	1.533	1.482	1.749	1.680	1.983	1.970
Betaine/ Adequate Methionine	1.110	1.137	1.525	1.484	1.728	1.694	1.969	1.961
SEM	0.0028	0.0036	0.0053	0.0040	0.0054	0.0052	0.0060	0.0051
P Value	0.8608	0.1601	0.3099	0.9963	0.1494	0.544	0.4734	0.7525

[1]Mortality weight was added to total live weight at each age for calculation of adjusted feed conversions

Table 20. Impact of Betaine and methionine levels on the 0-56 day cumulative feed conversion rates of male broilers vaccinated at day of age with coccivac [1]

Treatment	Day 15 Feed-to-Gain Ratios grams:grams		Day 31 Feed-to-Gain Ratios grams:grams		Day 42 Feed-to-Gain Ratios grams:grams		Day 56 Feed-to-Gain Ratios grams:grams	
Strain	**Cobb**	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**
Control – no coccidiostat	1.182	1.167	1.607	1.553	1.779	1.740	2.005	1.974
Reduced Methionine	1.212	1.178	1.609	1.545	1.778	1.677	2.006	1.961
Betaine/ reduced Methionine	1.158	1.174	1.596	1.523	1.742	1.718	1.985	1.983
Betaine/ Adequate Methionine	1.200	1.168	1.584	1.532	1.758	1.746	1.961	1.978
SEM	0.0124	0.0053	0.0125	0.0065	0.0079	0.0120	0.0107	0.0082
P Value	0.4565	0.8781	0.9046	0.3841	0.2904	0.1729	0.4309	0.8205

[1]Weight of mortality was added to total bird weight for calculation of mortality corrected feed conversions

Table 21. Impact of Betaine on the 0-56 day cumulative mortality (%) of male broilers provided a coccidiostat in the diet [1]

Treatment	Day 15 Mortality (%)		Day 31 Mortality (%)		Day 42 Mortality (%)		Day 56 Mortality (%)	
Strain	**Cobb**	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**
Control diet w/ Saccox	1.17	1.95	2.02	2.82	2.82	3.63	28.00	26.00
Reduced Methionine	1.95	3.52	2.82	4.03	3.23	4.44	37.50	25.00
Betaine/ reduced Methionine	1.95	1.56	2.42	1.61	2.42	2.02	34.50	10.05
Betaine/ Adequate Methionine	1.56	1.17	2.02	2.82	2.02	3.63	18.00	10.50
SEM	0.3433	0.4781	0.4156	0.5563	0.4898	0.6134	3.3828	3.0268
P Value	0.8422	0.3374	0.8962	0.5202	0.8520	0.5826	0.1806	0.0978

[1]Day 56 Mortality on 232 East elevated due to unanticipated Day 43 Mortality on East

Table 22. Impact of Betaine and methionine levels on the 0-56 day cumulative mortality

Treatment	Day 15 Mortality (%)		Day 31 Mortality (%)		Day 42 Mortality (%)		Day 56 Mortality (%)	
Strain	**Cobb**	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**
Control Diet	3.52	2.73	5.24	3.63	6.05	4.03	10.50	5.50
Reduced Methionine	1.56	1.95	2.02	3.23	3.23	4.44	4.50	7.50
Betafin/ reduced Methionine	3.13	1.56	3.63	2.02	4.84	2.82	8.50	5.50
Betafin/ Adequate Methionine	1.56	0.78	2.42	1.21	2.82	1.61	9.50	4.00
SEM	0.4378	0.3419	0.5700	0.4281	0.5872	0.5420	1.0463	0.7800
P Value	0.2537	0.2415	0.1835	0.1674	0.1849	0.2513	0.1931	0.4866

(%) of male broilers vaccinated at day of age with coccivac

Table 23. Impact of Betaine and methionine levels on the day 43 mortality (%) of male broilers when temperature is increased 5 degrees F above[1]

Treatment	Day 43 Mortality
Control diet with Biocox	4.81[ab]
Reduced Methionine	5.50[a]
Betaine/Reduced Methionine	4.25[ab]
Betaine/Adequate Methionine	2.25[b]

