# Effects of high dietary copper concentration on growth performance, processing characteristics, amino acid digestibility, and ileal microflora composition of broilers

by

Stephanie C. Philpot

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 3, 2019

Keywords: antibiotic free, broiler, copper, feed conversion, yield

Approved by

William A. Dozier, III, Chair, Professor of Poultry Science
Kenneth S. Macklin, Professor of Poultry Science
Wilmer J. Pacheco, Assistant Professor of Poultry Science

# ABSTRACT

Subtherapeutic antibiotics have been added to diets to improve growth performance of broilers. Pressure from the food service industry is reducing the use of this practice, so the broiler industry is evaluating alternatives to antibiotics. One potential alternative is feeding high concentrations of dietary Cu. Previous research has shown increases in BW gain and decreases in feed conversion ratio (**FCR**) when feeding high concentrations of Cu, but the mode of action is unknown. One hypothesis is that Cu may improve the digestibility of amino acids (**AA**). Two experiments were designed to examine the interaction between AA density and Cu concentration on growth performance, processing characteristics, and AA digestibility of broilers. In experiment 1, 1,600 chicks were grown until 33 d of age and fed 8 dietary treatments throughout experimentation. Positive and negative control treatments received moderate AA density diets with 14 mg/kg Cu. Positive control birds received diets supplemented with diclazuril and were not vaccinated against coccidiosis. All other birds were vaccinated against coccidiosis. Experimental diets were arranged as a 2 × 3 factorial of moderate (95% of primary breeder guidelines) and low (88% of primary breeder guidelines) AA density and 3 Cu programs, where birds were fed 135-135-135, 270-135-135, or 270-270-135 mg/kg of supplemental Cu in the starter, grower, and finisher periods, respectively. Broilers fed 270 mg/kg of Cu in the starter and grower periods and 135 mg/kg of Cu in the finisher period had lower FI and FCR ($P < 0.040$) and higher carcass weights and yields ($P < 0.035$) than broilers fed 135 mg/kg of Cu in all phases. Next generation sequencing showed no changes ($P \geq 0.07$) in ileal microbiota composition. In experiment 2, 672 male chicks were provided with 6 dietary treatments from 1 to 14 d of age. Experiment 2 employed the same positive and negative control treatments as experiment 1, and a 2 × 2 factorial arrangement of moderate or low AA density and supplementation with 135 or 270 mg/kg of Cu. Apparent digestibility of Cys was decreased ($P < 0.010$) from 63.4% in broilers fed no supplemental Cu to 51.2 and 56.0% in broilers fed diets supplemented with 135 or 270 mg/kg of Cu, respectively. An interaction between AA density and Cu concentration was observed where birds fed moderate AA diets had higher digestibility of Lys, Val, His, and Arg ($P < 0.040$) when fed diets containing 270 mg/kg of supplemental Cu, with no differences between the treatments receiving low AA density diets. Approximately 24% of broilers in the United States are raised to over 3.4 kg, but information is sparse on feeding high concentrations of Cu to these broilers, particularly from 2.8 to 4.0 kg BW. Therefore, experiment 3 was conducted to evaluate effects of feeding high concentrations of Cu to broilers from 29 to 53 d of age. All birds were provided common starter and grower diets containing 135 mg/kg of Cu in the starter and grower periods (1 to 19 and 20 to 28 d of age), and were given 0-0, 135-0, 270-0, 135-135, or 270-270 mg/kg of supplemental Cu in the finisher 1 and finisher 2 periods (29 to 41 and 42 to 53 d of age). No treatment differences ($P \geq 0.08$) were observed for growth performance. Total breast weight (pectoralis major and minor muscles) was increased by 32 g ($P = 0.010$) in birds fed 270-270 mg/kg of supplemental Cu compared with broilers fed 135-0 mg/kg of Cu, and total breast meat yield was increased 0.4% ($P = 0.017$) in birds fed 270-270 mg/kg of Cu compared with

birds fed 0-0 mg/kg of Cu. These studies indicated that feeding broilers high concentrations of Cu beyond the starter and grower periods may improve growth performance and carcass characteristics.

# ACKNOWLEDGMENTS

I would like to thank Dr. Bill Dozier for his diligent work in this process. His expertise has been valuable and his discipline and attention to detail has been a great example. I would also like to thank Dr. Ken Macklin and Dr. Wilmer Pacheco for their assistance and their willingness to serve on my committee, and Dr. Kurt Perryman and Micronutrients USA for their support of this research.

None of this work would have been possible without the hours of help from the farm and feed mill crews. Steve Martin and Denise Landers have been particularly important to me, and the past two years wouldn’t have been the same without their encouragement and support. Auburn would have been a lot more work and much less fun without the help and friendship of my lab mates Ruben Kriseldi, Klint McCafferty, Sara Cloft, and Tanner Wise. The undergrads in our lab, particularly Trevor Lee and Lindsey Pope, have also been invaluable and fun to work with. Additionally, I’ll always be grateful for Dr. Susan Watkins, Dr. David McCreery, and Dr. Mike Kidd for helping me get my start in the poultry industry, and for their continued support.

I’m so thankful to my mom, Lisa Philpot, for her unwavering encouragement, and to my dad, James Philpot, for his advice, commiseration, and sense of humor on every situation. Most importantly, no amount of love, encouragement, or support could compare to that of my God. I’m so blessed to have a Savior who makes it easy to try to work as if for God and not for man, and I hope that this thesis shows that.

## TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# I. INTRODUCTION

Broilers have commonly been fed diets containing nontherapeutic antibiotics to increase BW gain while decreasing feed conversion ratio. There is increasing pressure from consumers, particularly from the food service industry, to reduce the use of this practice. In 2017, approximately 40% of broilers in the United States were raised without antibiotics (Rennier, 2018). Therefore, the broiler industry is evaluating alternatives to antibiotic growth promotors that may help to maintain economic efficiency of live production.

One potential alternative is to feed broilers diets containing pharmacological concentrations of dietary Cu. Previous research has shown increases in BW gain and decreases in feed conversion ratio when dietary Cu concentrations exceed 100 mg/kg (Ewing et al., 1998). Data on the effects of pharmacological concentrations of Cu on processing yields are limited, but studies have observed increased carcass weight (Arias and Koutsos, 2006) or breast meat weight (Pekel et al., 2009) with higher Cu inclusions. The mode of action through which Cu increases BW gain and decreases feed conversion is unknown, but various hypotheses include positive modulation of the microflora in the gastrointestinal tract (Pang et al., 2009), alteration of nutrient digestibility (Aoyagi and Baker, 1995), improvements in immunological function (Koh et al, 1996), and increasing cell proliferation (Wlostowski, 1993). Additionally, previous studies have demonstrated interactions between amino acid (**AA**) density and Cu concentration on AA digestibility

(Rochell et al., 2017), as well as interactions approaching significance on growth performance and processing characteristics (Wang et al., 2014). Research evaluating effects of Cu feeding programs that vary Cu concentration by phase of production is sparse. Moreover, studies evaluating feeding pharmacological concentrations of Cu to broilers raised from 2.8 to 4.0 kg are limited.

A series of experiments was designed to evaluate different Cu feeding strategies addressing these knowledge gaps in the literature. Experiment 1 was designed to evaluate growth performance, processing characteristics, and ileal microbial profile of broilers provided diets varying in AA density and Cu concentrations to 32 d of age. Dietary Cu supplementation was provided in concentrations of 270-270-135, 270-135-135, or 135-135-135 mg/kg Cu during the starter, grower, and finisher periods, respectively. These treatments were employed to evaluate the effects of Cu supplementation of 270 vs. 135 mg/kg during the starter and grower periods with varying AA concentrations. Experiment 2 was conducted simultaneously with experiment 1 and assessed AA digestibility of broilers provided dietary treatments consisting of 2 AA densities and either 135 or 270 mg/kg Cu from 1 to 14 d of age. Experiment 3 was designed to assess the effects of increasing Cu concentration during the finisher phases of broilers raised to target weights of approximately 4.0 kg. Broilers were provided with common starter and grower diets containing 135 mg/kg Cu from 1 to 29 d of age, and with Cu concentrations of 0, 135, or 270 mg/kg in the finisher 1 and finisher 2 phases (from 29 to 40 and 41 to 53 d of age). These experiments should provide new information to develop feeding programs of Cu to optimize growth performance and meat yield and provide a better understanding on the effects of supplemental Cu on AA digestibility of corn and soybean-meal based diets.

# II. LITERATURE REVIEW

## FUNCTIONS OF DIETARY COPPER IN BROILER DIETS

The essentiality of Cu was discovered in 1928 when rats that were fed sufficient concentrations of Fe but deficient Cu concentrations developed pernicious anemia (Hart et al., 1928). These authors concluded that Cu is required for hemoglobin synthesis. While Cu is not a component of hemoglobin, it is required for hemoglobin synthesis as it is a component of ceruloplasmin. Ceruloplasmin acts as a ferroxidase to oxidize $Fe^{++}$ to $Fe^{+++}$ to allow Fe to bind to transferrin (Roeser et al., 1970). Transferrin facilitates the transfer of iron into the cell (Arredondo and Núñez, 2005), and a deficiency of Cu leads to anemia (Attieh et al., 1999). Another well-known function of Cu is its role as a cofactor in energy metabolism. Copper is a component of cytochrome C, which is an enzyme used in the electron transport chain for ATP synthesis (Klasing, 1998). Plasma Cu concentrations have been shown to increase when birds are under an immune challenge, indicating that Cu plays a role in immune function (Curtis and Butler, 1980; Turk, 1986). Copper is also a component of proteins of the acute phase response including ceruloplasmin through its ferroxidase and superoxide dismutase activities (Koh et al., 1996). Additionally, Cu is required for crosslinking of connective tissue, lipid metabolism, pigmentation, reproduction, and as an antioxidant (Leeson, 2009).

The NRC (1994) for poultry recommends a total dietary Cu concentration of 8 mg/kg. Moreover, McNaughton and Day (1979) reported that growth of broilers was optimized at a Fe:Cu ratio of 5:1. In the United States, broiler diets are primarily composed of corn and soybean meal, which contain approximately 4 mg/kg and 14 mg/kg of Cu, respectively (NRC, 2012). Hence, Cu is typically supplemented in diets as a component of the trace mineral premix at the concentration to meet the Cu requirement, above the Cu supplied by feed ingredients.

Copper is generally absorbed in the cupric (+2) state, although it can exist in 3 valence states (Schroeder et al., 1966). It is primarily absorbed in the duodenum, but also in the jejunum and ileum (Hill and Link, 2009). Absorption occurs through active and passive transport, with the protein Cu transporter 1 (**Ctr1**) being the most common transporter (Hill and Link, 2009). After absorption, Cu is transported to the liver, and most of circulatory Cu is bound to albumin (Linder and Hazegh-Azam, 1996). Copper is not easily recycled through the gall bladder, which is the main route for excretion (Rosenblum and Leach, 1985). The ability of the broiler to absorb Cu is partially determined by the Cu source used. Tribasic Cu chloride (**TBCC**) has 109% higher bioavailability relative to $CuSO_4$ based on liver Cu concentrations (Luo et al., 2005), and $CuSO_4$ is approximately twice as available compared with Cu oxide (McNaughton et al., 1974).

## PHARMACOLOGICAL DIETARY CONCENTRATIONS OF COPPER IN BROILER DIETS

### *Growth responses of broilers*

Early research reported growth performance benefits with nursery pigs fed high concentrations of Cu, with both BW gain and feed efficiency being improved when pigs were fed diets containing 250 mg/kg of Cu in the form of Cu sulfate (Bunch, 1961).

Similar benefits were observed in broilers fed pharmacological concentrations of Cu, with BW gain increases of 30 and 114 grams at 3 and 6 weeks of age, respectively, with supplementation of 250 mg/kg of Cu compared with the control-fed bids (Jenkins et al., 1970). These early studies indicated that feeding high concentrations of Cu can lead to increases in BW gain and decreases in feed conversion ratio.

Recent research has shown similar increases in BWG with Cu inclusion ranging from 100 to 200 mg/kg compared with control-fed broilers (Arias and Koutsos, 2006; Lim et al., 2006; Wang et al., 2014). Broilers provided with diets containing 100 mg/kg of Cu-Met chelate had a BWG of 1.415 kg vs. 1.379 kg ($P < 0.05$) in control-fed broilers when raised to 28 d of age (Kim et al., 2011) or of 1.568 kg with Cu supplementation vs. 1.488 kg in control-fed broilers at 35 d of age (Lim et al., 2006). In agreement, broilers fed diets containing 188 mg/kg of $CuSO_4$ to 31 d of age had a higher BW ($P < 0.05$; 1.056 vs 0.958 kg) than broilers provided control diets (Arias and Koutsos, 2006). At 40 d of age, Wang et al. (2014) observed that broilers fed diets containing 200 mg/kg of TBCC had higher BWG ($P = 0.01$; 2.64 vs. 2.58 kg) than broilers provided with diets containing 5 mg/kg of Cu. Furthermore, research has reported that Cu inclusion above 100 mg/kg can decrease feed conversion ratio as well as increase BWG (Pesti and Bakalli, 1996; Ewing et al., 1998; Pekel et al., 2009). Pekel et al. (2009) observed a 5 point decrease ($P = 0.008$; 1.53 vs. 1.58) in feed conversion ratio at 21 d of age in broilers fed diets containing 150 mg/kg of $CuSO_4$ compared with control-fed broilers. Pesti and Bakalli (1996) observed a 6 point reduction ($P < 0.05$; 1.89 vs. 1.95) in feed conversion ratio of broilers fed 250 mg/kg of cupric sulfate pentahydrate compared with control-fed broilers during a 42 d production period. Moreover, Ewing et al. (1998) fed broilers diets containing 125 mg/kg from either $CuSO_4$, cupric citrate, or Cu oxychloride, and found similar responses where broilers provided with supplemental Cu, regardless of source, had a reduced ($P < 0.05$) feed conversion ratio at 42 d of age compared with broilers receiving the control diets.

### ***Effect of age on growth responses***

Previous research has reported no significant differences during early growth phases, while finding differences in later phases (Pesti and Bakalli, 1996; Skrivan et al, 2000; Pekel et al, 2009; Kim et al., 2011). Kim et al. (2011) fed broilers a negative control diet containing 15 mg/kg of Cu, a positive control diet containing 15 mg/kg of Cu and avilamycin, and a diet containing 100 mg/kg of Cu-Met chelate. From 1 to 14 d of age birds had similar BW gain and feed conversion ratio ($P > 0.05$). From 15 to 28 d of age and cumulatively, broilers fed the Cu-Met chelate had higher BW gain than negative control-fed birds ($P < 0.045$), while maintaining a similar BW gain compared with positive control-fed broilers ($P > 0.05$), but feed conversion ratio was not affected ($P > 0.05$). Broilers may have to consume high concentrations of Cu for a specific duration before performance benefits can be observed. Despite these data, Cu is commonly supplemented in the starter phase in commercial practice, likely because of the antimicrobial-like properties of Cu. Karimi et al. (2011) demonstrated that broilers fed 250 mg/kg of Cu had a hepatic Cu concentration of 35.1 mg/kg, higher ($P < 0.0001$) than the concentrations of 3.7 and 9.8 mg/kg of control-fed broilers and broilers fed 125 mg/kg of Cu, respectively, from 1 to 20 d of age. Similarly, Hamdi et al. (2018) reported that broilers fed 300 mg/kg of Cu had a higher ($P < 0.0001$) hepatic Cu concentration at

6.3 mg/kg, compared with 2.7 and 3.2 mg/kg in broilers fed diets containing 15 or 150 mg/kg of Cu from 1 to 35 d of age.

### *Processing weights and yields*

Although dietary Cu is commonly used as a growth promotor, limited data are available on the effects of Cu on processing yields of broilers. Arias and Koutsos (2006) reported that broilers raised on used litter to 45 d of age and fed 188 mg/kg of either TBCC or $CuSO_4$ had similar ($P > 0.05$) carcass weights compared with control-fed broilers provided a diet containing an antibiotic but had heavier carcass weights than the negative control-fed broilers ($P < 0.05$). Wang et al. (2014) evaluated broilers diets varying in Lys concentrations and containing either 5 or 200 mg/kg of Cu on growth performance and processing yields from 1 to 40 d of age. Although BW gain was increased in broilers fed 200 mg/kg of Cu ($P = 0.01$; 2.64 kg vs 2.58 kg), Cu supplementation did not alter ($P > 0.05$) processing yields. In agreement, Pekel et al. (2009) determined that feeding broilers diets supplemented with 150 mg/kg of Cu increased ($P < 0.003$) BW, carcass weight, and breast weight compared with control-fed birds from 1 to 21 d of age. Additionally, numerical increases approaching significance were observed for birds fed diets supplemented with 150 mg/kg of Cu in carcass yield ($P = 0.06$; 64.2 vs. 63.4%) and breast meat yield ($P = 0.09$; 30.0 vs. 29.4%).

### *Sources of Copper*

The 2 Cu sources predominately used are Cu Sulfate and TBCC. Copper sulfate is a commonly used source that is considered the standard when conducting Cu studies (Leeson, 2009). Recently, TBCC has been gaining popularity as it has been reported to have higher Cu concentration, smaller particle size, more uniform mixing, and increased

Cu bioavailability compared with $CuSO_4$ (Miles et al., 1998; Luo et al., 2005). Copper sulfate contains 25.4% Cu, whereas TBCC contains between 55.6 to 56.7% Cu (Miles et al., 1998; Luo et al., 2005; Lu et al., 2010). This indicates that a lower inclusion of TBCC could be used to provide the same concentration of Cu compared with $CuSO_4$. Additionally, TBCC has a smaller particle size than $CuSO_4$. Miles et al. (1998) observed a modal particle size of 455 µm and that 33.3% and 65.9% of the $CuSO_4$ were retained on No. 20 and 100 screens, respectively, while TBCC had a modal particle size of 67 µm and none was retained in a No. 20 screen but 98.5% was retained in a No. 100 screen. This indicates that TBCC has a smaller and more uniform particle size than $CuSO_4$. Luo et al. (2005) reported that the smaller and more uniform particle size of TBCC led to a lower mixing CV than $CuSO_4$ (2.16 vs. 3.52). Other Cu sources include Cu oxide, Cu citrate, and organic Cu compounds such as Cu-lysine or Cu-methionine (Leeson, 2009). Cu oxide is approximately half as bioavailable as $CuSO_4$ (McNaughton et al., 1974). Copper-amino acid (**AA**) complexes have a similar bioavailability to $CuSO_4$ (Aoyagi and Baker, 1993a,b).

### ***Concerns in the use of high concentrations of copper***

Copper is highly abrasive, and thus is reported to cause gizzard erosion in broilers (Fisher et al., 1973; Poupoulis and Jensen, 1976). In 1973, Fisher et al. described extensive damage to gizzards when birds were fed 600 mg/kg of Cu in the form of $CuSO_4$, and milder damage occurred when fed 400 mg/kg of Cu. The authors reported a slight increase in cellular sloughing was observed in broilers fed 200 mg/kg of Cu. Poupoulis and Jensen (1976) reported similar results, with the additional observation that diet composition affects the concentration required to cause gizzard erosion. Copper supplementation in the form of $CuSO_4$ or $CuCl_2$ at 500 mg/kg in a corn and soybean meal-based diet was required to cause more erosion ($P < 0.05$) than in the control-fed broilers, whereas supplementation of 250 mg/kg caused greater erosion ($P < 0.05$) in broilers fed a diet composed of corn starch and soybean meal than the control-fed broilers. Chowdhury et al. (2004) found no adverse effects ($P > 0.05$) of feeding broilers up to 200 mg/kg of Cu-Met chelate on gizzard erosion.

Another concern in the utilization of pharmacological concentrations of Cu is that Cu can chelate with other minerals, as well as with phytate (Persson et al., 1998; Leeson and Summers, 2001; Pang and Applegate, 2006; Kim et al., 2011). Phytate, inositol, and inositol esters all have higher affinity for Cu than other minerals, although inositol phosphate 6 has only a slightly higher affinity for Cu than Zn (Persson et al., 1998). Conversely, Pang and Applegate (2006) reported that dietary Cu inclusion at 250 mg/kg did not reduce ($P > 0.05$) apparent P retention. Additionally, Cu has antagonistic relationships with Mo, Zn, P, and Fe (Leeson and Summers, 2001). Conversely, Kim et al. (2011) reported that feeding broilers diets supplemented with 100 mg/kg of Cu-Met did not alter ($P = 0.98$) hepatic Zn or Fe concentrations compared with the control-fed birds.

Two other concerns in feeding high concentrations of dietary Cu to poultry are toxicity and environmental pollution. Previous research has reported no toxic effects of Cu until dietary concentrations of 450 mg/kg are provided, which is beyond the concentrations typically fed to enhance growth performance. Hamdi et al. (2018) observed no decreases in BW or increases in feed conversion ratio when feeding 300 mg/kg of $Cu_2O$ or $CuSO_4$, but reduced feed intake and BW gain have been noted when broilers were fed diets supplemented with 450 mg/kg of $CuSO_4$ (Luo et al., 2005). In agreement, Miles et al. (1998) reported reduced feed intake when broilers were fed 450 mg/kg of $CuSO_4$ or TBCC. These authors also reported that supplementing diets with 400 mg/kg of Cu did not impair growth performance, but supplementation at 600 mg/kg impaired BW gain, feed intake, and feed conversion ratio. Additionally, there are concerns about environmental contamination from applying litter from broilers fed high concentrations of Cu to forage crops. One key concern of this is that Cu is highly toxic to sheep, and spreading broiler litter with a high Cu content can cause mortality in sheep (Christodoulopoulos and Roubies, 2007). Supplementing Cu above 25 mg/kg in diets fed to poultry is prohibited in Europe (EFSA, 2011), but no such regulations are currently in effect in the United States. Conversely, it has been reported that broilers fed diets containing approximately 14 mg/kg of Cu excreted 204 mg/kg of Cu, while birds fed 100 mg/kg or 200 mg/kg of Cu-Met chelate excreted 378 and 383 mg/kg of Cu, respectively (Chowdhury et al., 2004). While these excreta Cu concentrations are higher ($P < 0.01$) than the control, the concentrations are not proportional to the dietary treatment. In agreement, Dozier et al. (2003) reported no difference ($P > 0.10$) in the percentage of excretion of Cu relative to Cu intake when broilers were fed diets containing 4 to 12 mg/kg of Cu sulfate or a Cu-AA chelate from 1 to 17 d of age.

## MODE OF ACTION OF PHARMACOLOGICAL CONCENTRATIONS OF COPPER

### *Antimicrobial properties and regulation of microflora*

Several studies have evaluated the effects of Cu on the microbial profile of broilers with inconclusive results (Xia et al., 2004; Pang et al., 2009; Aydin et al., 2010;

Kim et al., 2011). Kim et al. (2011) fed broilers either negative-control diets, diets with 6 mg/kg of avilamycin or 50 or 100 mg/kg of supplemental Cu in the form of either Cu-Met or Cu-soy proteinate. Total bacteria counts in the ileum were reduced ($P < 0.05$) in broilers fed diets containing avilamycin compared with the negative control; however, inclusion of 100 mg/kg of Cu from either source increased ($P < 0.05$) the total bacteria. Lactobacilli displayed a similar response with inclusion of 100 mg/kg of Cu from either source increasing ($P < 0.05$) lactobacilli population compared with the negative control, but with no effect ($P < 0.05$) when supplemented with 50 mg/kg of Cu. The opposite effect was observed for *E. coli*, where 100 mg/kg of Cu decreased ($P < 0.05$) CFU. No differences ($P \geq 0.05$) were observed in the *C. perfringens* CFU for any Cu treatments. Xia et al. (2004) found no differences ($P < 0.05$) on total anaerobes, *Bifidobacteium, Lactobacillus, Clostridium,* or *E. coli* counts in either the small intestine or cecae between control-fed birds or birds fed 37 mg/kg of supplemental $CuSO_4$. Pang et al. (2009) indicated that Cu concentrations of 125 mg/kg or higher may be required to decrease bacterial growth. Aydin et al. (2010) found no significant reduction ($P > 0.05$) in the count of total anaerobic bacteria, *Campylobacter, E. coli, Enterococcus,* coliforms, lactic acid bacteria, *Staphylococcus,* or *Salmonella* in the ileum birds fed diets containing 250 mg/kg of supplemental Cu. Additionally, the pH was not affected ($P > 0.05$) by Cu concentration, with an ileal pH of 5.83 in the control birds and 5.81 in the birds fed 250 mg/kg of supplemental Cu. Moreover, no additive effects have been observed in pigs when feeding 50 or 250 mg/kg of Cu in diets supplemented penicillin (Barber et al., 1955; Barber et al., 1957).

### *Effects on digestion*

Copper affects digestibility of carbohydrates, lipids, and AA (Poupoulis and Jensen, 1976; Aoyagi and Baker, 1995; Pesti and Bakalli, 1996; Lim et al., 2006; Wang et al., 2014; Rochell et al., 2017). Aoyagi and Baker (1995) performed a precision-fed assay evaluating ground corn cobs, with or without 250 mg/kg of supplemental Cu. Roosters fed diets containing corn cobs and 250 mg/kg of supplemental Cu for the 3 weeks prior to collection had higher true dry matter digestibility ($P$ = 0.05; 11.1 vs. 6.2%) and higher true hemicellulose digestibility ($P$ = 0.01; 36.6 vs 22.1%) than the control birds. These authors hypothesized that the increased Cu stimulated an increase in lysosomal enzyme synthesis, but dearth data is available regarding this topic. Increased enzyme synthesis may enhance digestibility by increasing nutrient availability (Yuan et al., 2017).

Previous research has reported the effects of Cu on saturated and unsaturated fat content in liver and adipose tissue (Poupoulis and Jensen, 1976) and cholesterol in the plasma and breast muscle (Pesti and Bakalli,1996; Lim et al., 2006). Poupoulis and Jensen (1976) observed lower ($P < 0.05$) fatty acid concentrations in the liver and leg adipose tissue when broilers were provided feed with 500 or 1,000 mg/kg of supplemental Cu, but hypothesized that the effect was due to a reduced feed intake rather than by Cu effect. Pesti and Bakalli (1996) observed a decrease ($P < 0.05$) in cholesterol in breast meat in broilers at 21 or 42 d of age fed diets supplemented with 125 or 250 mg/kg compared with control-fed birds. Conversely, Lim et al. (2006) reported no differences ($P > 0.05$) in breast meat cholesterol between broilers fed 100 mg/kg of supplemental Cu compared with control-fed birds. Limited information is available on the mode of action of Cu affecting lipid digestion of broilers. Luo and Dove (1996) reported that weanling pigs fed diets supplemented with 250 mg/kg of Cu increased lipase ($P < 0.01$) and phospholipase A ($P < 0.05$) concentrations in the small intestine.

Interactions between AA density and Cu concentration have been reported on AA digestibility (Rochel et al., 2017), and interactions approaching significance have been observed for growth performance and processing characteristics (Wang et al., 2014). Wang et al. (2014) fed broilers diets containing either 5 or 200 mg/kg of Cu and either high, moderate, or low AA density (1.20, 1.10, or 1.00% digestible Lys in the starter period). No significant interaction effects ($P > 0.05$) were observed; however, the interaction of AA density and Cu concentration approached significance for BW gain ($P = 0.081$), abdominal fat percentage ($P = 0.100$), and carcass yield ($P = 0.066$). Rochell et al. (2017) performed a similarly designed study evaluating apparent ileal AA digestibility of broilers fed diets formulated with high or low AA density diets (1.20 or 1.20% dig Lys) and either 0 or 116 mg/kg of supplemental Cu, with the additional factor of a coccidiosis challenge. Interactions ($P < 0.05$) between AA density and Cu concentration were observed for Leu, Lys, Phe, Thr, Val, Ala, Asp, Glu, Ser, and Tyr. The general pattern observed was that Cu inclusion elicited an increase of 1.8% apparent ileal AA digestibility in birds fed low AA diets, and an equal decrease in digestibility for birds fed high AA diets. These authors hypothesized that this increase in AA digestibility could be due to the biologically elevated feed intake of broilers fed the low AA diets with 116 mg/kg of Cu compared with those provided with no supplemental Cu ($P > 0.05$; 715 vs 659 g) allowing for enhanced development and function of the gastrointestinal tract.

### *Other hypotheses of the mode of action of pharmacological copper*

Another hypothesis on the mode of action of Cu on growth promotion may be through increasing protein synthesis. Metallothionein (**MT**) is a Cys rich, low molecular weight protein that can bind 6 to 12 g Cu per mole (Nielson and Winge, 1983; Bremner and Beattie, 1990). It can be induced by many substances but is often through high contents of heavy metals, such as Cu (Wlostowski, 1993). Wlostowski (1993) hypothesized that MT causes cell proliferation by eliciting an increase of Cu in the nuclei, which is similar to that of rapidly growing tissues, particularly of the $G_1$ /S stage of the cell cycle. Nishimura et al. (1989) observed a much higher incidence of MT in rapidly differentiating cells such as neonatal rat cells and in the livers of rats recovering from hepatectomies than in normal adult tissues. Tsujikawa et al. (1991) suggested that the location of MT in the cell influences cell proliferation. Additionally, there is some evidence that feeding high concentrations of dietary Cu may increase serum mitogenic activity (Zhou et al., 1994; Apgar et al., 1995) or growth hormone mRNA concentration (Zhou et al., 1994) in pigs. Apgar et al. (1995) observed a liner effect ($P < 0.05$) where feeding supplemental Cu from either $CuSO_4$ or Cu-Lys at concentrations of 0, 100, 150, or 200 mg/kg increased serum mitogenic activity, indicating that Cu from either source may increase the circulating mitogenic compounds in pigs from 31 to 66 d of age. Zhou et al. (1994) reported similar responses of serum mitogenic activity in pigs in response to feeding high concentrations of Cu but also reported an increase ($P < 0.05$) of approximately 140% in growth hormone mRNA concentration of pigs fed diets containing 215 mg/kg of supplemental Cu compared with control-fed pigs, but this effect could have been due to the elevated feed intake of the pigs fed diets containing high concentrations of Cu.

Copper also plays a role in immune function; therefore, aiding in immunity by maintaining the gastrointestinal tract or as a component of acute phase proteins could be another mechanism of promoting growth (Klasing and Johnstone, 1991; Koh et al., 1996; Koutsos and Klasing, 2008). Copper directly influences gastrointestinal health through its bacteriostatic and anti-parasitic properties, as well as through altering the microflora and gastrointestinal morphology (Klasing, 2006). In addition, Cu influences the immune system of the bird through its role in acute phase proteins, and Cu is diverted from other processes to meet these needs when the bird is challenged (Koutsos and Klasing, 2008). Klasing and Johnstone (1991) reported that monokine release in response to immune challenges redirect Cu as well as other trace minerals to acute phase proteins. These proteins include ceruloplasmin and superoxide dismutase, which protect cells from damage by free radicals, correlated with higher plasma Cu and ceruloplasmin concentrations in birds experiencing an immune challenge compared with control-fed broilers (Koh et al., 1996).

## AMINO ACID DIGESTIBILITY

### ***Amino Acid Digestibility Assays***

The standardized ileal digestibility assay is a bioassay to assess digestibility of AA. Growing broilers are fed diets that contain an indigestible marker *ad libitum* and digesta is collected from the terminal end of the ileum. The digesta is analyzed using the marker to calculate the concentration of AA. Digesta and feed are analyzed for AA content using HPLC, and the concentration from the titanium dioxide assay is used to calculate the percentage of indigestible AA (Short et al., 1996). This method must be corrected for endogenous losses, calculated by feeding broilers a nitrogen-free diet or a diet containing a highly digestible protein, such as casein, and analyzing the AA content of the excreta (Adedokun et al., 2008). One disadvantage of the standardized ileal digestibility method is that it must be corrected for endogenous losses (Lemme et al., 2004). Endogenous losses are AA from digestive secretions, mucoproteins, intestinal sloughing, albumin, or amides that enter the gastrointestinal tract (Adedokun et al., 2011). Lemme et al. (2004) reported that if the calculated digestibility coefficient is not adjusted for endogenous losses, the assay is referred to as apparent ileal AA digestibility (AIAAD).

### ***Factors affecting amino acid digestibility***

Amino Acid digestibility is influenced by many factors including age, AA density, and feed ingredients (Cowieson and Ravindran, 2008; Huang et al., 2005). Huang et al. (2005) found that age affects ($P < 0.05$) AA digestibility, but the direction and magnitude of the effect is dependent on the AA in question and feed ingredients. Another factor that may influence AA digestibility is AA density of the diet: Cowieson and Ravindran (2008) reported that Asp, Ser, and Glu were more digestible ($P < 0.05$) when broilers were fed a higher AA density diet, while Leu was more highly digestible ($P = 0.05$) when birds were fed a lower AA density diet. One reason cited for that effect is that because of the role of AA in digestive enzymes such as pepsin, which is rich in aspartic acid, higher AA density may lead to higher protein digestion and AA absorption. Additionally, diets higher in AA typically have higher fat inclusion, which slows the rate of passage leading to enhanced digestion (Mateos et al., 1982). Another aspect by Cu may interact with AA digestibility is through regulating the intestinal microflora, which could increase AA availability to the broiler through reducing the microbial use of AA (Apajalahti and Vienola, 2016). This increase in AA digestibility could also be caused by a change in digestive enzyme activity or intestinal morphology. Xia et al. (2004) hypothesized that a change in the intestinal microflora could alter digestive enzyme expression, but no differences ($P > 0.05$) in the protease, amylase, or lipase concentrations in the pancreas or small intestine, or in intestinal morphology measurements were observed with the supplementation of 37 mg/kg of Cu. Similarly, Hedemann et al. (2006) reported no differences ($P > 0.24$) in the activity of amylase, caboxypeptidase A or B, chymotrypsin, trypsin, lipase, or carboxylester hydrolase in either the pancreas or small intestine of pigs fed either 0 or 175 mg/kg of supplemental Cu.

## AMINO ACIDS FOR PROTEIN SYNTHESIS

Amino Acids are the building blocks of protein, and a central role for AA is tissue development (Leeson and Summers, 2001). However, AA are also essential for cell signaling and gene expression, regulating food intake, the synthesis of hormones and nitrogenous substances, reproduction, immune function, cell growth and differentiation, and maintaining intestinal integrity (Wu, 2013). Amino acids are first used to maintain the intestine, and then used for other functions (Wijtten et al., 2010; Moran, 2016).

One of the main functions of AA is to serve building blocks of protein synthesis for muscle accretion. Protein accretion is the excess of protein synthesis over protein degradation (Leeson and Summers, 2001) This is due to the constant synthesis and breakdown of proteins, known as protein turnover (Bergen, 2008). Prenatal muscle synthesis is primarily through hyperplasia, an increase in the number of myocytes, while postnatal muscle growth is primarily through hypertrophy, an increase in the size of the myocytes (Rehfeldt et al., 2000). The growth hormone (**GH**) axis is key in stimulating both hyperplasia and hypertrophy (Florini et al., 1996). Growth hormone releasing hormone is released from the hypothalamus to the anterior pituitary, which stimulates the release of GH to the liver. Growth hormone has direct effects on metabolism, but primarily affects muscle growth through indirect effects mediated by insulin-like growth factor (**IGF**)-1 during the postnatal period and IGF-2 in the prenatal period, both of which are secreted from the liver and work similarly to promote muscle accretion by stimulating AA uptake and increasing protein synthesis (Florini et al., 1996). Previous research has established that IGF-1 concentrations are related to protein synthesis in broilers (Tomas et al., 1991). Additionally, both GH and IGF can cause an increase in muscle growth through increasing the activity of satellite cells (Halevy et al., 1995). Satellite cells can proliferate and differentiate into muscle fibers, so these cells play a central role in muscle accretion (Doumit et al., 1993).

Beyond providing sufficient AA available for protein synthesis, AA density influences muscle accretion by affecting IGF concentrations and satellite cell activity (Tesseraud et al., 1996; Rosebrough et al., 1996; Liao et al., 2015). Rosenbrough et al. (1996) reported that increasing the dietary crude protein in broiler feeds increased the concentration of IGF-I. More specifically, Lys concentration has been shown to have direct effects on the rate of protein synthesis (Tesseraud et al., 1996). This may be mediated through the direct effects of increasing Lys concentration eliciting increases in IGF-1 concentration (Liao et al., 2015). Additionally, Lys has been reported to have a more pronounced response with breast muscle accretion than other AA because Lys represents approximately a greater proportion relative to other individual AA in breast meat (Munks et al., 1945).

Because of these impacts on muscle accretion, increasing dietary AA density has been shown to improve growth performance of broilers by increasing BW gain while decreasing feed conversion ratio (Kidd et al., 2004; Kidd et al., 2005; Dozier et al., 2007). Maintaining a sufficient AA density is particularly important in modern broilers with improved feed conversion ratios, as they consume less feed while maintaining optimum growth (Dozier et al., 2008). Furthermore, dietary AA density can increase processing weights and yields of carcasses, breasts, and total breast meat (Kidd et al., 2004; Kidd et al., 2005; Dozier et al., 2007). Increased dietary AA density also improves uniformity within flocks (Corzo et al., 2004).

## KNOWLEDGE GAPS IN THE LITERATURE

There are several knowledge gaps in the feeding of high concentrations of dietary Cu to broilers. The optimum feeding strategy of Cu is unknown. Karimi et al. (2011) evaluated the use of 125 or 250 mg/kg of Cu during the starter period, but the majority of the literature utilizes treatments consisting of 1 concentration of Cu during the entire grow-out. Published literature is sparse on increasing or decreasing the Cu concentration during the entire production period. Despite this lack of scientific evidence, the industry typically utilizes a higher concentration of Cu during the starter period and reduces the concentration in subsequent growth periods to reduce cost while maintaining optimum growth performance. Hence, further research evaluating the effects of either increasing or decreasing pharmacological Cu concentrations on growth performance of broilers raised to different ages is warranted. A better understanding of the mode of action could provide understanding to optimize dietary Cu specifications.

Although many studies have evaluated possible modes of action of Cu, no consensus on the mechanism has been elucidated. Research is warranted to determine if Cu acts through regulating the microflora of the gastrointestinal tract, increasing digestibility of nutrients, altering protein synthesis, or through another mechanism. Additional work evaluating the effects of Cu on intestinal microflora could provide added insight into this mode of action. Another area deserving further research is the effects of Cu on AA digestibility. Previous research has indicated that high Cu inclusion may increase the digestibility of AA (Rochell et al., 2017), but data on this are sparse. Additionally, the mechanism by which Cu may increase AA digestibility is unknown. The impact of Cu enhancing AA digestibility may lead to improved growth performance and meat yield.

Information is sparse on the effects of Cu on birds grown > 3.0 kg. Ewing et al. (1998) reported on the effects of Cu on broilers at 56 d of age; however, this study was conducted in 1998 and the 56 d BW ranged from 3.2 to 3.5 kg, much lower than the expected weight of the modern broiler. Data are limited evaluating the effects of broilers raised to approximately 4.0 kg. Future research should determine the optimum concentrations of Cu during the final periods of growth of these broilers grown to 4.0 kg. In addition, research addressing the effects of Cu on processing characteristics of broilers is sparse. Published research is limited on the effects of Cu on yields of carcasses and parts of broilers raised to different target weights. These data would be particularly valuable for broilers raised to weights of over 3 kg as these broilers are raised to target the increased breast meat yield for the further processing market. Research addressing these knowledge gaps will aid in elucidating optimum Cu feeding strategies for broilers raised without the use of sub-therapeutic antibiotics.

# REFERENCES

Adedokun, S. A., O. Adeola, C. M. Parsons, Lilburn, and T. J. Applegate. 2008. Standardized ileal amino acid digestibility of plant feedstuffs in broiler chickens and turkey poults using a nitrogen-free or casein diet. Poult. Sci. 87:2535-2548.

Adedokun, S. A., O. Adeola, C. M. Parsons, M. S. Lilburn, and T. J. Applegate. 2011. Factors affecting endogenous amino acid flow in chickens and the need for consistency in methodology. Poult. Sci. 90:1737-1748.

Aoyagi, S. and D. H. Baker. 1993a. Nutritional evaluation of copper-lysine and zinc-lysine complexes for chicks. Poult. Sci. 72:165-171.

Aoyagi, S. and D. H. Baker, 1993b. Nutritional evaluation of a copper-methionine complex for chicks. Poult. Sci. 72: 2309-2315.

Aoyagi, S. and D. H. Baker, 1995. Effect of high copper dosing on hemicellulose digestibility in cecectomized cockerels. Poult. Sci. 74:208-211.

Apajalahti, J., and K. Vienola. 2016. Interaction between chicken intestinal microbiota and protein digestion. Anim. Feed Sci. Technol. 221:323-330.

Apgar, G. A., E. T. Kornegay, M. D. Lindemann, and D. R. Notter. 1995. Evaluation of copper sulfate and a copper lysine complex as growth promoters for weanling swine. J. Anim. Sci. 73:2640-2646.

Arias, V. J. and E. A. Koutsos. 2006. Effects of copper source and level on intestinal physiology and growth of broiler chickens. Poult. Sci. 85:999-1007.

Arredondo, M. and M. T. Núñez. 2005. Iron and copper metabolism. Molecular Aspects of Medicine. 26:313-327.

Attieh, Z. K., C. K. Mukhopadhyay, V. Seshadri, N. A. Tripoulas, and P. L. Fox. 1999. Ceruloplasmin ferroxidase activity stimulates cellular iron uptake by a trivalent cation-specific transport mechanism. J. Biol. Chem. 274:1116-1123.

Aydin, A., A. Y. Pekel, G. Issa, G. Demirel, and P. H. Patterson. 2010. Effects of dietary copper, citric acid, and microbial phytase on digesta pH and ileal and carcass microbiota of broiler chickens fed a low available phosphorus diet. J. Appl. Poult. Res. 19:422-431.

Barber, R. S., R. Braude, and K. G. Mitchell. 1955. Antibiotic and copper supplements for fattening pigs. Br. J. Nutr. 9:378-381.

Barber, R. S., R. Braude, K. G. Mitchell, J. A. Rook, and J. G. Rowell. 1957. Further studies on antibiotic and copper supplements for fattening pigs. Br. J. Nutr. 11:70-79.

Bergen, W.G. 2008. Measuring in vivo intracellular protein degradation rates in animal systems. J. Anim. Sci. 86:E3-E12.

Bremner, I. and J. H. Beattie. 1990. Metallothionein and the trace minerals. Annu. Rev. Nutr. 10:63-83.