[1] Means with different letters are statistically different at the $P<0.05$ level

Table 24. Impact of Betaine and dietary methionine levels on day 43 processing weights of male broilers provided a dietary coccidiostat

Treatment	Pre-slaughter Live weight (g)		Post-slaughter Hot WOG Weight (g)		Post-Chill Cold WOG Weight (g)	
	Genetic Strain					
	Cobb	Ross	Cobb	Ross	Cobb	Ross
Control diet w/ Biocox	2579.81	2640.40	1944.45	2010.55	1985.98	2053.03
Reduced Methionine	2565.39	2694.03	1917.07	2043.55	1965.88	2090.25
Betaine/Reduced Methionine	2549.90	2633.13	1925.78	1986.38	1973.83	2028.00
Betaine/Adequate Methionine	2555.73	2612.07	1922.33	1981.76	1970.73	2024.27
SEM	16.6553	18.1237	12.8539	14.3314	12.8082	14.7437
P Value	0.9275	0.4274	0.8860	0.4066	0.9533	0.3668

Table 25. Impact of Betaine and dietary methionine levels on the day 43 processing weight of male broilers treated with cocci vac at day of age

Treatment	**Pre-slaughter Live dock weight (g)**		**Post-slaughter Hot WOG Weight (g)**		**Post-Chill Cold WOG Weight (g)**	
	Genetic Strain					
	Cobb	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**
Control- no coccidiostat	2485.68	2545.58	1856.53	1919.90	1889.25	1957.33
Reduced Methionine	2533.15	2634.13	1884.54	1984.75	1922.28	2021.68
Betaine/Reduced Methionine	2509.30	2585.73	1880.40	1957.65	1912.70	1994.70
Betaine/Adequate Methionine	2533.43	2531.18	1885.83	1916.45	1922.60	1950.43
SEM	19.4395	22.4192	15.8372	18.6126	15.9911	18.7762
P Value	0.7962	0.3673	0.9061	0.5166	0.8705	0.5036

Table 26. Impact of Betaine and dietary methionine levels on the day 57 processing weights of male broilers provided a dietary coccidiostat

Treatment	Pre-slaughter Live weight (g)		Post-slaughter Hot WOG Weight (g)		Post-Chill Cold WOG Weight (g)	
	Genetic Strain					
	Cobb[1]	Ross	Cobb[1]	Ross	Cobb[1]	Ross
Control diet w/ Biocox	3223.38[a]	3515.342 9	2519.18[a]	2745.17	2571.78[a]	2798.26
Reduced Methionine	3157.43[ab]	3529.5	2451.28[ab]	2742.5	2505.33[ab]	2798.73
Betaine/Reduced Methionine	3031.9[b]	3467.8	2363.08[b]	2687.78	2414.25[b]	2748.68
Betaine/Adequat e Methionine	3181.13[a]	3452.466 7	2461.7[a]	2694.29	2517.68[a]	2753.69
SEM	0.02306	0.01280	17.5580	10.9550	17.7588	11.0986
P Value	0.0213	0.0925	0.0163	0.1221	0.0159	0.2105

[1] Means with different letters are statistically different at the $P<0.05$ level.

Table 27. Impact of Betaine and dietary methionine levels on the day 57 processing weights of male broilers treated with coccivac at day of age[1]

Treatment	Pre-slaughter Live dock weight (g)		Post-slaughter Hot WOG Weight (g)		Post-Chill Cold WOG Weight (g)	
	Genetic Strain					
	Cobb[1]	Ross	Cobb[1]	Ross	Cobb[1]	Ross
Control no coccidiostat	3307.88[b]	3519.25	2550.32[b]	2742.24	2609.51[b]	2799.26
Reduced Methionine	3316.68[b]	3500.925	2533.9[b]	2727.32	2587.97[b]	2786.65
Betaine/Reduced Methionine	3411.8[a]	3519.25	2634.05[a]	2752	2684.54[a]	2801.42
Betaine/Adequate Methionine	3285.23[b]	3503.675	2533.39[b]	2743.72	2581.61[b]	2794.94
SEM	14.1404	9.5291	12.2080	8.7183	12.2488	8.5480
P Value	0.0075	0.8517	0.0082	0.7914	0.0099	0.9335

[1]Means with different letters are statistically different at the $P<0.05$ level.