Bunch, R. J., V. C. Speer, V. W. Hays, J. H. Hawbaker and D. V. Catron. 1961. Effects of copper sulfate, copper oxide and chlortetracycline on baby pig performance. J. Anim. Sci. 20:723-726.

Chowdhury, S. D., I. K. Paik, H. Namkung, and H. S. Lim. 2004. Responses of broiler chickens to organic copper fed in the form of copper-methionine chelate. Anim. Feed Sci. Technol. 115:281-293.

Christodoulopoulos, G. and N. Roubies. 2007. Diagnosis and treatment of copper poisoning caused by accidental feeding on poultry litter in a sheep flock. Aust. Vet. J. 85:451-453.

Corzo, A., C. D. McDaniel, M. T. Kidd, E. R. Miller, B. B. Boren, and B. I. Fancher. 2004. Impact of dietary amino acid concentration on growth, carcass yield, and uniformity of broilers. Aus. J. Agri. Res. 55:1133-1138.

Cowieson, A. J. and V. Ravindran. 2008. Effect of exogenous enzymes in maize-based diets varying in nutrient density for young broilers: Growth performance and digestibility of energy, minerals and amino acids. Br. Poult. Sci. 49:37-44.

Curtis, M. J. and E. J. Butler. 1980. Response to ceruloplasmin to Escherichia coli endotoxins and adrenal hormones in the domestic fowl. Res. Vet. Sci. 28:217-222.

Doumit, M. E., D. R. Cook, and R. A. Merkel. 1993. Fibroblast growth factor, epidermal growth factor, insulin-like growth factors, and platelet-derived growth factor-BB stimulate proliferation of clonally derived porcine myogenic satellite cells. J. Cell. Phys. 157:326-332.

Dozier, W. A., III, A. J. Davis, M. E. Freeman, and T. L. Ward. 2003. Early growth and environmental implications of dietary zinc and copper concentrations and sources of broiler chicks. Br. J. Poult. Sci. 44:726-731.

Dozier, W. A., A. Corzo, M. T. Kidd, and S. L. Branton. 2007. Dietary apparent metabolizable energy and amino acid density effects on growth and carcass traits of heavy broilers. J. Appl. Poult. Res. 16:192-205.

Dozier, W. A., A. Corzo, M. T. Kidd, and M. W. Schilling. 2008. Dietary digestible lysine requirements of male and female broilers from forty-nine to sixty-three days of age. Poult. Sci. 87:1385-1391.

European Food Safety Authority (EFSA). 2011. Scientific Opinion on safety and efficacy of di copper chloride tri hydroxide (tribasic copper chloride, TBCC) as feed additive for all species. EFSA J. 9:2355.

Ewing, H. P., G. M. Pesti, R. I. Bakalli, and J. F. M. Menten. 1998. Studies on the feeding of cupric sulfate pentahydrate, cupric citrate and copper oxychloride to broiler chickens. Poult. Sci. 77:445-448.

Fisher, C., A. P. Laursen-Jones, K. J. Hill, and W. S. Hardy. 1973. The effect of copper sulphate on performance and the structure of the gizzard in broilers. Br. Poult. Sci. 14:55-68.

Florini, J. R., D. Z. Ewton, and S. A. Coolican. 1996. Growth hormone and the insulin-like growth factor system in myogenesis. Endocrinol. Rev. 17:481-517.

Halevy, O., V. Hodek, and A. Mett. 1995. The effects of growth hormone on avian skeletal muscle satellite cell proliferation and differentiation. Gen. Comp. Endocrinol. 101:43-52.

Hamdi, M., D. Sola, R. Franco, S. Durosoyc, A Roméo, and J. F. Perez. 2018. Including copper sulphate or dicopper oxide in the diet of broiler chickens affects performance and copper content in the liver. Anim. Feed Sci. Technol. 237:89-97.

Hart, E. B., H. Steenbock, J. Waddell, and C. A. Elvehjem. 1928. Iron in nutrition VII. Copper as a supplement to iron for hemoglobin building in the rat. J. Biol. Chem. 77:797-833.

Hedemann, M. S., B. B. Jensen, and H. D. Poulsen. 2006. Influence of dietary zinc and copper on digestive enzyme activity and intestinal morphology in weaned pigs. J. Anim. Sci. 84:3310-3320.

Hill, G. M. and J. E. Link. 2009. Transporters in the absorption and utilization of zinc and copper. J. Anim. Sci. 87:E85-9.

Huang, K. H., V. Ravindran, X. Li, and W. L. Bryden. 2005. Influence of age on the apparent ileal amino acid digestibility of feed ingredients for broiler chickens. Br. Poult. Sci. 46:236-245.

Jenkins, N. K., T. R. Morris and D. Valamotis, 1970. The effect of diet and copper supplementation on chick growth. Br. Poult. Sci. 11:241-248.

Karimi, A., G. H. Sadeghi, and A. Vaziry. 2011. The effect of copper in excess of the requirement during the starter period on subsequent performance of broiler chicks. J. Appl. Poult. Res. 20:203-209.

Kidd, M. T., C. D. McDaniel, S. L. Branton, E. R. Miller, B. B. Boren, and B. I. Fancher. 2004. Increasing amino acid density improves live performance and carcass yields of commercial broilers. J. Appl. Poult. Res. 13:593-604.

Kidd, M. T., A. Corzo, D. Hoehler, E. R. Miller, and W. A. Dozier. 2005. Broiler responsiveness (Ross x 708) to diets varying in amino acid density. Poult. Sci. 84:1389-1396.

Kim, G. B., Y. M. Seo, K. S. Shin, A. R. Rhee, J. Han, and I. K. Paik. 2011. Effects of supplemental copper-methionine chelate and copper-soy proteinate on the performance, blood parameters, liver mineral content, and intestinal microflora of broiler chickens. J. Appl. Poult. Res. 20:21-32.

Klasing, K. C. 1998. Minerals. Pages 234-276 in Comparative Avian Nutrition. CAB International, New York, USA.

Klasing, K. C. 2006. Micronutrient supply: influence on gut health and immunity. Pages 210-223 in Avian Gut Function in Health and Disease. G.C. Perry, ed. CAP International, Wallingford, Oxfordshire, United Kingdom.

Klasing, K. C. and B. J. Johnstone. 1991. Monokines in growth and development. Poult. Sci. 70:1781-1789.

Koh, T. S., R. K. Peng, and K. C. Klasing. 1996. Dietary copper level affects copper metabolism during lipopolysaccharide-induced immunological stress in chicks. Poult. Sci. 75:867-872.

Koutsos, E. A. and K. C. Klasing. 2008. Factors Modulating the avian immune system. Avian Immunology. 1:323-338.

Leeson, S. 2009. Copper metabolism and dietary needs. World’s Poult. Sci. J. 65:353-366.

Leeson, S. and J. D. Summers. 2001. Nutrition of the Chicken. 4th ed. Pages 394-397. University Books, Guelph, Ontario, Canada.

Lemme, A., V. Ravindran, and W. L. Bryden. 2004. Ileal digestibility of amino acids in feed ingredients for broilers. World's Poult. Sci. J. 60:423-438.

Liao, S. F., T. Wang, and N. Regmi. 2015. Lysine nutrition in swine and the related monogastric animals: muscle protein biosynthesis and beyond. SpringerPlus. 4:1-12.

Lim, H. S., I. K. Paik, T. I. Sohn and W. Y. Kim. 2006. Effects of supplementary copper chelates in the form of methionine, chitosan and yeast on the performance of broilers. Asian-Australas. J. Anim. Sci. 19:1322-1327.

Linder, M. C. and M. Hazegh-Azam. 1996. Copper biochemistry and molecular biology. Am. J. Clin. Nutr. 63:797S-811.

Lu, L., R. L. Wang, Z. J. Zhang, F. A. Steward, X. Luo, and B. Liu. 2010. Effect of dietary supplementation with copper sulfate or tribasic copper chloride on the growth performance, liver copper concentrations of broilers fed in floor pens, and stabilities of vitamin E and phytase in feeds. Biol. Trace Elem. Res. 138:181-189.

Luo, X. G., F. Ji, Y. X. Lin, F. A. Steward, L. Lu, B. Liu, and S. X. Yu. 2005. Effects of dietary supplementation with copper sulfate or tribasic copper chloride on broiler performance, relative copper bioavailability, and oxidation stability of vitamin E in feed. Poult. Sci. 84:888-893.

Luo, X. G. and C. R. Dove. 1996. Effect of dietary copper and fat on nutrient utilization, digestive enzyme activities, and tissue mineral levels in weanling pigs. J. Anim. Sci. 74:1888-1896.

Mateos, G. G., J. L. Sell, and J. A. Eastwood. 1982. Rate of food passage (transit time) as influenced by level of supplemental fat. Poult. Sci. 61:94-100.

McNaughton, J. L. and E. J. Day. 1979. Effect of dietary Fe to Cu ratios on hematological and growth responses of broiler chickens. J. Nutrition. 109:559-564.

McNaughton, J. L., E. J. Day, and B. C. Dilworth. 1974. Iron and copper availability from various sources. Poult. Sci. 53:1325-1330.

Miles, R. D., S. F. O'Keefe, P. R. Henry, C. B. Ammerman, and X. G. Luo. 1998. The effect of dietary supplementation with copper sulfate or tribasic copper chloride on broiler performance, relative copper bioavailability, and dietary prooxidant activity. Poult. Sci. 77:416-425.

Moran, Jr., E. T. 2016. Gastric digestion of protein through pancreozyme action optimizes intestinal forms for absorption, mucin formation and villus integrity. Anim. Feed Sci. Technol. 221:284-303.

Munks, B., A. Robinson, E. F. Beach, and H. H. Williams. 1945. Amino acids in the production of chicken egg and muscle. Poult. Sci. 24:459-464.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th Rev. Ed. National Academy Press, Washington, DC.

National Research Council. 2012. Nutrient Requirements of Swine: 11th Rev. Ed. National Academy Press, Washington, DC.

Nielson, K. B. and D. R. Winge. 1983. Order of metal binding in metallothionein. J. Biol. Chem. 258:13063-13069.

Nishimura, H., N. Nishimura, and C. Tohyama. 1989. Immunohistochemical localization of metallothionein in developing rat tissues. Journal of Histochemistry and Cytochemistry. 37:715-722.

Pang, Y., J. A. Patterson, and T. J. Applegate. 2009. The influence of copper concentration and source on ileal microbiota. Poult. Sci. 88:586-592.

Pang, Y. and T. J. Applegate. 2006. Effects of dietary copper supplementation and copper source on digesta pH, calcium, zinc, and copper complex size in the gastrointestinal tract of the broiler chicken. Poult. Sci. 86:531-537.

Pekel, A. Y., P. H. Patterson, R. M. Hulet, N. Acar, T. L. Cravener, D. B. Dowler, and J. M. Hunter. 2009. Dietary Camelina meal versus flaxseed with and without supplemental copper for broiler chickens: Live performance and processing yield. Poult. Sci. 88:2392-2398.

Persson, H., M. Türk, M. Nyman, and A. Sandberg. 1998. Binding of Cu2+, Zn2+, and Cd2+ to inositol tri-, tetra-, penta-, and hexaphosphates. J. Agric. Food Chem. 46:3194-3200.

Pesti, G. M. and R. I. Bakalli. 1996. Studies on the feeding of cupric sulfate pentahydrate and cupric citrate to broiler chickens. Poult. Sci. 75:1086-1091.

Poupoulis, C. and L. S. Jensen. 1976. Effect of high dietary copper on gizzard integrity of the chick. Poult. Sci. 55:113-121.

Rehfeldt, C., I. Fielder, G. Dietl, and K. Ender. 2000. Myogenesis and postnatal skeletal muscle cell growth as influenced by selection. Livestock Production Science. 66:177-188.

Rochell, S. J., J. L Usry, T. M. Parr, C. M Parsons, and R. N. Dilger. 2017. Effects of dietary copper and amino acid density on growth performance, apparent metabolizable energy, and nutrient digestibility in Eimeria acerviulina-challenged broilers. Poult Sci. 96:602-610.

Roeser, H. P., G. R. Lee, S. Nacht, and G. E. Cartwright. 1970. The role of ceruloplasmin in iron metabolism. J. Clin. Invest. 49:2408-2417.

Rosebrough, R. W., A. D. Mitchell, and J. P. McMurtry. 1996. Dietary crude protein changes rapidly alter metabolism and plasma insulin-like growth factor I concentrations in broiler chickens. J. Nutr. 126:2888-2898.

Rosenblum, C. I. and R. M. Leach. 1985. Biliary copper excretion in the chicken. Bio. Trace Elem. Res. 8:47-63.

Schroeder, H. A., A. P. Nason, I. H Tipton, and J. J. Balassa. 1966. Essential trace metals in man: copper. J. Chron. Dis. 19:1007-1034.

Short, F. J., P. Gorton, J. Wiseman, J., and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Tech. 59:215-221.

Skrivan, M., V. Skrivanova, M. Marounek, E. Tumova, and J. Wolf. 2000. Influence of dietary fat source and copper supplementation on broiler performance, fatty acid profile of meat and depot fat, and on cholesterol content in meat. Br. J. Poult. Sci. 41:608-614.

Tesseraud, S., R. Peresson, J. Lopes, and A. M. Chagneau. 1996. Dietary lysine deficiency greatly affects muscle and liver protein turnover in growing chickens. Br. J. Nutr. 75:853-865.

Tomas, F. M., R. A. Pym, and R. J. Johnson. 1991. Muscle protein turnover in chickens selected for increased growth rate, food consumption, or efficiency of food utilization: effects of genotype and relationship to plasma IGF-I and growth hormone. Br. Poult Sci. 32:363-376.

Tsujikawa, K., T. Imai, M. Kakutani, Y. Kayamori, T. Mimura, N. Otaki, M. Kimura, R. Fukuyama, and N. Shimizu. 1991. Localization of metallothionein in nuclei of growing primary cultured adult rat hepatocytes. FEBS Lett. 283:239-242.

Turk, D. E. 1986. Microelements in the circulation of coccidiosis-infected chicks. Poult. Sci. 65:2098-2103.

Wang, H., C. Zhang, Y. Mi, and M. T. Kidd. 2014. Copper and lysine amino acid density responses in commercial broilers. J. Appl. Poult. Res. 23:470-477.

Wijtten, P. J. A., E. Hangoor, J. Sparla, and M. W. A. Verstegen. 2010. Dietary amino acid levels and feed restriction affect small intestinal development, mortality, and weight gain of male broilers. Poult. Sci. 89:1424-1439.

Wlostowski, T. 1993. Involvement of metallothionein and copper in cell proliferation. BioMetals. 6:71-76.

Wu, G. 2013. Amino Acids: Biochemistry and Nutrition. CRC Press. Boca Raton, FL.

Xia, M. S., C. H. Hu, and Z. R. Xu. 2004. Effects of copper-bearing montmorillonite on growth performance, digestive enzyme activities, and intestinal microflora and morphology of male broilers. Poult. Sci. 83:1868-1875.

Yuan, L., M. Wang, X. Zhang, and Z. Wang. 2017. Effects of protease and non-starch polysaccharide enzyme on performance, digestive function, activity, and gene expression of endogenous enzyme of broilers. PLoS ONE 12:e0173941.

Zhou, W., E. T. Kornegay, H. Van Laar, J. W. G. M. Swinkels, E. A. Wong, and M. D. Lindemann. 1994. The role of feed consumption and feed efficiency in copper-stimulated growth. J. Anim. Sci. 72:2385-2394.

# III. GROWTH PERFORMANCE, CARCASS CHARACTERISTICS, ILEAL MICROBIOTA, AND AMINO ACID DIGESTIBILITY OF BROILERS FED DIETS VARYING IN SUPPLEMENTAL COPPER CONCENTRATIONS AND AMINO ACID DENSITY FROM 1 TO 32 DAYS OF AGE

## ABSTRACT

Two experiments were conducted simultaneously to determine interactive effects of dietary amino acid (**AA**) density and supplemental Cu concentrations on growth performance, carcass characteristics, intestinal microflora, and AA digestibility of broilers. In experiment 1, Yield Plus × Ross 708 male chicks were distributed to 64 floor pens (25 birds/pen) and provided 8 dietary treatments. Dietary treatments consisted of a factorial arrangement of 2 AA densities (moderate or low) and 3 Cu programs with positive and negative controls. Supplemental Cu was provided at concentrations of 135-135-135, 270-135-135, or 270-270-135 mg/kg from tribasic Cu chloride in the starter, grower, and finisher periods, respectively. Control birds were fed moderate AA diets with approximately 13 mg/kg Cu. Positive control broilers were unvaccinated and received diets containing diclazuril, whereas all other birds received Coccivac® B52 at hatch. Birds and feed were weighed at 1, 12, 25, and 32 d of age. At 33 d, 10 birds/pen were processed and deboned. At 35 d of age, ileal mucosa was collected for microbial sequencing. No interactive effects were observed for cumulative growth performance variables ($P > 0.05$). Birds fed moderate AA, 270-270-135 mg/kg Cu diets had lower cumulative feed intake and feed conversion ($P \leq 0.04$) than those fed moderate AA, 135-135-135 mg/kg Cu diets. Broilers fed moderate AA diets containing the 270-270-135 or 270-135-135 mg/kg Cu also had lower cumulative feed intake and feed conversion than negative control-fed birds ($P \leq 0.025$). There were no differences in breast meat weight or microflora composition based on Cu program ($P > 0.05$). In experiment 2, YP × Ross 708 male chicks were distributed to 48 floor pens (14 birds/pen) and were provided 6 dietary treatments containing $TiO_2$ as an indigestible marker. At 14 d of age, ileal digesta was collected. Cysteine digestibility was decreased for broilers fed supplemental Cu compared with negative control-fed birds ($P < 0.004$), while interactions were observed between AA density and Cu concentration ($P \leq 0.050$) for Lys, Val, Trp, His, and Arg. These results indicate that feeding high concentrations of Cu may enhance growth performance but did not improve AA digestibility or alter microflora.

## INTRODUCTION

In the United States, broiler production has utilized sub-therapeutic antibiotics as growth promotors, but the increasing demand for meat products from broilers raised without antibiotics is stimulating interest in identifying alternatives for enhancing broiler performance. High concentrations of dietary Cu may elicit growth promoting benefits to broilers fed antibiotic-free diets by altering gastrointestinal tract microbiota, or through increasing digestibility of amino acids (**AA**). Previous research has shown that supplementation of broiler diets with high concentration of Cu resulted in reductions of ileal and cecal *Clostridia* and *Escherichia coli* compared with control-fed broilers (Xia et al., 2004; Pang et al., 2009). Additionally, interactions between dietary AA density and supplemental Cu concentrations enhanced growth performance, processing characteristics, and ileal AA digestibility, with broilers fed low AA density diets benefiting more from the increased Cu concentration than those fed moderate AA density diets (Wang et al., 2014; Rochell et al., 2017). Changes in the microbiome or in AA digestibility may lead to increased BW gain (**BWG**) and decreased feed conversion ratio (**FCR**), as observed in broilers fed 125 mg/kg of supplemental Cu compared with control-fed broilers (Ewing et al., 1998).

Research is sparse on effects of varying Cu concentrations and dietary AA density on AA digestibility, growth performance, carcass characteristics, and intestinal microbiota. Furthermore, the optimum duration of increasing dietary Cu has not been well defined. The objectives of this study were to evaluate the interactive effects of supplemental Cu concentration and dietary AA density on growth performance, processing characteristics, AA digestibility, or ileal microbiota composition.

## MATERIALS AND METHODS

All procedures involving live birds were approved by Auburn University Institutional Animal Care and Use Committee (PRN 2018-3219).

### ***Bird Husbandry***

Experiments 1 and 2 were conducted simultaneously utilizing the same source of chicks and feed ingredients. Yield Plus × Ross 708 male chicks (Aviagen North America, Huntsville, AL) were procured from a commercial hatchery at day of hatch. All chicks were vaccinated against Marek’s disease, infectious bronchitis, and Newcastle disease. Additionally, all chicks except for the positive control (**PC**) treatments were also spray vaccinated against coccidiosis using a 1× dose of Coccivac B52® (Merck Animal Health, Summit, NJ). Broilers were vaccinated against coccidiosis as a mild challenge to be representative of commercial practice. Birds were housed in a solid-sided cross-ventilated house equipped with vent boards, exhaust fans, stir fans, evaporative cooling pads, forced-air heaters, and an electronic controller (Evolution 3000, Hired Hand Manufacturing, Inc., Bremen, AL). Each pen contained litter, nipple drinkers (5 nipples/pen), and a hanging pan feeder. Feed and water were available *ad libitum.* In experiment 1, 1,600 chicks were randomly distributed into 64 floor pens (25 birds per pen; 0.08 $m^2$ per bird). In experiment 2, 672 chicks were randomly distributed into 48 floor pens (14 birds per pen; 0.14 $m^2$ per bird). Ambient temperature was set at 33°C at placement in both experiments, and gradually decreased to final set points of 20°C and 29°C in experiments 1 and 2, respectively. Light intensity was 30 lux at placement with a photoperiod of 23:1 light:dark schedule, and changed to 5 lux and 20:4 light:dark schedule at 7 d of age in both experiments. In experiment 1, birds and feed were weighed at 1, 12, 25, and 32 d of age to determine BWG, feed intake (**FI**), and FCR. In experiment 2, birds and feed were weighed at 1 and 14 d of age. In both experiments incidence of mortality was recorded daily. Starter diets were provided as crumbles, and subsequent diets presented as whole pellets.

### *Dietary Treatments*

Experiment 1 consisted of 8 dietary treatments, arranged as a 3 × 2 factorial arrangement of Cu supplementation programs and dietary AA densities, with PC and negative control (**NC**) (Tables 3.1, 3.2, and 3.3). The 3 Cu programs were created by supplementing Cu (tribasic Cu chloride) at 270 or 135 mg/kg over the different feeding phases. The 270-270-135 supplemented 270 mg/kg of Cu during the starter and grower phases and 135 mg/kg in the finisher phase. The 270-135-135 program supplemented 270 mg/kg of Cu during the starter phase and 135 mg/kg of Cu for subsequent phases. The 135-135-135 program provided 135 mg/kg of supplemental Cu during all phases. The supplementation for the Cu programs is in addition to the Cu provided in the trace mineral premix and in feed ingredients for total Cu concentrations of 149 and 284 mg/kg in the starter phase, 148 or 283 mg/kg in the grower phase, and 148 mg/kg in the finisher phase. The moderate and low dietary AA densities were set at 95 and 88% of the recommended guidelines for Ross 708 broilers (Aviagen, 2014) respectively. This resulted in digestible Lys concentrations of 1.22, 1.09, and 1.01% in the moderate diets and 1.13, 1.01, and 0.93% in the low diets in the starter, grower, and finisher phases. All diets were formulated using Met+Cys, Thr, Val, and Ile ratios relative to Lys of 0.74, 0.67, 0.75, an 0.69, respectively. The PC and NC received moderate AA diets with no supplemental Cu above that in the trace mineral premix and feed ingredients, for dietary Cu concentrations of 14, 13, and 12 mg/kg in the starter, grower, and finisher phases. The PC contained diclazuril at 0.05% during the starter and grower phases.

Experiment 2 consisted of 6 treatments with a 2 × 2 factorial of Cu programs and dietary AA densities, and a PC and NC. The diets were formulated identically to the starter diets for experiment 1, except for the inclusion of 0.5% titanium dioxide at the expense of sand as an indigestible marker for the digestibility assay (Table 3.1). Additionally, the diets were made with ingredients from the same source as those in experiment 1. The birds received the crumble starter diet for the duration of experiment 2.

Diets were analyzed in triplicate at the University of Missouri Agricultural Extension Station Chemical Laboratories. High performance liquid chromatography was used to analyze the AA concentration of the diets (method 982.30 E (a,b,c); AOAC International, 2006), and inductively coupled plasma optical emission spectroscopy was used to analyze the Cu content (method 985.01 A, B, D; AOAC International, 2006).

### *Intestinal Lesion Scores*

In experiment 1, intestinal lesion scoring was performed on 4 birds per pen at 21 d of age. Birds were euthanized via $CO_2$ asphyxiation and the gastrointestinal tract was extracted, opened, and rinsed with water. Lesion scoring was conducted according to the method described by Johnson and Reid (1970) using the regions defined by Conway and McKenzie (1991).

### *Microbial Analysis*

In experiment 1, ileal mucosa scrapings were analyzed for microbiome analysis. At 35 d of age, 4 birds per pen were selected for necropsy and were euthanized using $CO_2$ asphyxiation. The ileum was flushed with sterile PBS, and mucosa was aseptically scraped using slides and stored in microcentrifuge tubes. The mucosa was stored on ice until transported to the lab, where it was pooled by pen and stored in a -80°C freezer until analysis.

The microbiome profile was analyzed using 16s ribosomal ribonucleic acid (**rRNA**) sequencing. Extraction of DNA, PCR amplifications, next generation sequencing, and basic bioinformatics were performed by the DNA Services Facility Research Resources Center at the University of Illinois at Chicago. Extraction was performed according to manufacturer's instructions for the QIAamp PowerFecal DNA kit (Qiagen, Inc., Valencia, CA) to produce genomic DNA for amplification. Proteinase K (Thermo Fisher Scientific, Waltham, MA) was used to lyse the cells, and then the DNA was bound to a membrane that allowed other materials to pass through. Genomic DNA was tested for purity using a Qubit Fluorometer (Thermo Fisher Scientific, Waltham, MA). Stage 1 and 2 PCR performed as described by Greene et al. (2015). Briefly, 28 rounds of stage 1 PCR were performed in order to amplify the V3-V4 section of the 16S rRNA gene, using the 515F/806R universal target primers (Integrative DNA Technologies, Coralville, IA), as described by Caporaso et al. (2010). An aliquot of the product was used in stage 2 PCR, where 8 rounds were performed in order to attach adaptors (barcodes) for sequencing using Fluidigm Access Array™ primers (Fluidigm, South San Francisco, CA). After amplification, yield was assessed using agarose gel electrophoresis. Stage 2 yields were purified using SequalPrep plates (Thermo Fisher Scientific, Waltham, MA), and qPCR was used to quantify the purified amplicons using Qubit quantification (Thermo Fisher Scientific, Waltham, MA). An Illumina Miseq (Illumina, Inc., San Diego, CA) was used to read the samples with 300 read cycles.

Basic bioinformatics were also performed by the DNA Services Facility Research Resources Center. The software program Paired-end read merger (**PEAR**) merged reads, and ambiguous nucleotides were discarded (Zhang et al., 2014). The Smith-Watermann alignment was utilized to identify primer sequences and nucleotides without primer sequences were also discarded. Quality was assessed using a modified Mott algorithm and sequences with fewer than 250 bases were discarded. The program Quantitative Insights into Microbial Ecology (**QIIME**) was used to produce operational taxonomic units clusters using a 97% similarity threshold, and taxonomic summaries with a similarity threshold of 90% (Caporaso et al., 2010; Edgar et al., 2010; McDonald et al., 2012).

### ***Processing***

In experiment 1, 10 birds per pen were individually weighed and selected for processing. All birds selected had a BW within ± 10% of the average of the pen weight. At 32 d of age, feed was withdrawn 12 hours prior to processing. At 33 d of age, birds were placed in coops and transported to the Auburn University Pilot Processing Plant. Broilers were shackled, electrically stunned, exsanguinated, scalded, and manually eviscerated. Carcasses were chilled in an ice bath for 3 hours and then allowed to drip for 3 minutes. Carcasses and fat pads were weighed to determine carcass yield and abdominal fat percentage. Carcasses were stored on ice for 18 hours, and then were deboned to calculate the weights and yields of fillets, tenders, wings, boneless thighs, and drums. Yields were based on 32 d of age live weight.

### ***Digestibility***

In experiment 2, an apparent ileal AA digestibility assay was conducted from 1 to 14 d of age. Digesta was collected from 8 birds per pen at 14 d of age. Birds were allowed access to feed and water until immediately prior to being euthanized by $CO_2$ asphyxiation. The intestine was extracted, and a section from the terminal third of the ileum to 2 cm from the ileo-cecal junction was cut. The section was gently flushed with distilled, deionized water, pooled by pen, and stored on ice until transported to the laboratory. Samples were frozen at -20 C° until analysis. Digesta and diets were lyophilized in a Virtis Genesis Pilot Lyophilizer (SP Industries, Warminster, PA), and then ground in an electric coffee grinder (Hamilton Beach, Glen Allen, VA). The dried digesta was analyzed in duplicate and dried diets were analyzed in quaduplicate for $TiO_2$ concentration using the method described by Short et al. (1996). After allowing sufficient time for color to develop, absorbance was measured on a spectrophotometer (Shimadzu, Kyoto, Japan), using 1.5 mL of solution in a cuvette reader. A standard curve was used to create a regression equation ($R^2$=0.999) to calculate $TiO_2$ concentration. Digesta samples were also analyzed in duplicate for AA profile using HPLC (method 982.30 E (a,b,c); AOAC International, 2006) by the University of Missouri Extension Station Chemical Laboratory. These values were used to calculate apparent ileal AA digestibility using the following formula:

$$Apparent\ Ileal\ Digestibility, (\%) = \left[1 - \left(\frac{Ti_i}{Ti_o}\right) \times \left(\frac{AA_o}{AA_i}\right)\right] \times 100$$

where $Ti_i$ represents the $TiO_2$ concentration in the input (diet), $Ti_o$ represents the $TiO_2$ concentration in the output (digesta), $AA_o$ represents the concentration of the AA in the output, and $AA_i$ represents the concentration of the AA in the input (Dilger et al., 2004).

### ***Statistical Analyses***

All analyses were performed using PROC MIXED procedure of SAS 9.4 (2017). Both experiments were analyzed as a randomized complete block design with pen location as the blocking factor. Pen was considered the experimental unit. Experiment 1 consisted of 2 control treatments and a 2-way factorial arrangement of 3 Cu programs and 2 AA densities, and experiment 2 consisted of 2 control treatments and a 2-way factorial arrangement between 2 Cu programs and 2 AA densities. The factorial arrangements were analyzed for main and interactive effects. In both experiments, treatments were represented by 8 replicate pens. Statistical significance was considered at a $P$-value ≤ 0.05.

The factorial arrangement in both experiments was analyzed according to the following model:

$$y_{ijk} = \mu ... + \rho_i + \tau_i + B_j + (\tau\beta)_{ij} + \epsilon_{ijk}$$

where $\mu$ ... is the overall mean; the ρi are identically and independently normally distributed random block effects with mean 0 and variance $\sigma^2$; $\tau_i$ are the factor level effects of the *i*th AA density such that $\Sigma\tau_i = 0$; $B_j$ are the identically and independently normally distributed effects of the *j*th level effect of the Cu program factor such that $\Sigma B_j = 0$; $(\tau\beta)_{ij}$ represents the interaction of AA density and Cu program at the *i*th and *j*th levels such that $\Sigma_j$ $(\tau\beta)$ $ji = 0$ and $\Sigma i$ $(\tau\beta)$ $ji = 0$; and the random error $\varepsilon_{ijk}$ are identically and independently normally distributed with mean 0 and variance $\sigma^2$.

Additionally, preplanned orthogonal contrasts were conducted. In experiment 1, a contrast compared the PC with the NC to determine the effect of the vaccination. Additional contrasts compared the NC individually with each of the treatments receiving the moderate AA density diets to determine the effects of Cu supplementation. In experiment 2, a contrast was employed to determine the vaccination effect between the PC and the NC, and contrasts were employed between the NC and treatments receiving moderate AA density diets with Cu supplementation. Statistical significance was considered at a *P*-value $\leq 0.05$.

## RESULTS AND DISCUSSION

### *Diet Analysis*

The Cu concentrations during the starter period in experiment 1 were close to the expected values, as shown in Table 3.1, with the exception of the PC diet, which had analyzed Cu concentration of 37 mg/kg of Cu, elevated above the expected 24 mg/kg of Cu. However, this is lower than the concentration required to induce a growth promoting effect (Kim et al., 2011). Analyzed total AA densities in the starter diets were higher than calculated values, which may be due to the utilization of soybean-meal with a higher AA content than expected. All grower and finisher diets had analyzed Cu concentrations in agreement with the calculated values (Tables 3.2 and 3.3), and total AA density was close to the calculated values.

In experiment 2, diets also had elevated AA concentrations similar to those in the growth performance study, but consistent among treatments (Table 3.4). This was likely due to the utilization of soybean-meal with a higher AA concentration than expected, as the same source of soybean-meal was used in starter diets in experiments 1 and 2. Dietary Cu concentrations were also close to the calculated values, with the exception of the PC diet, with an analyzed Cu of 61 mg/kg but a calculated Cu concentration of 14 mg/kg. This is likely not high enough to elicit a growth performance response (Kim et al., 2011). Titanium dioxide recovery ranged from 0.45 to 0.54%, compared with the expected concentration of 0.50%.

### ***Growth Performance, Experiment 1***

From 1 to 12 d of age, an interaction was observed between AA density and Cu concentration was FCR ($P = 0.001$), where broilers fed the low AA-270 mg/kg of Cu diets had the highest FCR and broilers fed the low AA-135 mg/kg of Cu or moderate AA 270 mg/kg of Cu diets had the lowest FCR (Table 3.5). Main effects of Cu concentration elicited a response ($P < 0.001$) where broilers fed diets containing 135 mg/kg of Cu had increased BW, BWG, and FI compared with broilers fed diets containing 270 mg/kg of Cu. Broilers fed NC diets had a 12 g higher ($P = 0.030$) FI than those fed moderate AA, 270 mg/kg of Cu diets. The main effect of AA density reduced feed intake ($P = 0.017$) in broilers fed the moderate AA diets compared with birds fed the low AA diets.

The lack of response to Cu during the starter period agrees with the literature, which often cites no significant increase in BWG or reduction in FCR from 1 to approximately 14 d of age (Pesti and Bakalli, 1996; Skrivan et al., 2000; Pekel et al., 2009; Kim et al., 2011). The lack of response to AA density is not in agreement with previous research, as reduced dietary AA density generally leads to reduced BW and BWG during the starter period (Kidd et al., 2004, 2005). The lack of response could be due to the diets being higher in AA density than expected, causing the Low AA diets to not be sub-marginal in AA concentrations. The total Lys content in the starter diet was 1.44% in the moderate AA density diets and 1.34% in the low AA density diets, compared with calculated total Lys of 1.34 and 1.24%, respectively. This is likely due to the soybean-meal having a higher AA content than expected.

From 1 to 25 d of age, no interactions ($P \geq 0.29$) were observed for the growth performance variables measured (Table 3.6). Feed intake was reduced in broilers fed the 270-270 mg/kg of Cu diets compared with those fed the 135-135 mg/kg of Cu diets ($P$ = 0.025; 1.880 vs. 1.935 kg). The reduction of FI in broilers fed high concentrations of Cu, however, has not been frequently cited in the literature. It is unclear as to why these broilers had lower FI. It is not likely due to a possible toxicity, as reductions in BWG and FCR have not been reported in broilers fed Cu concentrations of 400 mg/kg, while they are cited when broilers are provided with feed supplemented with 600 mg/kg of Cu (Miles et al., 1998). Feed conversion ratio was reduced in broilers fed the moderate AA diets compared with the low AA diets ($P$ = 0.001; 1.378 vs. 1.411 points), as was mortality ($P$ = 0.017; 0.667 vs. 2.167%). The AA response observed from 1 to 25 d of age is consistent with the literature, as increased dietary AA density has been shown to reduce FCR (Kidd et al., 2004, 2005; Dozier et al., 2007). Although BWG was not significantly increased in broilers fed the moderate AA diets compared with the broilers fed the low AA diets, the trend of 24 g increase in BWG was also consistent with previous research (Kidd et al., 2004, 2005). No significant differences ($P > 0.05$) were observed for lesions caused by coccidiosis between treatments, though lesions consistent with vaccination were observed.

From 1 to 32 d of age, no interactions ($P \geq 0.45$) were detected, but main effects of dietary AA density and Cu supplementation were observed for growth performance (Table 3.7). Dietary Cu concentration did not affect ($P \geq 0.34$) BW, BWG, or mortality from 1 to 32 d of age. However, FI was reduced ($P = 0.017$; 3.006 vs. 3.097 kg) by 91g in broilers fed diets containing 270-270-135 mg/kg of Cu compared with those fed the 270-135-135 mg/kg of Cu diets. This led to a 1.7 point reduction ($P = 0.039$) in FCR of broilers fed the 135-135-135 mg/kg of Cu diets compared with broilers fed the 270-270-270 mg/kg of Cu diets. Similarly, both FI and FCR were reduced ($P \leq 0.025$) in broilers fed moderate AA diets containing either 270-135-135 mg/kg of Cu or 270-270-135 mg/kg of Cu compared with the broilers fed moderate AA density diets with no supplemental Cu. Broilers fed diets containing 270-270-135 mg/kg of Cu had 100 g reduction ($P = 0.018$) in FI, and 3.3 points reduction ($P = 0.001$) in FCR compared with the birds receiving the NC treatment. Broilers provided with diets containing 270-135-135 mg/kg of Cu had a 94 g decrease ($P = 0.025$) in FI and a 2.3 point decrease ($P = 0.013$) in FCR compared with the NC-fed birds. Broilers fed the moderate AA diets had higher BWG ($P < 0.001$; 2.111 vs. 2.029 kg), lower FCR ($P < 0.0001$; 1.432 vs. 1.498), and reduced mortality (*P* = 0.042; 1.00 vs. 2.50%) than the broilers provided with low AA diets. Feed intake was not affected (*P* = 0.41) by AA density.

No significant interactions between AA density and Cu concentration were observed for cumulative growth performance. Wang et al. (2014) reported no interactions at 40 d of age between birds fed either 5 or 200 mg/kg of Cu and varying dietary Lys concentrations on BWG, FCR, or livability. The reduction in FCR is consistent with previous research, which has shown decreases in FCR with Cu inclusion above 100 mg/kg (Pesti and Bakalli, 1996; Ewing et al., 1998; Pekel et al., 2009). However, those results were primary elicited through an increase in BWG, rather than a decrease in FI, as in the current study. The increase in BWG and decrease in FCR and mortality with increased dietary AA density is consistent with previous research (Kidd et al., 2004, 2005; Dozier et al., 2007).

### ***Processing, Experiment 1***

In this experiment, increased Cu supplementation decreased ($P \leq 0.031$) carcass weight and yield but had no effect ($P \geq 0.10$) on total breast meat weight or yield, while broilers fed moderate AA density diets had higher ($P \leq 0.0001$) carcass and total breast meat weights and yields (Table 3.8). The Cu program affected carcass weight and yield ($P \leq 0.031$), drum weight and yield ($P \leq 0.041$), and wing weight (*P* = 0.027). Broilers provided with the diets containing 270-270-135 mg/kg of Cu had reduced ($P \leq 0.031$) carcass weights and yields and drum and wing weights compared with the broilers fed the diets containing 135-135-135 mg/kg of Cu. This represented a decrease of 32 g carcass weight (1.525 vs. 1.557 kg) and 1.00% decrease in carcass yield (71.34 vs. 72.06%) in the broilers fed diets containing 270-270-135 mg/kg of Cu compared with those fed the diets containing 135-135-135 mg/kg of Cu. Negative control-fed broilers exhibited reduced ($P \leq 0.04$) thigh weight and yield compared with broilers fed diets containing 270-270-135 mg/kg of Cu. For the main effect of dietary AA density, carcass weight and yield, total breast meat weight and yield, drum, wing, and thigh weights were all increased ($P < 0.001$) in broilers fed the moderate AA diets compared with those fed the low AA diets. This represented a 90 g increase in carcass weight and a 35 g increase in total breast meat weight. Abdominal fat weight and percentage were decreased ($P < 0.0001$) in broilers fed the moderate AA density diets compared with those fed the low AA diets, while drum, wing, and thigh yield were not affected ($P \geq 0.10$) by AA density. Broilers fed the moderate AA, 270-270-135 mg/kg of Cu diets had reduced ($P = 0.047$) abdominal fat percentage compared with the birds fed the low AA, 270-270-135 mg/kg of Cu or low AA 270-135-135 mg/kg of Cu diets, while the birds receiving the other treatments had intermediary abdominal fat percentages that were not significantly different.

The reduction in carcass yield in broilers fed 270-135-135 mg/kg of Cu may have been caused by the reduction in FI, which led to a decrease in digestible AA intake. Digestible Met and Lys intake were reduced ($P = 0.015$) by approximately 3% in broilers fed 270-270-135 mg/kg of Cu diets compared with those fed the 270-135-135 mg/kg of Cu diets, with other essential AA exhibiting the same effect. This translated to a 0.933 g decrease in digestible Lys in broilers fed the 270-270-135 mg/kg of Cu diets compared with those fed the 270-135-135 mg/kg of Cu diets. Sparse information is available regarding the effect of dietary Cu concentration on processing yields. Wang et al. (2014) evaluated the effects of feeding broilers diets varying in Lys concentrations and containing either 5 or 200 mg/kg of Cu from 1 to 40 d of age. Although BWG was increased in broilers fed 200 mg/kg of Cu (2.64 kg vs 2.58 kg; $P = 0.01$), neither Cu supplementation nor the interaction between Cu and Lys density altered ($P > 0.05$) processing yields. Conversely, Pekel et al. (2009) determined that feeding broilers diets supplemented with 150 mg/kg of Cu increased ($P < 0.003$) carcass weight compared with control-fed birds from 1 to 21 d of age. The reduction in carcass weight and yield and in wing and drum weight could be due to the alterations in AA digestibility elicited through supplementation of high concentrations of Cu. Previous research has noted that Cu concentration interacts with AA density to affect AA digestibility (Rochell et al., 2017). The reduced AA digestibility leading to reduced AA absorption could reduce AA available for protein synthesis. The effect of AA density on carcass and breast meat yield and weight in this study agree with the literature (Kidd et al., 2004, 2005; Dozier et al., 2007), as increased dietary AA density can lead to increases in carcass and total breast meat weight and yield.

### ***Microbial Analysis, Experiment 1***

No differences ($P \geq 0.07$) were observed between the proportions of bacteria analyzed for either an interaction between AA density and Cu concentration, the main effects of AA density or Cu concentration, or the contrasts evaluated (Figure 3.1). Biological differences were apparent; however, this did not translate to statistical differences due to the high variation within treatments. Broilers fed diets containing moderate AA density and 270-135-135 or 270-270-135 mg/kg of Cu during the starter, grower, and finisher periods had higher numerical proportions of clostridiaceae and lower proportions of lactobacillacea and enterococcaceae than broilers fed NC diets or fed 135 mg/kg of supplemental Cu from 1 to 32 d of age.