Table 28. Impact of Betaine and dietary methionine levels on the day 43 parts yield of male broilers provided a dietary coccidiostat[1]

Treatment	Carcass Yield (%)		Breast Yield (%)		Tender Yield (%)		Wing Yield (%)		Leg Yield (%)	
	Genetic Strain									
	Cobb	Ross	Cobb	Ross[2]	Cobb	Ross	Cobb	Ross[2]	Cobb	Ross
Control diet w/ Biocox	75.39	76.10	23.76	26.59[a]	5.05	5.58	10.31	9.95[b]	30.92	29.93
Reduced Methionine	74.75	75.89	23.01	25.32[b]	5.06	5.50	10.39	10.24[a]	31.64	30.58
Betaine/Reduced Methionine	75.51	75.55	23.72	26.87[a]	5.11	5.69	10.20	10.03[ab]	31.30	29.70
Betaine/Adequate Methionine	75.17	75.83	24.12	26.37[a]	5.15	5.55	10.32	10.10[ab]	31.14	29.74
SEM	0.1075	0.1482	0.1505	0.1567	0.0334	0.0368	0.0386	0.0379	0.1206	0.1349
P Value	0.0635	0.6256	0.0712	0.0025	0.6726	0.3075	0.3916	0.0461	0.1853	0.0769

[1] Carcass Yield expressed as Hot WOG:Pre-slaughter Live weight. Parts Yields expressed as Part weight:Cold WOG weight.
[2] Means within columns with different letters are statistically different at the $P<0.05$ level.

Table 29. Impact of Betaine and dietary methionine levels on the day 43 parts yield of male broilers vaccinated with coccivac at day of age [1]

Dietary Treatment	Carcass Yield (%)		Breast Yield (%)		Tender Yield (%)		Wing Yield (%)		Leg Yield (%)	
	Genetic Strain									
	Cobb	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**
Control no coccidiostat	74.66	75.35	23.05^{b}	25.30^{a}	5.10	5.41	10.42	10.22	30.78	29.61^{b}
Reduced Methionine	74.31	75.21	21.98^{c}	23.67^{b}	5.06	5.26	10.61	10.39	31.18	30.68^{a}
Betaine/Reduced Methionine	74.93	75.65	23.99^{a}	25.82^{a}	5.09	5.38	10.46	10.11	30.85	29.73^{b}
Betaine/Adequate Methionine	74.34	75.65	23.29^{ab}	25.44^{a}	5.09	5.42	10.38	10.26	30.83	29.77^{b}
SEM	0.1285	0.1448	0.1746	0.1736	0.0418	0.0482	0.0542	0.05	0.1294	0.1326
P Value	0.2811	0.6212	0.0005	<.0001	0.9812	0.6264	0.4616	0.2623	0.7023	0.0144

[1] Carcass Yield expressed as Hot WOG:Pre-slaughter Live weight. Parts Yields expressed as Part weight:Cold WOG weight.

Table 30. Impact of Betaine and dietary methionine levels on the day 57 parts yield of male broilers provided a dietary coccidiostat[1]

Treatment	Carcass Yield (%)		Breast Yield (%)		Tender Yield (%)		Wing Yield (%)		Leg Quarter Yield (%)	
	Genetic Strain									
	Cobb[2]	Ross	Cobb	Ross	Cobb	Ross	Cobb	Ross	Cobb	Ross[2]
Control diet w/ Biocox	78.16^{a}	78.10	24.28	25.53	5.20	5.37	10.21	9.91	31.65	31.09^{a}
Reduced Methionine	77.67^{ab}	77.69	23.28	25.15	5.13	5.42	10.25	9.99	31.87	30.45^{ab}
Betaine/Reduced Methionine	77.98^{a}	77.50	24.32	26.24	5.07	5.56	10.30	10.03	31.89	30.10^{b}
Betaine/Adequate Methionine	77.39^{b}	78.03	24.10	26.31	5.22	5.27	10.33	9.72	31.47	29.31^{c}
SEM	0.09	0.11	0.17	0.18	0.04	0.07	0.04	0.05	0.17	0.15
P Value	0.0179	0.1678	0.1072	0.0571	0.6359	0.5346	0.8053	0.0580	0.7926	0.0001