The lack of differing ileal microbiota proportions could be due to the high variability of the microflora of the broiler gastrointestinal tract (Stanley et al., 2013). Despite being contrary to a common hypothesis for the mechanism of action of Cu, previous studies have inconclusive results regarding the effects of Cu on intestinal bacteria. Aydin et al. (2010) observed no changes in the ileal counts of various bacteria in broilers provided with diets containing 250 mg/kg of Cu compared with those fed control diets. Conversely, Kim et al. (2011) reported reductions in ileal *E. coli* counts but increases in lactobacilli and total bacteria in broilers fed diets containing 100 mg/kg of Cu compared with control-fed broilers. Additionally, the current study analyzed only the relative proportion of ileal microflora, rather than the total bacterial counts, which could have been reduced in broilers fed diets containing higher concentrations of Cu due to the bacteriostatic properties of Cu (Borkow and Gabbay, 2005).

### ***Amino Acid Digestibility, Experiment 2***

Interactions ($P \leq 0.050$) between Cu concentration and AA density for AA digestibility were observed for Lys, Val, Trp, His, and Arg (Table 3.9). Lysine, Val, His, and Arg were more digestible in broilers fed moderate AA density diets containing 270 mg/kg of Cu compared with those fed 135 mg/kg of Cu, while the broilers fed the low AA diets had intermediary digestibility. Isoleucine was more digestible in broilers fed moderate AA density diets with 270 mg/kg of Cu than those fed 135 mg/kg of Cu at either AA density, and the broilers fed the low AA density diets with 270 mg/kg of Cu had greater Ile digestibility than birds fed the moderate AA, 135 mg/kg of Cu diets.

Broilers fed diets containing 270 mg/kg of Cu had greater digestibility ($P \leq 0.033$) of Lys, Thr, Val, Ile, Trp, Leu, Phe, His, and Arg compared with broilers fed diets supplemented with 135 mg/kg of Cu. Broilers fed diets containing either 135 or 270 mg/kg of supplemental Cu had reduced ($P \leq 0.009$) Cys and Met+Cys digestibility compared with the NC-fed broilers. Broilers fed diets with 135 mg/kg of supplemental Cu had reductions ($P \leq 0.021$) in Met, Cys, Met+Cys, Lys, Thr, Val, Ile, Trp, Leu, Phe, His, and Arg, while broilers fed diets supplemented with 270 mg/kg Cu only exhibited reductions ($P \leq 0.009$) in Met, Cys, and Met+Cys, and had increased Trp ($P = 0.046$) digestibility compared with NC-fed birds. Dietary AA density had no effect ($P \geq 0.19$) on digestibility for any essential AA. Broilers provided vaccination against coccidiosis had no differences ($P \geq 0.09$) in AA digestibility compared with birds that were provided a coccidiostat. No differences were observed for BWG, FI, or FCR between the treatments.

The interaction observed for Lys, Val, Trp, His, and Arg had higher digestibility in the moderate AA diets with 270 mg/kg of Cu compared with the broilers fed the moderate AA, 135 mg/kg of Cu diets, with no differences observed for broilers fed the low AA diets. These results are contrary to those delineated by Rochell et al. (2016), who reported an interaction where AA were most highly digestible in broilers fed low AA diets with 116 mg/kg of Cu than in broilers fed low AA diets without supplemental Cu or in moderate AA diets with or without Cu inclusion. Therefore, the results of the current research do not support the hypothesis that higher inclusions of dietary Cu could help to ameliorate the effects of feeding diets low in AA density. The increased digestibility of Thr, Ile, Leu, and Phe may be due to the regulation of the intestinal microflora, which could increase AA availability to the broiler through reducing the microbial use of AA

(Apajalahti and Vienola, 2016). Additionally, the increased Thr digestibility in broilers fed 270 mg/kg of Cu compared with those fed 135 mg/kg of Cu could be due to less mucin production in broilers fed higher concentrations of Cu, as Thr is utilized heavily for mucin production (Azzam et al., 2011).

Broilers fed moderate AA diets supplemented with Cu had decreased Cys digestibility compared with NC-fed birds, likely due to the role of Cys as a component of metallothionein (Wlostowski, 1993). Metallothionein is a protein used in the metabolism of heavy metals such as Cu (Nielson and Winge, 1983). Thus, an increase in dietary Cu could bind Cys, reducing the digestibility of both Cys and Met+Cys. Although high concentrations of Cu may decrease Cys digestibility through the inclusion of Cys in metallothionein, this reduction in digestibility did not translate to a reduction in growth performance. This could be due to the dietary Met concentration being sufficient to meet the Cys requirement, as Cys can be synthesized from Met (Bunchasak, 2009). There is evidence (Nishimura et al., 1989; Tsujikawa et al., 1991) that this may be due to metallothionein potentially increasing cell proliferation. It is unclear why the broilers fed the moderate AA, 135 mg/kg of Cu diet had a reduction in digestibility of many AA in comparison to the NC, while the broilers fed the moderate AA, 270 mg/kg of Cu diet did not exhibit a similar response. The lack of increased BW or BWG in response to increased AA density was unexpected (Kidd et al., 2004, 2005), but was likely due to the soybean meal being higher in AA than the calculated values used in formulation. One reason for increased AA digestibility when broilers are fed diets higher in AA density may be due to the higher fat content of these diets. Higher fat inclusion has been shown to reduce the rate of passage (Mateos et al., 1982). Reduced rate of passage may increase AA digestibility.

The results of these experiments indicate that high concentrations of dietary Cu can be effective in enhancing growth performance of broilers, as feeding higher concentrations of Cu reduced FCR by maintaining a similar BWG at a lower FI. The higher Cu concentrations did lead to a reduction in carcass weight and yield, but this did not translate to a reduction in breast meat weight and yield. This could be due to the reduced FI leading to reduced AA intake, which could lead to reductions in yield. Additionally, the different Cu feeding programs affected AA digestibility, but the response varied based on the individual AA. The results of these experiments indicate that high concentrations of dietary Cu may be effective in reducing the FCR of broilers through a reduction in FI.

# REFERENCES

Apajalahti, J., and K. Vienola. 2016. Interaction between chicken intestinal microbiota and protein digestion. Anim. Feed Sci. Technol. 221:323-330.

Association of Official Analytical Chemists International. 2006a. Official Methods of Analysis. 18th ed. Association of Official Analytical Chemists, Arlington, VA. 985.01 A, B, D.

Association of Official Analytical Chemists International. 2006b. Official Methods of Analysis. 18th ed. Association of Official Analytical Chemists, Arlington, VA. 982.30 E (a, b, c).

Aviagen. 2014. Ross 708: Broiler nutrition specifications. Aviagen, Huntsville, Alabama, USA. www.aviagen.com.

Aydin, A., A. Y. Pekel, G. Issa, G. Demirel, and P. H. Patterson. 2010. Effects of dietary copper, citric acid, and microbial phytase on digesta pH and ileal and carcass microbiota of broiler chickens fed a low available phosphorus diet. J. Appl. Poult. Res. 19:422-431.

Azzam, M. M., X. T. Zou, X. Y. Dong, P. Xie. 2011. Effect of supplemental l-threonine on mucin 2 gene expression and intestine mucosal immune and digestive enzyme activities of laying hens in environments with high temperature and humidity. Poult. Sci. 90:2251-2256.

Borkow, G. and J. Gabbay. 2005. Copper as a biocidal tool. Curr. Med. Chem.. 12:2163-2175.

Bunchasak, C., 2009. Role of dietary methionine in poultry production. J. Poult. Sci. 46:169-179.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G Peña, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, and R. Knight. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods. 7:335-336.

Conway, D. P., and M. E. McKenzie. 1991. Poultry Coccidiosis Diagnostic and Testing Procedures 2nd edn. New York: Pfizer.

Dilger, R. N., J. S. Sands, D. Ragland, and O. Adeola. 2004. Digestibility of nitrogen and amino acids in soybean meal with added soyhulls. J. Anim. Sci. 82:715-724.

Dozier, W. A., A. Corzo, M. T. Kidd, and S. L. Branton. 2007. Dietary apparent metabolizable energy and amino acid density effects on growth and carcass traits of heavy broilers. J. Appl. Poult. Res, 16:192-205.

Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 26:2460-2461.

Ewing, H. P., G. M. Pesti, R. I. Bakalli, and J. F. M. Menten. 1998. Studies on the feeding of cupric sulfate pentahydrate, cupric citrate and copper oxychloride to broiler chickens. Poult. Sci. 77: 445-448

Green, S. J., R. Venkatramanan, and A. Naqib. 2015. Deconstructing the polymerase chain reaction: Understanding and correcting bias associated with primer degeneracies and primer-template mismatches. PLOS ONE. 10:e0128122. doi:10.1371/journal.pone.0128122.

Johnson, J., and W. H. Reid. 1970. Anticoccidial drugs: lesion scoring techniques in battery and floor-pen experiments with chickens. Exp. Parasitol. 29:30-36.

Kidd, M. T., C. D. McDaniel, S. L. Branton, E. R. Miller, B. B. Boren, and B. I. Fancher. 2004. Increasing amino acid density improves live performance and carcass yields of commercial broilers. J. Appl. Poult. Res. 13:593-604.

Kidd, M. T., A. Corzo, D. Hoehler, E. R. Miller, and W. A. Dozier. 2005. Broiler responsiveness (Ross x 708) to diets varying in amino acid density. Poult. Sci. 84:1389-1396.

Kim, G. B., Y. M. Seo, K. S. Shin, A. R. Rhee, J. Han, and I. K. Paik. 2011. Effects of supplemental copper-methionine chelate and copper-soy proteinate on the performance, blood parameters, liver mineral content, and intestinal microflora of broiler chickens. J. Appl. Poult. Res. 20:21-32.

Luo, X. G., and C. R. Dove. 1996. Effect of dietary copper and fat on nutrient utilization, digestive enzyme activities, and tissue mineral levels in weanling pigs. J. Anim. Sci. 74:1888-1896.

Mateos, G. G., J. L. Sell, and J. A. Eastwood. 1982. Rate of food passage (transit time) as influenced by level of supplemental fat. Poult. Sci. 61:94-100.

McDonald, D., J. C. Clemente, J. Kuczynski, J. R. Rideout, J. Stombaugh, D. Wendel, A. Wilke, S. Huse, J. Hufnagle, F. Meyer, R. Knight, J. G. Caporaso. 2012. The Biological Observation Matrix (BIOM) format or: how I learned to stop worrying and love the omeome. J. Nanobiotechnol. 1:1-6.

Miles, R. D., S. F. O’Keefe, P. R. Henry, C. B. Ammerman, and X. G. Luo. 1998. The effect of dietary supplementation with copper sulfate or tribasic copper chloride on broiler performance, relative copper bioavailability, and dietary prooxidant activity. Poult. Sci. 77:416-425.

Nielson, K. B. and D. R. Winge. 1983. Order of metal binding in metallothionein. J. Biol. Chem. 258:13063-13069.

Nishimura, H., N. Nishimura, and C. Tohyama. 1989. Immunohistochemical localization of metallothionein in developing rat tissues. J. Histochem. Cytochem. 37:715-722.

Pang, Y., J. A. Patterson, and T. J. Applegate. 2009. The influence of copper concentration and source on ileal microbiota. Poult. Sci. 88:586-592.

Pekel, A. Y., P. H. Patterson, R. M. Hulet, N. Acar, T. L. Cravener, D. B. Dowler, and J. M. Hunter. 2009. Dietary Camelina meal versus flaxseed with and without supplemental copper for broiler chickens: Live performance and processing yield. Poult. Sci. 88:2392-2398.

Pesti, G. M. and R. I. Bakalli. 1996. Studies on the feeding of cupric sulfate pentahydrate and cupric citrate to broiler chickens. Poult. Sci. 75:1086-1091.

Rochell, S. J., J. L Usry, T. M. Parr, C. M Parsons, and R. N. Dilger. 2017. Effects of dietary copper and amino acid density on growth performance, apparent metabolizable energy, and nutrient digestibility in Eimeria acerviulina-challenged broilers. Poult. Sci. 96:602-610.

SAS Institute. 2017. SAS® User’s Guide. Version 9.4 ed. SAS Institute Inc., Cary, NC.

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59:215-221.

Skrivan, M., V. Skrivanova, M. Marounek, E. Tumova, and J. Wolf. 2000. Influence of dietary fat source and copper supplementation on broiler performance, fatty acid profile of meat and depot fat, and on cholesterol content in meat. Br. J. Poult. Sci. 41:608-614.

Stanley, D., M. S. Geier, R. J. Hughes, S. E. Denman, and R. J. Moore. 2013. Highly variable microbiota development in the chicken gastrointestinal tract. PLoS ONE 8:e84290. doi:10.1371/journal.pone.0084290.

Tsujikawa, K., T. Imai, M. Kakutani, Y. Kayamori, T. Mimura, N. Otaki, M. Kimura, R. Fukuyama, and N. Shimizu. 1991. Localization of metallothionein in nuclei of growing primary cultured adult rat hepatocytes. Eur. J. Biochem. 283:239-242.

Wang, H., C. Zhang, Y. Mi, and M. T. Kidd. 2014. Copper and lysine amino acid density responses in commercial broilers. J. Appl. Poult. Res. 23:470-477.

Wlostowski, T. 1993. Involvement of metallothionein and copper in cell proliferation. BioMetals. 6:71-76.

Xia, M. S., C. H. Hu, and Z. R. Xu. 2004. Effects of copper-bearing montmorillonite on growth performance, digestive enzyme activities, and intestinal microflora and morphology of male broilers. Poult. Sci. 83:1868-1875.

Zhang J., K. Kobert, T. Flouri, and A. Stamatakis. 2014. PEAR: A fast and accurate Illumina Paired-End read merger. Bioinformatics. 30:614-620.

**Table 3.1.** Ingredient and calculated nutrient composition of starter diets fed from 1 to 12 d of age (experiment 1) and from 1 to 14 d of age (experiment 2)

| | 0 mg/kg supplemental Cu[1] | | 135 mg/kg supplemental Cu[1] | | 270 mg/kg supplemental Cu[1] | |
|---|---|---|---|---|---|---|
| Ingredient, % | Mod[2] PC[3] | Mod[2] NC[3] | Mod[2] | Low[2] | Mod[2] | Low[2] |
| Corn | 53.39 | 53.39 | 53.39 | 58.37 | 53.39 | 58.37 |
| Soybean Meal[4] | 36.06 | 36.06 | 36.06 | 31.87 | 36.06 | 31.87 |
| DDGS[5] | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 |
| Corn Oil | 2.50 | 2.50 | 2.50 | 1.66 | 2.50 | 1.66 |
| Calcium Carbonate | 1.13 | 1.13 | 1.13 | 1.14 | 1.13 | 1.14 |
| Dicalcium Phosphate | 1.13 | 1.13 | 1.13 | 1.17 | 1.13 | 1.17 |
| Sand/$TiO_2$[6] | 0.55 | 0.60 | 0.57 | 0.57 | 0.55 | 0.55 |
| Sodium Chloride | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| DL-Methionine 99% | 0.30 | 0.30 | 0.30 | 0.27 | 0.30 | 0.27 |
| L-Lysine•HCl | 0.20 | 0.20 | 0.20 | 0.21 | 0.20 | 0.21 |
| AU Mineral Premix[7] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| AU Vitamin Premix[8] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| L-Threonine | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Choline[9] | 0.07 | 0.07 | 0.07 | 0.09 | 0.07 | 0.09 |
| Phytase[10] | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Econase[11] | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| TBCC[12] | 0.00 | 0.00 | 0.03 | 0.03 | 0.05 | 0.05 |
| Diclazuril[13] | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | | | | | | |
| Calculated Nutrient Content (% unless otherwise indicated) | | | | | | |
| Crude Protein | 22.38 | 22.38 | 22.38 | 20.77 | 22.38 | 20.77 |
| $AME_n$ (kcal/kg)[14] | 3,000 | 3,000 | 3,000 | 3,000 | 3,000 | 3,000 |
| Digestible Lys | 1.22 | 1.22 | 1.22 | 1.13 | 1.22 | 1.13 |
| Digestible Met | 0.60 | 0.60 | 0.60 | 0.55 | 0.60 | 0.55 |
| Digestible Cys | 0.30 | 0.30 | 0.30 | 0.29 | 0.30 | 0.29 |
| Digestible Thr | 0.82 | 0.82 | 0.82 | 0.76 | 0.82 | 0.76 |
| Digestible Trp | 0.23 | 0.23 | 0.23 | 0.21 | 0.23 | 0.21 |
| Digestible Arg | 1.34 | 1.34 | 1.34 | 1.22 | 1.34 | 1.22 |
| Digestible Ile | 0.85 | 0.85 | 0.85 | 0.78 | 0.85 | 0.78 |
| Digestible Val | 0.91 | 0.91 | 0.91 | 0.84 | 0.91 | 0.84 |
| Digestible TSAA[15] | 0.90 | 0.90 | 0.90 | 0.84 | 0.90 | 0.84 |
| Calcium | 0.96 | 0.96 | 0.96 | 0.96 | 0.96 | 0.96 |
| Non-phytate P | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 |
| Sodium | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 |
| Choline (mg/kg) | 1,700 | 1,700 | 1,700 | 1,700 | 1,700 | 1,700 |
| Copper (mg/kg)[1] | 14 | 14 | 149 | 149 | 284 | 284 |
| | | | | | | |
| Analyzed Cu Content (mg/kg)[16] | 37 | 19 | 133 | 136 | 264 | 270 |

---

[1] Copper was provided at 14 mg/kg in all diets in the mineral premix and ingredients. Supplemental Cu was added at an additional 0, 135, or 270 mg/kg, depending on treatment, for total concentrations of 14, 149, or 284 mg/kg.
[2] Mod represents 95% of recommended dietary amino acid density for the Ross 708, and Low represents 88% of recommendations (Aviagen North America, Huntsville, AL).
[3] The positive control (PC) diet was fed to birds not vaccinated against coccidiosis. The negative control diet and all other diets were fed to birds that received Coccivac B52® (Merck and Co., Kenilworth, NJ).
[4] Soybean meal contained 47.5% crude protein.
[5] istillers dried grains with solubles contained 5% ether extract.
[6] $TiO_2$ was added as an indigestible marker at 0.5% in all diets at the expense of sand for the diets for experiment 2.
[7] Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic Cu chloride), 8 mg; I (ethylenediaminedihydroxide), 1.4mg; Se (sodium selenite), 0.3 mg.
[8] Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 9,370 IU; Vitamin D (cholecalciferol), 3,300 IU; Vitamin E (DL-alpha tocopheryl acetate), 33 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.3 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 11 mg; niacin (niacinamide), 44 mg; thiamin (thiamin mononitrate), 2.7 mg; D-biotin (biotin), 0.09 mg; and pyridoxine (pyridoxine hydrochloride), 3.8 mg.
[9] Choline chloride-60 (Balchem Corporation, New Hamptopn, NY).
[10] Quantum ® Blue 5G (AB Vista Feed Ingredients, Marlborough, UK) provides 1,000 FTU/kg phytase activity / kg diet.
[11] Econase® XT (AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 16,000 BXU of xylanase activity / 100 gram per ton inclusion.
[12] TBCC = Tribasic Cu chloride as IntelliBond (Micronutrients, Indianapolis, IN) provides 59.21% Cu.
[13] Diclazural is a coccidiostat (Eli Lilly and Company, Indianapolis, IN)
[14] $AME_n$ = nitrogen-corrected apparent metabolizable energy.
[15] TSAA = total sulfur amino acids.
[16] Analyzed Cu concentration was analyzed in triplicate according to AOAC 985.01 A, B, D.