[1] Carcass Yield expressed as Hot WOG:Pre-slaughter Live weight. Parts yields are expressed as Part weight:Cold WOG weight.
[2] Means within columns with different letters are statistically different at the *P*<0.05 level.

Table 31. Impact of Betaine and dietary methionine levels on the day 57 parts yield of male broilers vaccinated with coccivac at day of age [1]

Treatment	Carcass Yield (%)		Breast Yield (%)		Tender Yield (%)		Wing Yield (%)		Leg Yield (%)	
	Genetic Strain									
	Cobb	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross**
Control no coccidiostat	77.52[a]	77.90	24.22[ab]	25.46[ab]	4.99	5.43	10.10	9.94[b]	30.82	29.41
Reduced Methionine	76.47[b]	78.02	23.37[b]	24.64[b]	5.08	5.57	10.28	10.20[a]	30.59	29.53
Betaine/Reduced Methionine	77.44[a]	78.19	24.94[a]	26.02[a]	5.19	5.28	10.14	9.85[b]	30.65	28.92
Betaine/Adequate Methionine	77.19[a]	78.36	24.14[ab]	25.95[a]	5.01	5.32	10.32	9.79[b]	30.81	29.80
SEM	0.13	0.10	0.18	0.18	0.05	0.05	0.05	0.04	0.15	0.13
P Value	0.0104	0.3590	0.0214	0.0261	0.4312	0.2321	0.2695	0.0020	0.9353	0.1070

[1]Carcass Yield expressed as Hot WOG:Pre-slaughter Live weight. Parts Yields are expressed as Part weight:Cold WOG weight.

[2] Means within columns with different letters are statistically different at the *P*<0.05 level.

Table 32. Impact of Betaine and dietary methionine levels on the day 57 combined breast and tender yield (%) of male broilers provided either a dietary coccidiostat or vaccinated with coccivac at day of age[1]

Cocci Control	Diet	Day 43 Breast and Tender Yield (%)		Day 57 Breast and Tender Yield (%)	
	Strain	**Cobb**	**Ross[2]**	**Cobb[2]**	**Ross**
Biocox	Control	28.807	32.171^{a}	29.481^{ab}	30.827
	Reduced Methionine	28.070	30.817^{bc}	28.403^{c}	30.573
	Betaine/Reduced Methionine	28.822	32.552^{a}	29.392^{abc}	31.843
	Betaine/Adequate Methionine	29.274	31.926^{a}	29.316^{abc}	31.581
Coccivac	Control	27.722	30.026^{c}	29.158^{abc}	30.890
	Reduced Methionine	28.098	28.923^{d}	28.453^{bc}	30.209
	Betaine/Reduced Methionine	29.072	31.545^{ab}	30.123^{a}	31.294
	Betaine/Adequate Methionine	28.402	30.855^{bc}	29.147^{abc}	31.273
SEM		0.1409	0.1485	0.1338	0.1412
P Value		0.0702	<.0001	0.0346	0.0721

[1] Yields are expressed as Part weight:Cold WOG weight.

[2] Means within columns with different letters are statistically different at the $P<0.05$ level.

Table 33. Impact of Betaine and dietary methionine levels on the day 43 parts weights of male broilers treated with a dietary coccidiostat[1]

Treatment	Breast weight (g)		Tender weight (g)		Wing weight (g)		Leg weight (g)	
	Genetic Strain							
	Cobb	**Ross**	**Cobb**	**Ross**	**Cobb**	**Ross[1]**	**Cobb**	**Ross[1]**
Control diet w/ Biocox	473.50	546.78	100.40	114.15	204.45	203.73[b]	612.90	614.30[ab]
Reduced Methionine	455.45	529.85	99.80	114.65	204.55	213.85[a]	623.28	639.33[a]
Betaine/ Reduced Methionine	469.44	545.88	100.98	115.33	201.15	203.10[b]	617.07	602.03[b]
Betaine/ Adequate Methionine	475.83	534.60	101.45	112.17	202.93	204.02[b]	614.10	601.8[b]
SEM	5.0252	5.5449	0.9624	0.9920	1.2611	1.3654	4.1310	5.1785
P Value	0.4899	0.6383	0.9401	0.7052	0.7538	0.0132	0.8191	0.0326

[1] Means within columns with different letters are statistically different at the $P<0.05$ level.

Table 34. Impact of Betaine on parts weights of male broilers grown to 43 days of age and vaccinated with coccivac at day of age[1]

Dietary Treatment	Breast weight (g)		Tender weight (g)		Wing weight (g)		Leg weight (g)	
	Genetic Strain							
	Cobb	Ross	Cobb	Ross	Cobb	Ross	Cobb	Ross
Control no coccidiostat	437.58	497.10	96.60	105.80	196.23	199.28	580.48	578.38^{b}
Reduced Methionine	424.92	480.68	97.49	106.23	203.33	209.30	597.69	619.49^{a}
Betaine/Reduced Methionine	460.43	516.78	97.38	106.90	199.73	200.85	588.90	591.38^{ab}
Betaine/Adequate Methionine	451.10	498.73	98.15	105.73	200.00	199.08	591.60	579.83^{b}
SEM	6.1358	6.7098	1.2389	1.3078	1.5384	1.6792	4.6149	5.6037
P Value	0.1887	0.3063	0.9783	0.9886	0.4481	0.0988	0.6209	0.0338

[1] Means within columns with different letters are statistically different at the *P*<0.05 level.

Table 35. Impact of Betaine on the day 57 parts weight of male broilers treated with a dietary coccidiostat[1]

Treatment	Breast weight (g)		Tender weight (g)		Wing weight (g)		Leg weight (g)	
	Genetic Strain							
	Cobb	Ross	Cobb	Ross	Cobb	Ross	Cobb	Ross
Control w/ Biocox	625.33	715.06	133.75[a]	150.03	262.28[a]	277.14[a]	812.90[a]	869.29[a]
Reduced Methionine	583.53	703.98	128.38[ab]	151.88	256.60[ab]	279.00[a]	797.13[ab]	851.30[ab]
Betaine/ Reduced Methionine	587.68	722.40	122.23[b]	153.21	248.58[b]	275.58[ab]	766.80[b]	827.25[bc]
Betaine/ Adequate Methionine	609.38	725.67	131.60[a]	145.24	259.10[a]	267.67[b]	791.55[ab]	806.42[c]
SEM	6.7005	6.2893	1.5136	2.0834	1.7424	1.4774	5.8334	4.8007
P Value	0.0938	0.6165	0.0395	0.5218	0.0357	0.0256	0.0430	<0.0001

[1]Means within columns with different letters are statistically different at the $P<0.05$ level.

Table 36. Impact of Betaine on the day 57 parts weight of male broilers vaccinated with coccivac at day of age[1]

Treatment	Breast weight (g)		Tender weight (g)		Wing weight (g)		Leg weight (g)	
	Genetic Strain							
	Cobb	Ross	Cobb	Ross	Cobb	Ross	Cobb	Ross
Control no coccidiostat	632.84[b]	713.16	130.36[b]	152.08	263.22	278.05[ab]	803.81	822.76
Reduced Methionine	605.16[b]	687.35	131.53[b]	155.35	265.74	284.19[a]	791.08	822.62
Betaine/ Reduced Methionine	670.54[a]	728.89	139.39[a]	147.79	272.00	275.92[b]	821.43	810.24
Betaine/ Adequate Methionine	624.42[b]	726.22	129.34[b]	148.64	265.70	273.50[b]	794.87	832.14
SEM	6.3735	6.0211	1.3714	1.6066	1.3553	1.3099	4.6866	3.9698
P Value	0.0025	0.0578	0.0400	0.3262	0.1263	0.0272	0.1002	0.2824

[1] Means within columns with different letters are statistically different at the $P<0.05$ level.