**Table 3.2.** Ingredient and calculated nutrient composition of grower diets fed from 13 to 25 d of age (experiment 1)

| | 0 mg/kg supplemental Cu[1] | | 135 mg/kg supplemental Cu[1] | | 270 mg/kg supplemental Cu[1] | |
|---|---|---|---|---|---|---|
| Ingredient, % | Mod[2] PC[3] | Mod[2] NC[3] | Mod[2] | Low[2] | Mod[2] | Low[2] |
| Corn | 60.52 | 60.63 | 60.57 | 65.09 | 60.52 | 65.04 |
| Soybean Meal[4] | 30.61 | 30.59 | 30.60 | 26.80 | 30.61 | 26.81 |
| DDGS[5] | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 |
| Corn Oil | 1.71 | 1.67 | 1.69 | 0.93 | 1.71 | 0.95 |
| Calcium Carbonate | 1.05 | 1.05 | 1.05 | 1.06 | 1.05 | 1.06 |
| Dicalcium Phosphate | 0.93 | 0.93 | 0.93 | 0.97 | 0.93 | 0.97 |
| Sodium Chloride | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| DL-Methionine 99% | 0.27 | 0.27 | 0.27 | 0.24 | 0.27 | 0.24 |
| L-Lysine•HCl | 0.20 | 0.20 | 0.20 | 0.21 | 0.20 | 0.21 |
| AU Mineral Premix[6] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| L-Threonine | 0.09 | 0.09 | 0.09 | 0.08 | 0.09 | 0.08 |
| AU Vitamin Premix[7] | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 |
| Choline[8] | 0.07 | 0.07 | 0.07 | 0.09 | 0.07 | 0.09 |
| Phytase[9] | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Econase[10] | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| TBCC[11] | 0.00 | 0.00 | 0.03 | 0.03 | 0.05 | 0.05 |
| Diclazuril[12] | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | | | | | | |
| Calculated Nutrient Content (% unless otherwise indicated) | | | | | | |
| Crude Protein | 20.32 | 20.32 | 20.32 | 18.87 | 20.32 | 18.87 |
| $AME_n$ (kcal/kg)[13] | 3,100 | 3,100 | 3,100 | 3,100 | 3,100 | 3,100 |
| Digestible Lys | 1.09 | 1.09 | 1.09 | 1.01 | 1.09 | 1.01 |
| Digestible Met | 0.55 | 0.55 | 0.55 | 0.50 | 0.55 | 0.50 |
| Digestible Cys | 0.28 | 0.28 | 0.28 | 0.27 | 0.28 | 0.27 |
| Digestible Thr | 0.73 | 0.73 | 0.73 | 0.68 | 0.73 | 0.68 |
| Digestible Trp | 0.21 | 0.21 | 0.21 | 0.19 | 0.21 | 0.19 |
| Digestible Arg | 1.19 | 1.19 | 1.19 | 1.09 | 1.19 | 1.09 |
| Digestible Ile | 0.76 | 0.76 | 0.76 | 0.70 | 0.76 | 0.70 |
| Digestible Val | 0.83 | 0.83 | 0.83 | 0.77 | 0.83 | 0.77 |
| Digestible TSAA[14] | 0.83 | 0.83 | 0.83 | 0.77 | 0.83 | 0.77 |
| Calcium | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 |
| Non-phytate P | 0.44 | 0.44 | 0.44 | 0.44 | 0.44 | 0.44 |
| Sodium | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 |
| Choline (mg/kg) | 1,600 | 1,600 | 1,600 | 1,600 | 1,600 | 1,600 |
| Copper (mg/kg)[1] | 13 | 13 | 148 | 148 | 283 | 283 |
| | | | | | | |
| Analyzed Cu content (mg/kg)[15] | 13 | 15 | 145 | 152 | 272 | 279 |

[1] Copper was provided at 13 mg/kg in all diets in the mineral premix and ingredients. Supplemental Cu was added at an additional 0, 135, or 270 mg/kg, depending on treatment, for total concentrations of 13, 148, or 283 mg/kg.

[2] Mod represents 95% of recommended dietary amino acid density for the Ross 708, and Low represents 88% of recommendations (Aviagen North America, Huntsville, AL).
[3] The positive control (PC) diet was fed to birds not vaccinated against coccidiosis. The negative control diet and all other diets were fed to birds that received Coccivac B52® (Merck and Co., Kenilworth, NJ).
[4] Soybean meal contained 47.5% crude protein.
[5] Distillers dried grains with solubles contained 5% ether extract.
[6] Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic Cu chloride), 8 mg; I (ethylenediaminedihydroxide), 1.4mg; Se (sodium selenite), 0.3 mg.
[7] Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 9,370 IU; Vitamin D (cholecalciferol), 3,300 IU; Vitamin E (DL-alpha tocopheryl acetate), 33 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.3 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 11 mg; niacin (niacinamide), 44 mg; thiamin (thiamin mononitrate), 2.7 mg; D-biotin (biotin), 0.09 mg; and pyridoxine (pyridoxine hydrochloride), 3.8 mg.
[8] Choline chloride-60 (Balchem Corporation, New Hamptopn, NY).
[9] Quantum ® Blue 5G (AB Vista Feed Ingredients, Marlborough, UK) provides 1,000 FTU/kg phytase activity / kg diet.
[10] Econase® XT (AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 16,000 BXU of xylanase activity / 100 gram per ton inclusion.
[11] TBCC = Tribasic Cu chloride as IntelliBond (Micronutrients, Indianapolis, IN) provides 59.21% Cu.
[12] Diclazural is a coccidiostat (Eli Lilly and Company, Indianapolis, IN)
[13] $AME_n$ = nitrogen-corrected apparent metabolizable energy.
[14] TSAA = total sulfur amino acids.
[15] Analyzed Cu concentration was analyzed in triplicate according to AOAC 985.01 A, B, D.

**Table 3.3.** Ingredient and calculated nutrient composition of finisher diets fed from 26 to 32 d of age (experiment 1)

| Ingredient, % | Mod[1], 0 mg/kg supplemental Cu[2] | Mod[1], 135 mg/kg supplemental Cu[2] | Low[1], 270 mg/kg supplemental Cu[2] |
|---|---|---|---|
| Corn | 63.42 | 63.36 | 67.59 |
| Soybean Meal[3] | 27.27 | 27.28 | 23.74 |
| DDGS[4] | 4.00 | 4.00 | 4.00 |
| Corn Oil | 2.43 | 2.45 | 1.74 |
| Calcium Carbonate | 0.99 | 0.99 | 1.00 |
| Dicalcium Phosphate | 0.81 | 0.81 | 0.84 |
| Sodium Chloride | 0.31 | 0.31 | 0.31 |
| DL-Methionine 99% | 0.26 | 0.26 | 0.23 |
| L-Lysine•HCl | 0.19 | 0.19 | 0.20 |
| AU Mineral Premix[5] | 0.10 | 0.10 | 0.10 |
| L-Threonine | 0.08 | 0.08 | 0.07 |
| Choline[6] | 0.07 | 0.07 | 0.08 |
| AU Vitamin Premix[7] | 0.05 | 0.05 | 0.05 |
| Phytase[8] | 0.01 | 0.01 | 0.01 |
| Econase[9] | 0.01 | 0.01 | 0.01 |
| TBCC[10] | 0.00 | 0.03 | 0.03 |
| | | | |
| Calculated Nutrient Content (% unless otherwise indicated) | | | |
| Crude Protein | 18.96 | 18.96 | 17.60 |
| $AME_n$ (kcal/kg)[11] | 3,175 | 3,175 | 3,175 |
| Digestible Lys | 1.01 | 1.01 | 0.93 |
| Digestible Met | 0.52 | 0.52 | 0.48 |
| Digestible Cys | 0.27 | 0.27 | 0.25 |
| Digestible Thr | 0.68 | 0.68 | 0.63 |
| Digestible Trp | 0.19 | 0.19 | 0.17 |
| Digestible Arg | 1.10 | 1.10 | 1.00 |
| Digestible Ile | 0.70 | 0.70 | 0.64 |
| Digestible Val | 0.77 | 0.77 | 0.71 |
| Digestible TSAA[12] | 0.79 | 0.79 | 0.73 |
| Calcium | 0.81 | 0.81 | 0.81 |
| Non-phytate P | 0.41 | 0.41 | 0.41 |
| Sodium | 0.18 | 0.18 | 0.18 |
| Choline (mg/kg) | 1,500 | 1,500 | 1,500 |
| Copper (mg/kg)[1] | 12 | 148 | 147 |
| | | | |
| Analyzed Cu concentration (mg/kg)[13] | 12 | 143 | 148 |

[1] Mod represents 95% of recommended dietary amino acid density for the Ross 708, and Low represents 88% of recommendations (Aviagen North America, Huntsville, AL).

[2] Copper was provided at 12 mg/kg in all diets in the mineral premix and ingredients. Supplemental Cu was added at an additional 0, 135, or 270 mg/kg, depending on treatment, for total concentrations of 12, 147, or 282 mg/kg.

[3] Soybean meal contained 47.5% crude protein.

[4] Distillers dried grains with solubles contained 5% ether extract.

[3] Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic Cu chloride), 8 mg; I (ethylenediaminedihydroxide), 1.4mg; Se (sodium selenite), 0.3 mg.

[4] Choline chloride-60 (Balchem Corporation, New Hamptopn, NY).

[5] Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 9,370 IU; Vitamin D (cholecalciferol), 3,300 IU; Vitamin E (DL-alpha tocopheryl acetate), 33 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.3 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 11 mg; niacin (niacinamide), 44 mg; thiamin (thiamin mononitrate), 2.7 mg; D-biotin (biotin), 0.09 mg; and pyridoxine (pyridoxine hydrochloride), 3.8 mg.

[6] Quantum ® Blue 5G (AB Vista Feed Ingredients, Marlborough, UK) provides 1,000 FTU/kg phytase activity / kg diet.

[7] Econase® XT (AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 16,000 BXU of xylanase activity / 100 gram per ton inclusion.

[8] TBCC = Tribasic Cu chloride as IntelliBond (Micronutrients, Indianapolis, IN) provides 59.21% Cu.

[9] $AME_n$ = nitrogen-corrected apparent metabolizable energy.

[10] TSAA = total sulfur amino acids.

[11] Analyzed Cu concentration was analyzed in triplicate according to AOAC 985.01 A, B, D.

**Table 3.4.** Analyzed total amino acid percentages, copper concentrations, and titanium dioxide concentrations of starter diets fed from 1 to 14 d of age[1] (experiment 2)

| | Calculated | | Analyzed | | | | | |
|---|---|---|---|---|---|---|---|---|
| Percent, unless otherwise noted | Mod AA | Low AA | Mod PC | Mod NC | Mod 135 mg/kg | Low 135 mg/kg | Mod 270 mg/kg | Low 270 mg/kg |
| Crude protein | 22.42 | 20.77 | 22.35 | 22.45 | 22.02 | 21.45 | 22.91 | 21.20 |
| Met + Cys[2] | 0.99 | 0.92 | 0.94 | 0.96 | 0.99 | 0.89 | 0.96 | 0.90 |
| Methionine[2] | 0.63 | 0.58 | 0.57 | 0.59 | 0.61 | 0.54 | 0.58 | 0.54 |
| Cysteine[2] | 0.37 | 0.34 | 0.38 | 0.37 | 0.39 | 0.35 | 0.38 | 0.35 |
| Lysine[2] | 1.34 | 1.24 | 1.43 | 1.42 | 1.44 | 1.33 | 1.45 | 1.27 |
| Threonine[2] | 0.93 | 0.86 | 0.92 | 0.92 | 0.93 | 0.85 | 0.93 | 0.87 |
| Isoleucine[2] | 0.93 | 0.86 | 1.00 | 0.99 | 1.01 | 0.93 | 1.02 | 0.91 |
| Valine[2] | 1.01 | 0.94 | 1.10 | 1.10 | 1.11 | 1.04 | 1.12 | 1.01 |
| Tryptophan[2] | 2.20 | 2.04 | 0.28 | 0.28 | 0.27 | 0.26 | 0.29 | 0.25 |
| Arginine[2] | 1.44 | 1.33 | 1.43 | 1.44 | 1.47 | 1.34 | 1.50 | 1.34 |
| $TiO_2$[3] | 0.50 | 0.50 | 0.45 | 0.52 | 0.54 | 0.54 | 0.54 | 0.53 |
| Copper (mg/kg)[4] | 14 | 14 | 61 | 20 | 152 | 153 | 260 | 280 |

[1] Treatments consisted of moderate (**Mod**) or Low amino acid density, where Mod represents 95% of recommended dietary amino acid density for the Ross 708, and Low represents 88% of recommendations (Aviagen North America, Huntsville, AL). Copper was provided at 14 mg/kg in all diets in the mineral premix and ingredients. Supplemental Cu was added at an additional 0, 135, or 270 mg/kg, depending on treatment, for total calculated concentrations of 14, 149, or 284 mg/kg. Positive control (**PC**) birds received diclazuril in the feed, while all other birds received a 1× dose of Coccivac B52®.

[2] Total amino acid concentrations were analyzed in triplicate according to AOAC 982.30 E (a,b,c).

[3] Titanium dioxide concentrations were analyzed in quadruplicate according to the method described by Short et al. (1996).

[4] Copper concentrations were analyzed in triplicate according to AOAC 985.01 A, B, D.

**Table 3.5.** Growth performance of Yield Plus × Ross 708 broilers fed diets varying in amino acid density and supplemental copper concentration from 1 to 12 days of age[1] (experiment 1)

| AA density[2] | Supplemental Cu[3], mg/kg | Coccidiosis Vaccination[4] | BW, kg | BWG, kg | FI, kg | FCR[5], kg:kg | Mortality[6], % |
|---|---|---|---|---|---|---|---|
| Moderate[7] | 0 | No | 0.437 | 0.396 | 0.469 | 1.199 | 0.5 |
| Moderate[7] | 0 | Yes | 0.431 | 0.390 | 0.471 | 1.209 | 0.5 |
| Moderate | 135 | Yes | 0.432 | 0.391 | 0.477 | $1.221^{ab}$ | 0.5 |
| Low | 135 | Yes | 0.443 | 0.402 | 0.482 | $1.200^{b}$ | 0.0 |
| Moderate | 270 | Yes | 0.425 | 0.383 | 0.459 | $1.201^{b}$ | 0.5 |
| Low | 270 | Yes | 0.424 | 0.383 | 0.473 | $1.227^{a}$ | 0.5 |
| SEM[8] | | | 0.005 | 0.005 | 0.005 | 0.008 | 0.5 |
| *Amino Acid Density Main Effects* | | | | | | | |
| Moderate | | | 0.428 | 0.387 | 0.468 | 1.211 | 0.3 |
| Low | | | 0.434 | 0.392 | 0.477 | 1.213 | 0.9 |
| SEM[8] | | | 0.003 | 0.003 | 0.003 | 0.005 | 0.3 |
| *Copper Concentration Main Effects* | | | | | | | |
| | | 135 | 0.438 | 0.396 | 0.480 | 1.211 | 0.5 |
| | | 270 | 0.424 | 0.383 | 0.466 | 1.214 | 0.6 |
| | | SEM[8] | 0.004 | 0.004 | 0.004 | 0.005 | 0.4 |
| | | | *Probabilities* | | | | |
| Amino Acid × Copper | | | 0.14 | 0.12 | 0.24 | 0.001 | 0.13 |
| Amino Acid Density | | | 0.15 | 0.14 | 0.017 | 0.79 | 0.13 |
| Copper Concentration | | | <0.001 | <0.001 | <0.001 | 0.61 | 0.76 |
| *Preplanned Orthogonal Contrasts* | | | | | | | |
| PC vs. NC | | | 0.34 | 0.35 | 0.78 | 0.43 | 1.00 |
| NC vs. Mod AA, 135 mg/kg Cu | | | 0.82 | 0.90 | 0.35 | 0.32 | 1.00 |
| NC vs. Mod AA, 270 mg/kg Cu | | | 0.23 | 0.21 | 0.03 | 0.44 | 0.39 |

[1] Values are least-square means of 8 replicate pens, with each pen having 25 chicks at placement.

[2] Amino acid densities were calculated at 95% and 88% of the Ross recommended guidelines for Ross 708 for the moderate and low density diets, respectively (Aviagen, 2014).

[3] Copper was supplied in the mineral premix at 14 mg/kg to all birds. Supplemental Cu was added at 0, 135, or 270 mg/kg, depending on treatment.

[4] All chicks except for treatment 1 received Coccivac B52 at the hatchery. Positive control was supplemented with 0.05% diclazuril.

[5] Feed conversion ratio (FCR) was corrected for mortality.

[6] Mortality values were arcsine transformed.

[7] Positive and negative controls were only used for the orthogonal contrasts and are excluded from the main and interactive effect means and standard errors.

[8] Pooled standard error

**Table 3.6.** Growth performance of Yield Plus × Ross 708 broilers fed diets varying in amino acid density and supplemental copper concentration from 1 to 25 days of age[1] (experiment 1)

| AA density[2] | Supplemental Cu[3], mg/kg | Coccidiosis Vaccination[4] | BW, kg | BWG, kg | FI, kg | FCR[5], kg:kg | Mortality[6], % |
|---|---|---|---|---|---|---|---|
| Moderate[7] | 0-0 | No | 1.442 | 1.401 | 1.938 | 1.384 | 1.5 |
| Moderate[7] | 0-0 | Yes | 1.423 | 1.381 | 1.927 | 1.395 | 1.0 |
| Moderate | 135-135 | Yes | 1.424 | 1.382 | 1.912 | 1.384 | 0.5 |
| Low | 135-135 | Yes | 1.416 | 1.375 | 1.959 | 1.421 | 2.0 |
| Moderate | 270-135 | Yes | 1.403 | 1.368 | 1.905 | 1.379 | 1.0 |
| Low | 270-135 | Yes | 1.374 | 1.332 | 1.895 | 1.424 | 2.5 |
| Moderate | 270-270 | Yes | 1.423 | 1.381 | 1.884 | 1.372 | 0.5 |
| Low | 270-270 | Yes | 1.374 | 1.332 | 1.895 | 1.424 | 2.5 |
| SEM[8] | | | 0.020 | 0.020 | 0.023 | 0.012 | 0.7 |
| *Amino Acid Density Main Effects* | | | | | | | |
| Moderate | | | 1.419 | 1.377 | 1.900 | 1.378 | 0.7 |
| Low | | | 1.394 | 1.353 | 1.910 | 1.411 | 2.2 |
| SEM[8] | | | 0.014 | 0.014 | 0.014 | 0.007 | 0.4 |
| *Copper Concentration Main Effects* | | | | | | | |
| | 135-135 | | 1.420 | 1.379 | $1.935^{a}$ | 1.402 | 1.3 |
| | 270-135 | | 1.392 | 1.350 | $1.899^{ab}$ | 1.402 | 1.8 |
| | 270-270 | | 1.407 | 1.366 | $1.880^{b}$ | 1.380 | 1.3 |
| | SEM[8] | | 0.016 | 0.016 | 0.017 | 0.009 | 0.5 |
| | | | *Probabilities* | | | | |
| Amino Acid × Copper | | | 0.66 | 0.65 | 0.29 | 0.48 | 0.97 |
| Amino Acid Density | | | 0.08 | 0.08 | 0.53 | 0.001 | 0.017 |
| Copper Concentration | | | 0.23 | 0.22 | 0.025 | 0.09 | 0.83 |
| *Preplanned Orthogonal Contrasts* | | | | | | | |
| PC vs. NC | | | 0.37 | 0.37 | 0.71 | 0.50 | 0.61 |
| NC vs. Mod AA, 135-135 mg/kg Cu | | | 0.96 | 0.97 | 0.63 | 0.51 | 0.61 |
| NC vs. Mod AA, 270-135 mg/kg Cu | | | 0.99 | 1.00 | 0.16 | 0.18 | 0.61 |
| NC vs. Mod AA, 270-270 mg/kg Cu | | | 0.52 | 0.52 | 0.49 | 0.35 | 1.00 |

[1] Values are least-square means of 8 replicate pens, with each pen having 25 chicks at placement.

[2] Amino acid densities were calculated at 95% and 88% of the Ross recommended guidelines for Ross 708 for the moderate and low density diets, respectively (Aviagen, 2014).

[3] Copper was supplied in the mineral premix at 14 mg/kg to all birds. Supplemental Cu was added at 0, 135, or 270 mg/kg, depending on treatment.

[4] All chicks except for the positive control (PC) received Coccivac B52 at the hatchery. Positive control diets were supplemented with 0.05% diclazuril.
[5] Feed conversion ratio (FCR) was corrected for mortality.
[6] Mortality values were arcsine transformed.
[7] Positive and negative controls (NC) were only used for the orthogonal contrasts and are excluded from the main and interactive effect means and standard errors.
[8] Pooled standard error

**Table 3.7.** Growth performance of Yield Plus × Ross 708 broilers fed diets varying in amino acid density and supplemental copper concentration from 1 to 32 days of age[1] (experiment 1)

| AA density[2] | Supplemental Cu[3], mg/kg | Coccidiosis Vaccination[4] | BW, kg | BWG, kg | FI, kg | FCR[5], kg:kg | Mortality[6], % |
|---|---|---|---|---|---|---|---|
| Moderate[7] | 0-0 | No | 2.172 | 2.131 | 3.086 | 1.450 | 2.0 |
| Moderate[7] | 0-0 | Yes | 2.172 | 2.131 | 3.115 | 1.455 | 1.5 |
| Moderate | 135-135-135 | Yes | 2.168 | 2.127 | 3.068 | 1.443 | 0.5 |
| Low | 135-135-135 | Yes | 2.100 | 2.058 | 3.127 | 1.506 | 2.5 |
| Moderate | 270-135-135 | Yes | 2.138 | 2.100 | 3.021 | 1.432 | 2.0 |
| Low | 270-135-135 | Yes | 2.063 | 2.022 | 3.045 | 1.497 | 2.5 |
| Moderate | 270-270-135 | Yes | 2.151 | 2.110 | 3.015 | 1.422 | 0.5 |
| Low | 270-270-135 | Yes | 2.049 | 2.008 | 2.996 | 1.492 | 2.5 |
| SEM[8] | | | 0.027 | 0.027 | 0.033 | 0.007 | 0.9 |
| *Amino Acid Density Main Effects* | | | | | | | |
| Moderate | | | 2.153 | 2.111 | 3.035 | 1.432 | 1.0 |
| Low | | | 2.071 | 2.029 | 3.056 | 1.498 | 2.5 |
| SEM[8] | | | 0.017 | 0.017 | 0.021 | 0.005 | 0.5 |
| *Copper Concentration Main Effects* | | | | | | | |
| | 135-135-135 | | 2.134 | 2.093 | $3.097^{a}$ | $1.474^{a}$ | 1.5 |
| | 270-135-135 | | 2.101 | 2.059 | $3.033^{ab}$ | $1.464^{ab}$ | 2.3 |
| | 270-270-135 | | 2.100 | 2.059 | $3.006^{b}$ | $1.457^{b}$ | 1.5 |
| | SEM[8] | | 0.020 | 0.020 | 0.024 | 0.006 | 0.6 |
| | | | *Probabilities* | | | | |
| Amino Acid × Copper | | | 0.79 | 0.79 | 0.45 | 0.84 | 0.66 |
| Amino Acid Density | | | <0.001 | <0.001 | 0.41 | <0.0001 | 0.042 |
| Copper Concentration | | | 0.34 | 0.34 | 0.017 | 0.039 | 0.66 |
| *Preplanned Orthogonal Contrasts* | | | | | | | |
| PC vs. NC | | | 0.99 | 0.99 | 0.50 | 0.57 | 0.47 |
| NC vs. Mod AA, 135-135-135 mg/kg Cu | | | 0.90 | 0.90 | 0.25 | 0.15 | 0.51 |
| NC vs. Mod AA, 270-135-135 mg/kg Cu | | | 0.52 | 0.52 | 0.018 | 0.001 | 0.51 |
| NC vs. Mod AA, 270-270-135 mg/kg Cu | | | 0.31 | 0.30 | 0.025 | 0.013 | 0.65 |

[1] Values are least-square means of 8 replicate pens, with each pen having 25 chicks at placement.