Table 37. Impact of Betaine and methionine levels on the day 36 litter temperature for male broilers treated with either a dietary coccidiostat or coccivac at one day of age[1]

Cocci Control	Diet	Litter Temperature (°F)	
	Strain	**Cobb**	**Ross**
Biocox	Control diet	85.13[a]	84.00[a]
	Reduced Methionine	84.50[ab]	84.50[a]
	Betaine/Reduced Methionine	85.13[a]	84.00[a]
	Betaine/Adequate Methionine	84.13[abc]	84.13[a]
Coccivac	Control	79.50[e]	80.63[b]
	Reduced Methionine	81.25[de]	82.00[ab]
	Betaine/Reduced Methionine	81.75[cde]	81.13[b]
	Betaine/Adequate Methionine	82.50[bcd]	82.13[ab]
SEM		0.3730	0.3577
P Value		<.0001	0.0184

[1]Means within columns with different letters are statistically different at the $P<0.05$ level.

Table 38. Impact of Betaine and methionine levels on the day 36 litter ammonia emission for male broilers treated with either a dietary coccidiostat or coccivac at one day of age

Cocci Control	Diet	Initial Ammonia Reading (ppm)		Ammonia Reading After Five Minutes (ppm)	
	Strain	**Cobb**	**Ross**	**Cobb**	**Ross**
Biocox	Control diet	10.50[a]	8.00[a]	9.63[a]	7.25[a]
	Reduced Methionine	7.63[ab]	7.38[a]	8.50[a]	7.63[a]
	Betaine/Reduced Methionine	8.63[a]	6.63[a]	8.75[a]	6.88[a]
	Betaine/Adequate Methionine	9.50[a]	7.50[a]	9.38[a]	7.00[a]
Coccivac	Control	5.13[b]	4.38[b]	5.25[b]	4.25[b]
	Reduced Methionine	5.25[b]	4.25[b]	5.38[b]	4.13[b]
	Betaine/Reduced Methionine	4.88[b]	4.50[b]	5.25[b]	4.38[b]
	Betaine/Adequate Methionine	5.25[b]	4.25[b]	4.88[b]	4.00[b]
SEM		0.4306	0.3069	0.4146	0.2937
P Value		0.0002	<.0001	0.0005	<.0001

[1] Means within columns with different letters are statistically different at the $P<0.05$ level.

Table 39. Impact of Betaine and methionine levels on the day 36 litter ammonia flux for broilers treated with either a dietary coccidiostat or coccivac at one day of age[1]

Cocci Control	Diet	Ammonia Flux (ppm / 5 min)	
	Strain	**Cobb**	**Ross**
Biocox	Control diet	0.50	0.25
	Reduced Methionine	1.50	1.00
	Betaine/Reduced Methionine	1.38	0.38
	Betaine/Adequate Methionine	1.00	0.38
Coccivac	Control	0.13	0.13
	Reduced Methionine	0.38	0.13
	Betaine/Reduced Methionine	0.63	0.00
	Betaine/Adequate Methionine	0.13	0.13
SEM		0.2013	0.1213
P Value		0.5376	0.5792

[1]Ammonia Flux value is the difference between the Initial Ammonia reading and Ammonia reading after 5 minutes.