[2] Amino acid densities were calculated at 95% and 88% of the Ross recommended guidelines for Ross 708 for the moderate and low density diets, respectively (Aviagen, 2014).

[3] Copper was supplied in the mineral premix at 14 mg/kg to all birds. Supplemental Cu was added at 0 or 135 mg/kg, depending on treatment.

[4] All chicks except for the positive control (PC) treatment received Coccivac B52 at the hatchery.
[5] Feed conversion ratio (FCR) was corrected for mortality.
[6] Mortality values were arcsine transformed.
[7] Positive and negative control (NC) treatments were only used for the orthogonal contrasts and are excluded from the main and interactive effect means and standard errors.
[8] Pooled standard error

**Table 3.8.** Carcass characteristics of Yield Plus × Ross 708 male broilers fed diets varying in amino acid density and supplemental copper concentration from 1 to 33 days of age[1] (experiment 1)

| | | | Carcass | | Abdominal Fat | | Drums | | Wings | | Thigh | | Total Breast | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Treatments* | | | Wt (kg) | Yield (%) | Wt (kg) | Percent (%) | Wt (kg) | Yield (%) | Wt (kg) | Yield (%) | Wt (kg) | Yield (%) | Wt (kg) | Yield (%) |
| AA[2] | Supplemental Cu[3] | Vac[4] | | | | | | | | | | | | |
| Mod[5] | 0-0-0 | No | 1.602 | 72.59 | 0.019 | 0.85 | 0.192 | 8.68 | 0.171 | 7.79 | 0.207 | 9.39 | 0.561 | 25.44 |
| Mod[5] | 0-0-0 | Yes | 1.598 | 72.35 | 0.018 | 0.83 | 0.189 | 8.59 | 0.170 | 7.73 | 0.203 | 9.21 | 0.558 | 25.28 |
| Mod | 135-135-135 | Yes | 1.596 | 72.34 | 0.018 | $0.82^{bc}$ | 0.191 | 8.66 | 0.171 | 7.77 | 0.200 | 9.10 | 0.552 | 25.09 |
| Low | 135-135-135 | Yes | 1.518 | 71.78 | 0.019 | $0.89^{ab}$ | 0.182 | 8.64 | 0.164 | 7.78 | 0.195 | 9.15 | 0.520 | 24.52 |
| Mod | 270-270-135 | Yes | 1.568 | 71.94 | 0.017 | $0.77^{c}$ | 0.187 | 8.60 | 0.166 | 7.64 | 0.202 | 9.24 | 0.545 | 25.08 |
| Low | 270-270-135 | Yes | 1.481 | 70.74 | 0.020 | $0.98^{a}$ | 0.178 | 8.55 | 0.161 | 7.72 | 0.188 | 8.98 | 0.504 | 24.05 |
| Mod | 270-135-135 | Yes | 1.576 | 71.97 | 0.017 | $0.77^{bc}$ | 0.189 | 8.66 | 0.168 | 7.73 | 0.195 | 8.96 | 0.542 | 24.78 |
| Low | 270-135-135 | Yes | 1.502 | 71.48 | 0.021 | $0.98^{a}$ | 0.184 | 8.74 | 0.165 | 7.87 | 0.190 | 9.08 | 0.511 | 24.23 |
| SEM[6] | | | 0.013 | 0.231 | 0.001 | 0.031 | 0.002 | 0.053 | 0.002 | 0.055 | 0.003 | 0.101 | 0.006 | 0.215 |
| *Amino Acid Density Main Effects* | | | | | | | | | | | | | | |
| Mod | | | 1.580 | 72.08 | 0.017 | 0.79 | 0.189 | 8.64 | 0.169 | 7.72 | 0.199 | 9.10 | 0.546 | 24.99 |
| Low | | | 1.500 | 71.33 | 0.020 | 0.95 | 0.182 | 8.64 | 0.163 | 7.78 | 0.191 | 9.07 | 0.511 | 24.27 |
| SEM[6] | | | 0.008 | 0.002 | <0.001 | 0.018 | 0.001 | 0.030 | 0.001 | 0.031 | 0.002 | 0.072 | 0.004 | 0.146 |
| *Copper Concentration Main Effects* | | | | | | | | | | | | | | |
| | 135-135-135 | | $1.557^{a}$ | $72.06^{a}$ | 0.019 | 0.86 | $0.187^{a}$ | $8.65^{ab}$ | $0.1674^{a}$ | 7.78 | 0.197 | 9.12 | 0.536 | 24.81 |
| | 270-270-135 | | $1.525^{b}$ | $71.34^{b}$ | 0.019 | 0.87 | $0.183^{b}$ | $8.57^{a}$ | $0.1638^{b}$ | 7.68 | 0.195 | 9.11 | 0.524 | 24.56 |
| | 270-135-135 | | $1.539^{ab}$ | $71.72^{ab}$ | 0.019 | 0.88 | $0.186^{ab}$ | $8.70^{b}$ | $0.1668^{al}$ | 7.80 | 0.193 | 9.02 | 0.526 | 24.51 |
| | SEM[6] | | 0.009 | 0.002 | 0.001 | 0.022 | 0.002 | 0.037 | 0.001 | 0.038 | 0.002 | 0.080 | 0.005 | 0.166 |

|  | Probabilities |  |  |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amino acid × copper | 0.87 | 0.13 | 0.08 | 0.047 | 0.51 | 0.41 | 0.36 | 0.48 | 0.09 | 0.06 | 0.60 | 0.34 |
| Amino acid density | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.98 | <0.0001 | 0.10 | <0.0001 | 0.66 | <0.0001 | <0.0001 |
| Copper program | 0.031 | 0.001 | 0.91 | 0.83 | 0.029 | 0.041 | 0.027 | 0.06 | 0.14 | 0.41 | 0.10 | 0.23 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Preplanned Orthogonal Contrasts* |  |  |  |  |  |  |  |  |  |  |  |  |
| PC vs. NC | 0.82 | 0.39 | 0.46 | 0.56 | 0.19 | 0.22 | 0.48 | 0.38 | 0.27 | 0.16 | 0.64 | 0.57 |
| NC vs. Mod AA, 135-135-135 mg/kg Cu | 0.90 | 0.98 | 0.96 | 0.92 | 0.33 | 0.30 | 0.57 | 0.51 | 0.33 | 0.35 | 0.50 | 0.48 |
| NC vs. Mod AA, 270-135-135 mg/kg Cu | 0.08 | 0.13 | 0.16 | 0.17 | 0.22 | 0.95 | 0.07 | 0.27 | 0.74 | 0.85 | 0.12 | 0.45 |
| NC vs. Mod AA, 270-270-135 mg/kg Cu | 0.21 | 0.16 | 0.25 | 0.23 | 0.99 | 0.32 | 0.47 | 0.93 | 0.014 | 0.04 | 0.06 | 0.06 |

[1] Values are least-square means of 8 replicate pens, with 10 birds processed per pen. All yields were calculated using the live weight of the individual bird at 32 days of age.

[2] Amino acid densities were calculated at 95% and 88% of the Ross recommended guidelines for the Ross × Ross 708 for the moderate and low density diets (Aviagen, 2014).

[3] The Cu program is represented as the mg/kg of supplemental Cu in the starter, grower, and finisher diets.

[4] All chicks except for the positive control (PC) treatment received Coccivac B52 at the hatchery. Neither diet for the control treatments contained supplemental Cu. The PC was supplemented with 0.05% diclazuril during the starter and grower phases.

[5] Positive and negative controls (NC) are only used for orthogonal contrasts, and are excluded from the SEM and means of interactive and main effects.

[6] Pooled standard error

**Table 3.9.** Apparent ileal amino acid digestibility of Yield Plus × Ross 708 male broilers fed varying amino acid density and supplemental copper concentrations from 1 to 14 days of age[1] (experiment 2)

| *Treatment*[2] |  | Apparent ileal digestibility, % |  |  |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AA density[3] | Supplemental Cu[4], mg/kg | Met | Cys | Met + Cys | Lys | Thr | Val | Ile | Trp | Leu | Phe | His | Arg |
| Mod[5] | 0 | 91.67 | 66.81 | 81.74 | 85.24 | 75.76 | 77.53 | 80.77 | 83.93 | 81.62 | 81.06 | 82.46 | 87.55 |
| Mod[5] | 0 | 91.14 | 63.40 | 80.36 | 83.94 | 73.37 | 75.21 | 78.82 | 82.50 | 79.74 | 79.36 | 80.88 | 86.64 |
| Mod | 135 | 89.15 | 51.22 | 74.33 | 80.92$^{b}$ | 68.73 | 71.15$^{b}$ | 75.61 | 78.96$^{c}$ | 76.42 | 76.22 | 76.35$^{b}$ | 84.32$^{b}$ |
| Low | 135 | 90.40 | 55.22 | 76.49 | 83.13$^{ab}$ | 71.56 | 74.21$^{ab}$ | 77.86 | 81.60$^{bc}$ | 78.71 | 78.57 | 78.77$^{ab}$ | 85.64$^{ab}$ |
| Mod | 270 | 90.48 | 56.00 | 76.72 | 84.65$^{a}$ | 74.66 | 76.68$^{a}$ | 80.04 | 84.53$^{a}$ | 80.31 | 80.66 | 80.23$^{a}$ | 87.66$^{a}$ |
| Low | 270 | 90.51 | 53.32 | 75.89 | 82.71$^{ab}$ | 73.54 | 74.85$^{ab}$ | 78.29 | 82.00$^{ab}$ | 79.79 | 79.44 | 78.94$^{ab}$ | 86.54$^{ab}$ |
| SEM[6] |  | 0.50 | 2.05 | 1.10 | 0.71 | 1.12 | 1.07 | 0.99 | 0.78 | 1.03 | 1.01 | 0.93 | 0.65 |
| *Amino Acid Density Main Effect* |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Mod |  | 89.81 | 53.61 | 75.54 | 82.79 | 71.69 | 73.92 | 77.82 | 81.74 | 78.36 | 78.44 | 78.29 | 85.99 |
| Low |  | 90.46 | 54.27 | 76.19 | 82.92 | 72.55 | 74.53 | 78.07 | 81.80 | 79.25 | 79.00 | 78.85 | 86.09 |
| *Copper Concentration Main Effect* |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 135 | 89.77 | 53.22 | 75.42 | 82.02 | 70.14 | 72.68 | 76.73 | 80.28 | 77.56 | 77.39 | 77.56 | 84.98 |
|  | 270 | 90.50 | 54.66 | 76.31 | 83.68 | 74.10 | 75.76 | 79.16 | 83.27 | 80.05 | 80.05 | 79.58 | 87.10 |
|  | SEM[6] | 0.39 | 1.62 | 0.86 | 0.53 | 0.82 | 0.81 | 0.73 | 0.53 | 0.78 | 0.75 | 0.72 | 0.50 |
|  |  | Probabilities |  |  |  |  |  |  |  |  |  |  |  |
| Amino Acid × Copper |  | 0.23 | 0.07 | 0.14 | 0.008 | 0.07 | 0.024 | 0.06 | 0.001 | 0.18 | 0.09 | 0.050 | 0.050 |
| Amino Acid Density |  | 0.21 | 0.71 | 0.51 | 0.85 | 0.41 | 0.55 | 0.81 | 0.93 | 0.39 | 0.58 | 0.53 | 0.87 |
| Copper Concentration |  | 0.16 | 0.42 | 0.37 | 0.029 | <0.001 | 0.006 | 0.023 | <0.001 | 0.022 | 0.015 | 0.033 | 0.002 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Preplanned Orthogonal Contrasts* | | | | | | | | | | | | |
| PC vs. NC | 0.40 | 0.16 | 0.30 | 0.16 | 0.09 | 0.08 | 0.14 | 0.15 | 0.16 | 0.20 | 0.18 | 0.27 |
| NC vs. Mod AA, 135 mg/kg Cu | 0.004 | <0.001 | <0.001 | 0.002 | 0.002 | 0.004 | 0.017 | <0.001 | 0.016 | 0.021 | <0.001 | 0.007 |
| NC vs. Mod AA, 270 mg/kg Cu | 0.314 | 0.004 | 0.009 | 0.44 | 0.36 | 0.27 | 0.35 | 0.046 | 0.67 | 0.32 | 0.57 | 0.21 |

[1] Values are least-square means of 8 replicate pens, with each pen having 12 chicks at placement. Significant interaction effects are separated with different superscripts.

[2] Amino acid densities were calculated at 95% and 88% of the Ross recommended guidelines for 708 for the moderate and low density diets, respectively.

[3] Copper was supplied in the mineral premix at 14 mg/kg to all birds. Supplemental Cu was added at 0, 135, or 270 mg/kg, depending on treatment.

[4] All chicks except for the first treatment received Cocci-vac B52® at the hatchery. The broilers receiving the first treatment were fed diets supplemented with 0.05% diclazuril.

[5] The first two treatments are the negative (NC) and positive controls (PC) and were only used for the orthogonal contrasts and are excluded from the main and interactive effect means and standard errors.

[6] Pooled standard error

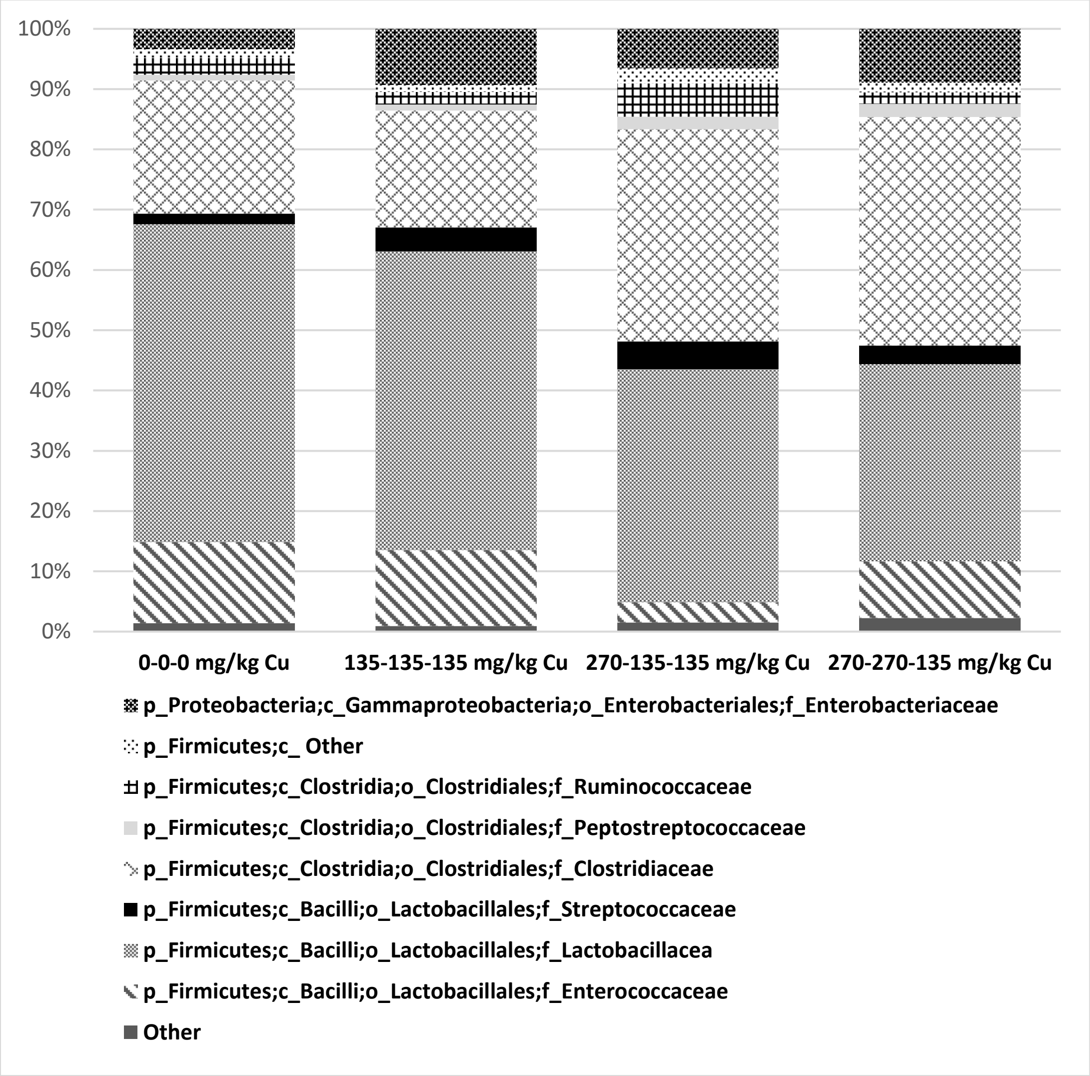

**Figure 3.1.** Relative bacterial community composition according to phyla, class, order and family of 16S rRNA sequences of ileal mucosa samples from 35 d of age broilers fed moderate amino acid density diets containing either 0-0-0, 135-135-135, 270-135-135, or 270-270-135 mg/kg of supplemental Cu during the starter, grower, and finisher periods, respectively. No treatment differences ($P \geq 0.14$; SEM = 5.6) were observed between proportions of bacteria in ileal mucosa.

# IV. GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS OF BROILERS FED DIETS VARYING IN SUPPLEMENTAL COPPER CONCENTRATIONS FROM 29 TO 53 DAYS OF AGE

## ABSTRACT

Previous research indicates that high concentrations of supplemental Cu increases BW gain of broilers raised to approximately 2 kg. An experiment was conducted to determine the effects of supplemental Cu concentration on growth performance and carcass characteristics of Yield Plus × Ross 708 broilers fed from 32 to 53 d of age. Male chicks were randomly distributed to 60 floor pens (22 birds/pen, 0.1 $m^2$ /bird). Broilers were provided common starter and grower diets containing 135 mg/kg of supplemental Cu from 1 to 29 d of age. At 29 d of age, birds were assigned to 1 of 5 dietary treatments. Treatments contained either 0-0, 135-0, 270-0, 135-135, or 270-270 mg/kg in the finisher 1 (from 29 to 41 d of age) and finisher 2 (from 41 to 53 d of age) periods. Body weight gain, feed intake, feed conversion ratio, and mortality were determined at 29, 41, and 53 d of age. At 54 d of age, 14 birds per pen were processed and front-half deboned to determine total breast meat (**TBM**) weight and yield, and fillets (pectoralis major muscles) were scored for white striping and wooden breast. No differences ($P \geq 0.08$) were observed for growth performance characteristics. Broilers fed diets containing 270 mg/kg of Cu in the finisher 1 and finisher 2 diets had higher TBM yield than the broilers fed no supplemental Cu from 29 to 53 d of age (28.1 vs. 27.6%; $P = 0.017$). This

represented a 32 g increase in fillet weight ($P = 0.003$) compared with birds fed diets supplemented with 0-0 or 135-0 mg/kg of Cu in the finisher 1 and finisher 2 periods. Severity of white striping or wooden breast was not affected ($P \geq 0.08$) by Cu supplementation. These data indicate that feeding high concentrations of dietary Cu from 29 to 53 d of age increases TBM weight and yield without adverse effects on meat quality.

## INTRODUCTION

In 2019, 24% of birds were grown to 3.5 kg or greater (USDA, 2019). This is driven by demand for desired product mix and elicits increased processing yields and efficiency in the plant compared with small birds (Brewer, 2012). Many of these broilers are raised in antibiotic-free programs, so integrators are evaluating alternatives to antibiotic growth promoters. One option may be feeding high (> 100 mg/kg) concentrations of dietary Cu. It has been demonstrated that feeding Cu concentrations above 125 mg/kg may improve growth performance of broilers raised to approximately 2 to 2.5 kg (Ewing et al., 1998; Wang et al., 2014). Philpot et al. (2018) reported reductions in feed conversion ratio (**FCR**) at 32 d of age in broilers weighing approximately 2.1 kg when maintaining 270 mg/kg of dietary Cu throughout the starter and grower periods compared with feeding 135 mg/kg of Cu during the same periods. Additionally, Cu supplementation may increase processing yields (Pekel et al., 2009). Previous research observed Cu having a delayed effect, often reporting no difference in the starter period but having cumulative growth benefits. (Pekel et al., 2009; Kim et al., 2011). Kim et al. (2011) reported that broilers fed diets with 100 mg/kg of supplemental Cu had increased

BW from 1 to 28 d of age ($P \leq 0.05$) compared with negative control-fed birds and similar ($P > 0.05$) BW to birds fed diets containing antibiotics.