Table 40. Impact of Betaine and methionine levels on the day 23 Cocci Lesion Scores of male broilers treated with either a dietary coccidiostat or with coccivac at one day of age[1]

Cocci Control	Diet	Cocci Score (Mean)		
		Combined Strains	**Cobb**	**Ross**
Biocox	Control diet	0.125[bc]	0.250[ab]	0.000
	Reduced Methionine	0.292[ab]	0.417[a]	0.167
	Betaine/Reduced Methionine	0.104[c]	0.042[bc]	0.167
	Betaine/Adequate Methionine	0.313[a]	0.375[a]	0.250
Coccivac	Control	0.00[c]	0.000[c]	0.000
	Reduced Methionine	0.00[c]	0.000[c]	0.000
	Betaine/Reduced Methionine	0.00[c]	0.000[c]	0.000
	Betaine/Adequate Methionine	0.00[c]	0.000[c]	0.000
SEM		0.06359	0.08145	0.0964
P Value		0.0003	0.0002	0.3403

[1] Means within columns with different letters are statistically different at the *P*<0.05 level.

Table 41. Impact of Betaine and methionine levels on the day 42 and day 56 paw lesion scores of male broilers treated with either a dietary coccidiostat or with coccivac at day of age [1]

Cocci Control	Diet	Day 42 Paw Score		Day 56 Paw Score	
	Strain	**Cobb**	**Ross**	**Cobb**	**Ross**
Biocox	Control diet	1.915[a]	1.732[bc]	1.394[c]	1.950[a]
	Reduced Methionine	1.914[a]	1.932[a]	1.452[c]	1.977[a]
	Betaine/Reduced Methionine	1.500[c]	1.136[d]	1.658[b]	1.744[c]
	Betaine/Adequate Methionine	1.915[a]	1.724[bc]	1.864[a]	1.936[ab]
Coccivac	Control	1.983[a]	1.879[ab]	1.936[a]	1.978[a]
	Reduced Methionine	1.867[a]	1.820[ab]	1.870[a]	1.830[bc]
	Betaine/Reduced Methionine	1.702[b]	1.559[c]	1.767[ab]	1.894[ab]
	Betaine/Adequate Methionine	1.983[a]	1.850[ab]	1.936[a]	2.000[a]
SEM		0.0176	0.0257	0.0254	0.0152
P Value		<.0001	<.0001	<.0001	<0.0001

[1]Means within columns with different letters are statistically different at the $P<0.05$ level.

Image 1. Vials from Coccidiosis vaccine administered to chicks on University of Arkansas Poultry Science Farm at day of hatch and placed in 232 West. Malea Frank, used with permission.

UNIVERSITY OF ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Susan Watkins

FROM: Craig N. Coon, Chairman
Institutional Animal Care
And Use Committee

DATE: July 12, 2011

SUBJECT: IACUC PROTOCOL **APPROVAL**
Expiration date : **July 11, 2014**

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** Protocol **#11056- "EVALUATION OF METHODS USED TO IMPROVE THE GROWTH EFFICIENCY AND CARCASS CHARACTERISTICS OF MEAT BIRDS**". You may begin this study immediately.

The IACUC encourages you to make sure that you are also in compliance with other UAF committees such as Biosafety, Toxic Substances and/or Radiation Safety if your project has components that fall under their purview.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes in the protocol during the research, please notify the IACUC in writing [Modification Request form] **prior** to initiating the changes. If the study period is expected to extend beyond **07-11-2014,** you must submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

cnc/car

cc: Animal Welfare Veterinarian

Administration Building 210 • 1 University of Arkansas • Fayetteville, AR 72701-1201 • 479-575-4572
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UNIVERSITY OF ARKANSAS
DIVISION OF AGRICULTURE

Center of Excellence for Poultry Science
1260 W. Maple • POSC 0-114 • Fayetteville, Arkansas 72701
(479) 575-4952 • FAX (479) 575-8775

Attn: University of Arkansas Graduate School

November 3, 2014

Dear Sir,

I attest that Malea Frank was first author of the manuscript cited below and completed at least 51% of the work for the paper.

M. Frank, T. Clark, P. Maharjan, J. Thompson, C. Eagleson, S. Watkins. Evaluation of betaine and methionine replacement for improving performance and meat quality for broilers reared under elevated temperature conditions.

Yours Sincerely,

Susan E. Watkins,
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Fayetteville,
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