Sparse information is available about the effects of diets containing supplemental Cu fed to broilers from 2.8 to 4.0 kg on growth performance and processing yields. Additionally, information is limited on the effects of supplemental Cu on the incidence and severity of wooden breast or white striping. Therefore, the objective of this study was to evaluate the effects of increasing dietary Cu concentrations fed to male broilers from 29 to 53 d of age on growth performance and processing characteristics.

## MATERIALS AND METHODS

All procedures involving live birds were approved by Auburn University Institutional Animal Care and Use Committee (PRN 2018-3386).

### ***Bird Husbandry***

A total of 1,320 Yield Plus × Ross 708 male chicks (Aviagen, Huntsville, AL, USA) was purchased from a commercial hatchery at day of hatch. All birds were vaccinated against Marek’s, Newcastle, and infectious bronchitis.  Additionally, the chicks received a 1× dosage of vaccination against coccidiosis via spray cabinet at the hatchery. Chicks were randomly distributed into 60 floor pens (22 chicks/pen, 0.09 $m^2$/chick) in a solid-sided cross-ventilated house equipped with vent boards, exhaust fans, stir fans, evaporative-cooling pads, forced-air heaters, and an electronic controller system (Evolution 3000, Hired Hand Manufacturing, Inc., Bremen, AL). Each pen contained used litter, nipple drinkers (5 nipples/pen), and a hanging pan feeder.  Feed and water were available *ad libitum*. Photoperiod was set at 23 hours of light and 1 hour of darkness from 1 to 7 d of age, and 20 hours of light and 4 hours of darkness for the remainder of the grow-out period. Light intensity was set at 30 lux from 1 to 7 d of age, 5 lux from 8 to 14 d of age, and 3 lux from 15 to 54 d of age. Light intensity was verified at bird level using a photometer (LI-250A light meter, LI-COR Bioscience, Lincoln, NE). Ambient temperature was set at 33˚C at placement and decreased to a final set point of 20˚C based on bird comfort. Birds and feed were weighed at 1, 28, 40, and 53 d of age to determine BW, BW gain (**BWG**), feed intake (**FI**), and FCR. Mortality was recorded daily. Mortality percentages were arcsine transformed prior to analysis, and FCR were adjusted for mortality using chick days.

### ***Processing***

At 53 d of age, 14 birds per pen were selected for processing. All birds selected for processing had a BW within ± 10% of the average of the pen weight. Feed was withdrawn 12 hours prior to processing. At 54 d of age, birds were placed in coops and transported to the Auburn University Pilot Processing Plant. Birds were shackled, electrically stunned, exsanguinated, scalded, plucked, and manually eviscerated. Carcasses were chilled in ice water for 3 hours and then rehung and allowed to drip for approximately 3 minutes. Carcasses and fat pads were weighed to determine carcass yield and abdominal fat pad percentage. Carcasses were split into front and back halves and the weight of the back half was recorded. Front halves were stored on ice for 18 hours, and then were deboned to calculate the weights and yields of breast fillets (pectoralis major muscles) and tenders (pectoralis minor muscles). Yields were based on 53 d live weight. After deboning, breasts were scored on a scale of 0 to 3 for wooden breast and white striping where 0 indicated the absence of defects, 1 had mild defects, 2 had moderately severe defects, and 3 indicated severe defects (Cruz et al., 2017).

### ***Treatments***

From 1 to 29 d of age, birds received common starter (from 1 to 19 d of age) and grower (from 20 to 29 d of age) diets that were formulated to meet or exceed the recommendations of the primary breeder guide with the exception of amino acid (**AA**) density, which was set at 95% of recommendations (Aviagen, 2014), as shown in Table 4.1. Starter and grower diets were formulated to contain 135 mg/kg of supplemental Cu above that provided by the trace mineral premix and feed ingredients from tribasic Cu chloride (Micronutrients, USA, Indianapolis, IN). The starter diet was provided in crumble form, while subsequent diets were fed as whole pellets

At 29 d of age, birds and feed were weighed and bird number was equalized between pens (21 birds/pen, 0.1 $m^2$/bird). Three finisher 1 diets (from 30 to 41 d of age) and 3 finisher 2 diets (from 42 to 53 d of age) were arranged to create 5 dietary treatments. Diets contained either 0, 135, or 270 mg/kg of supplemental Cu as tribasic Cu chloride (Micronutrients, USA, Indianapolis, IN) in addition to approximately 12 mg/kg of Cu supplied by the trace mineral premix and feed ingredients (Table 4.1). Treatment 1 (**0-0**) was formulated to contain 0 mg/kg of supplemental Cu during both finisher phases. Treatments 2 (**135-0**) and 3 (**270-0**) received 135 and 270 mg/kg of supplemental Cu during the finisher 1 period, respectively, and 0 mg/kg of supplemental Cu during the finisher 2 period. Treatments 4 (**135-135**) and 5 (**270-270**) received 135 and 270 mg/kg of supplemental Cu, respectively, through both finisher periods. All experimental diets were formulated to meet or exceed the recommendations of the primary breeder, with the exception of AA density, which was set a 95% of recommendations (Aviagen, 2014). Supplemental fat at 0.5% was added in the mixer, and the remainder of the supplemental fat was applied post pelleting to minimize adverse effects on pellet quality. All experimental diets were pelleted and were tested for pellet durability (Pfost and Allen, 1962). Experimental diets were analyzed for Cu by the University of Missouri Agricultural Experiment Station Chemical Laboratories using the AOAC Official Method 975.03B(b) (AOAC, 2006).

### *Statistical Analyses*

Data were analyzed as a randomized complete block design and the pen location was the blocking factor. Pen was considered the experimental unit. Five dietary treatments were fed from 29 to 53 d of age, and each treatment was represented by 12 replicate pens. A one-way analysis of variance of growth performance and carcass characteristics was performed using PROC MIXED in SAS (2017), by the following model:

$$y_{ij} = \mu_{\dots} + \tau_i + \beta_j + e_{ij}$$

where $\mu_{\dots}$ is the overall mean; $\tau_i$ is the effect of the $i^{th}$ inclusion of Cu such that $\Sigma\ \tau_i 0$; $\beta_j$ is the identically and independently normally distributed effect of the $j^{th}$ block with mean 0 and variance $\sigma^2$; and random error $e_{ij}$ are identically and independently normally distributed with mean 0 and variance $\sigma^2$. Tukey's honestly significant difference was used to separate means, and statistical significance was considered at a $P$-value at $\leq 0.05$.

## RESULTS AND DISCUSSION

Analyzed dietary Cu concentrations were in good agreement to the calculated values (Table 4.1). In the common starter and grower periods, the analyzed Cu content was 139 and 146 mg/kg, compared to the expected concentrations of 149 and 149 mg/kg, respectively. From 29 to 41 d of age, the analyzed Cu concentrations were 14, 149, and

292 mg/kg, compared with the expected 12, 147, and 292 mg/kg. The diets with no supplemental Cu or with a calculated 135 mg/kg of Cu both deviated 2 mg/kg from expected values, while the diet formulated to contain 270 mg/kg of Cu had an analyzed Cu content 10 mg/kg above calculated values. From 42 to 53 d of age, the diet containing no supplemental Cu contained 18 mg/kg of Cu, compared with the calculated 12 mg/kg. The diets formulated to contain 135 or 270 mg/kg of Cu were 2 mg/kg lower and 2 mg/kg higher than expected, respectively. The analyzed Cu concentration of all diets was within 10 mg/kg of the calculated Cu concentrations.

### ***Growth Performance***

During the period from 1 to 29 d of age, birds were provided with common diets, and no differences ($P > 0.05$) were observed for any growth performance variables. From 29 to 41 d of age, no differences were observed ($P \geq 0.16$) in BWG, FI, or FCR. (Table 4.2). The lack of significant response of growth performance characteristics to Cu was likely due to variance. A 5.5 point numerical decrease in FCR was observed between broilers fed 135 mg/kg of supplemental Cu from 29 to 41 d of age compared with control-fed broilers, but the pooled standard error was 2.7 points. A larger number of replicates likely would have yielded statistical significance in this study.

From 41 to 53 d of age, BW, FI, and the incidence of mortality were not affected by dietary Cu inclusion ($P \geq 0.12$), as shown in Table 4.3. However, FCR was influenced ($P = 0.010$) where broilers fed the 135-0 or 270-0 diets had higher FCR of 12.9 and 13.9 points, respectively, compared with broilers provided with the 135-135 diets. The increase in FCR of the broilers fed the 135-0 or 270-0 diets could be explained the removal of supplemental Cu during the finisher 2 period. Additionally, BWG increased

($P = 0.030$) from 1.508 kg in broilers fed the 135-0 diets to 1.631 kg in birds provided the 135-135 diets. From 29 to 53 d of age, no differences ($P \geq 0.08$) were observed for growth performance variables (Table 4.4). Overall, the incidence of mortality was higher than expected, which may have been due to the accelerated growth rate (sudden death syndrome or ascities). However, the incidence of these metabolic disorders was not determined. As these broilers were raised to a final average BW of 4.86 kg, the variance in BWG, FI, and FCR of these birds was greater than would be expected from birds raised to lower target weights. This increased variance likely influenced the lack of significance found in this study.

Cumulative growth performance was not influenced ($P \geq 0.12$) by Cu supplementation (Table 4.5). Total Cu intake of the birds from 1 to 53 d of age was 0.32, 0.65, 0.99, 1.09, and 1.83g for the broilers receiving the 0-0, 135-0, 135-135, 270-0, and 270-270 treatments, respectively. Ewing et al. (1998) observed higher BWG and lower FCR ($P \leq 0.05$) when broilers were fed either 125 mg/kg of $CuSO_4$ or Cu oxychloride or 63 mg/kg of Cu citrate compared with the control-fed broilers from 1 to 56 d of age. The authors may have observed significant differences because the control-fed broilers in the study received no supplemental Cu at any period, whereas in the current study, broilers fed the 0-0 mg/kg of Cu diets did receive Cu supplemented at 135 mg/kg from 1 to 28 d of age.

### ***Processing Characteristics***

Dietary Cu concentration influenced ($P \leq 0.017$) processing characteristics of broilers (Table 4.6). Carcass weight was not affected ($P = 0.07$); however, carcass yield was increased ($P = 0.001$) with higher Cu concentration where broilers fed the 270-270 treatment had a 0.78% higher carcass yield than birds provided with the 0-0 treatment. Abdominal fat had an unexpected response where broilers fed the 0-0 diets had increased ($P \leq 0.016$) abdominal fat weight and percentage compared with broilers fed the 135-0 diets, despite having similar ($P \geq 0.12$) BWG and FI. Breast fillet weight and yield were increased ($P \leq 0.005$) in the broilers fed the 270-270 diets compared with birds fed the 0-0 or 135-0 diets. Total breast meat weight and yield (breast fillet and tender) was also optimized in birds fed the 270-270 diets. Total breast meat weight was increased ($P = 0.010$) compared with the broilers receiving the 135-0 treatment. Total breast meat yield was increased ($P = 0.017$) compared with the birds receiving the 0-0 diets. No differences ($P \geq 0.23$) were observed in the weights or yields of tenders, wings or leg quarters. Dietary treatments were similar ($P \geq 0.08$) for the incidence of severity of wooden breast or white striping (Table 4.7).

In agreement, Arias and Koutsos (2006) observed an increase ($P < 0.05$) of 909 g in carcass weight in broilers fed diets formulated to contain 188 mg/kg of Cu from tribasic Cu chloride compared with the control-fed birds from 1 to 45 d of age. Pekel et al. (2009) reported that broilers fed diets containing 150 mg/kg of Cu had increased ($P \leq 0.012$) carcass and breast weights compared with control-fed broilers at 21 d of age. In contrast, Wang et al. (2014) reported no differences ($P \geq 0.19$) in carcass or part yields between broilers fed either 5 or 200 mg/kg of Cu from 1 to 40 d of age.

The mode of action by which Cu increased breast meat weight and yield has not been elucidated. Metallothionein concentrations increase in response to high concentrations of heavy metals (Wlostowski, 1993). In turn, metallothionein could be involved in increasing cell proliferation by eliciting an increase in the Cu concentration in the nuclei, similar to that of rapidly growing tissues, particularly of the gap 1 and synthesis stages of the cell cycle (Wlostowski, 1993). Nishimura et al. (1989) lended support to this hypothesis by reporting a much higher incidence of metallothionein in rapidly differentiating cells such as neonatal rat kidney cells and hepatocytes of rats recovering from hepatectomies than in normal adult tissues. Additionally, Cherian (1994) observed that metallothionein is present in some tumor cells going through cell synthesis. In the current study, potential increases in cell proliferation from Cu supplementation may have resulted in increased breast meat yield. High concentrations of dietary Cu have also been shown to increase serum mitogenic activity (Zhou et al., 1994; Apgar et al., 1995) and growth hormone mRNA concentrations (Zhou et al., 1994) in pigs, which could lead to increased muscle growth.

Additionally, Cu may improve growth performance through enhancing nutrient utilization by altering the microflora in the gastrointestinal tract of broilers (Pang et al., 2009). High concentrations of Cu may also lead to a beneficial shift in the microbiota in the gastrointestinal tract, which could decrease the incidence of pathogens and, thus, decrease lymphocyte recruitment (Arias and Koutsos, 2006). Additionally, certain bacteria are more susceptible to pharmacological Cu concentrations. Moreover, Xia et al. (2004) reported reduced *Escherichia coli* and Clostridia in broilers that were fed 37 mg/kg of Cu in the form of Cu-bearing montmorillonite compared with control-fed broilers. This shift in the gastrointestinal microbiota may reduce the production of growth-reducing metabolites, or may allow for increased absorption of nutrients. Additionally, the bacteriostatic activity of Cu may decrease the bacterial utilization of feed (Arias and Koutsos, 2006; Pang and Applegate, 2007). Furthermore, providing birds with 135 mg/kg of Cu throughout the growout period may minimize disruptions to the diversity of the gastrointestinal microflora associated with changing feed (Petersen et al., 1999). Greater diversity in the microflora generally allows the broiler to have a more stable gastrointestinal environment (Chee et al., 2010).

Another mechanism associated with dietary Cu to enhance breast meat yield could be through altering AA digestibility (Rochell, 2017). Rochell et al. (2017) fed broilers high and low AA density diets (1.20 or 1.05% digestible Lys) and either 0 or 116 mg/kg of supplemental Cu from 1 to 14 d of age. An interaction ($P < 0.05$) was observed where apparent ileal digestibility of Leu, Lys, Phe, Thr, Val, Ala, Asp, Glu, Ser, and Tyr was increased with Cu inclusion on average by 1.8 percentage units for birds fed the low AA density diet, but decreased by the same amount in the broilers fed the high AA density diet with Cu inclusion. An interaction was observed ($P \leq 0.05$) where broilers fed the low AA diets had increased Lys digestibility (88.3 vs. 86.7%) when provided diets supplemented with 116 mg/kg of Cu compared with birds provided no supplemental Cu, but birds fed the high AA diets with 116 mg/kg of Cu had a depressed Lys digestibility (86.5 vs. 87.3%) compared with broilers fed no supplemental Cu. This indicates that supplemental Cu inclusion may increase AA digestibility, which would increase breast meat yield. Lysine can induce an increase in IGF-1 concentration, which increase the rate of muscle synthesis (Tesseraud et al., 1996; Liao et al., 2015). Increased Lys digestibility could lead to higher muscle accretion, and, thus, increased breast meat yield. Wang et al. (2014) fed broilers diets containing high or low Lys (1.20 or 1.00% digestible Lys from 1 to 18 d of age; 1.00 or 0.80% digestible Lys from 19 to 40 d of age) and either 5 or 200 mg/kg of supplemental Cu. No significant interactions between Cu concentration and AA density were observed for growth performance variables or processing characteristics; however, BWG and wing yield had numerical interactions ($P < 0.09$). The mode by which Cu concentration affects AA digestibility is unknown.

In the research reported herein, no differences due to Cu supplementation were observed for BWG or FCR from 29 to 53 d of age. Carcass yield and total breast yield were increased in the broilers fed diets containing 270 mg/kg of Cu from 29 to 53 d of age compared with those fed no supplemental Cu from 29 to 53 d of age, while broilers fed diets containing 270 mg/kg of Cu from 29 to 53 d of age had increased total breast weight compared with broilers fed diets containing 135 mg/kg of Cu from 29 to 41 d of age. This increase in breast meat yield may have occurred due to a reduction in the microbial challenge in the intestine, altered AA digestibility, or through the effects of metallothionein. Dietary Cu inclusion did not appear to influence the severity of wooden breast or white striping. These data indicate that there are benefits of maintaining high dietary concentrations of Cu from 29 to 53 d of age, primarily through the increase in breast meat yield.

# REFERENCES

Apgar, G. A., E. T. Kornegay, M. D. Lindemann, and D. R. Notter. 1995. Evaluation of copper sulfate and a copper lysine complex as growth promoters for weanling swine. J. Anim. Sci. 73:2640-2646.

Arias, V. J. and E. A. Koutsos. 2006. Effects of copper source and level on intestinal physiology and growth of broiler chickens. Poult. Sci. 85:999-1007.

Association of Official Analytical Chemists International. 2006. Official Methods of Analysis. 18th ed. Association of Official Analytical Chemists, Arlington, VA. 975.03B(b).

Aviagen. 2014. Ross 708: Broiler nutrition specifications. Aviagen, Huntsville, Alabama, USA. www.aviagen.com.

Brewer, V. B., V. A. Kuttappan J. L. Emmert J. F. C. Meullenet, and C. M. Owens. 2012. Big-bird programs: Effect of strain, sex, and debone time on meat quality of broilers. Poult. Sci. 91: 248-254.

Chee, S. H., P. A. Iji, M. Choct, L. L. Mikkelsen, and A. Kocher. 2010. Characterisation and response of intestinal microflora and mucins to manno-oligosaccharide and antibiotic supplementation in broiler chickens, Br. J. Poult. Sci. 51:368-380.

Cherian, M. G. 1994. The significance of the nuclear and cytoplasmic localization of metallothionein in human liver and tumor cells. Environ. Health Perspec. 102:131-135.

Cruz, R. F. A., S. L. Vieira, L. Kindlein, M. Kipper, H. S. Cemin, and S. M. Rauber. 2017. Occurrence of white striping and wooden breast in broilers fed grower and finisher diets with increasing lysine levels. Poult. Sci. 96:501-510.

Ewing, H. P., G. M. Pesti, R. I. Bakalli, and J. F. M. Menten. 1998. Studies on the feeding of cupric sulfate pentahydrate, cupric citrate and copper oxychloride to broiler chickens. Poult. Sci. 77:445-448.

Kim, G. B., Y. M. Seo, K. S. Shin, A. R. Rhee, J. Han, and I. K. Paik. 2011. Effects of supplemental copper-methionine chelate and copper-soy proteinate on the performance, blood parameters, liver mineral content, and intestinal microflora of broiler chickens. J. Appl. Poult. Res. 20:21-32.

Liao, S. F., T. Wang, and N. Regmi. 2015. Lysine nutrition in swine and the related monogastric animals: muscle protein biosynthesis and beyond. SpringerPlus. 4:1-12.

Nishimura, H., N. Nishimura, and C. Tohyama. 1989. Immunohistochemical localization of metallothionein in developing rat tissues. J. Histochem. Cytochem. 37:715-722.

Pang, Y. and T. J. Applegate. 2007. Effects of dietary copper supplementation and copper source on digesta pH, calcium, zinc, and copper complex size in the gastrointestinal tract of the broiler chicken. Poult. Sci. 86:531-537.

Pang, Y., J. A. Patterson, and T. J. Applegate. 2009. The influence of copper concentration and source on ileal microbiota. Poult. Sci. 88:586-592.

Pekel, A. Y., P. H. Patterson, R. M. Hulet, N. Acar, T. L. Cravener, D. B. Dowler, and J. M. Hunter. 2009. Dietary Camelina meal versus flaxseed with and without supplemental copper for broiler chickens: Live performance and processing yield. Poult. Sci. 88:2392-2398.

Petersen, S. T., J. Wiseman, and M. R. Bedford. 1999. Effects of age and diet on the viscosity of intestinal contents in broiler chicks. Br. Poult. Sci. 40:364-370.

Pfost, H. B. and R. N. Allen. 1962. A standard method of measuring pellet durability. Proceedings of Feed Production School, Midwest Feed Manuf. Assoc.

Philpot, S., K. Perryman, K. Macklin, and W. Dozier, III. 2018. Growth performance and carcass characteristics of broilers fed diets varying in amino acid densities and supplemental copper concentrations from 1 to 33 days of age. Poult. Sci. 97 (E-suppl 1):9.

Rochell, S. J., J. L Usry, T. M. Parr, C. M Parsons, and R. N. Dilger. 2017. Effects of dietary copper and amino acid density on growth performance, apparent metabolizable energy, and nutrient digestibility in Eimeria acerviulina-challenged broilers. Poult Sci. 96:602-610.

SAS Institute. 2017. SAS® User's Guide. Statistics. Version 9.4 ed. SAS Institute Inc., Cary, NC.

Tesseraud, S., R. Peresson, J. Lopes, and A. M. Chagneau. 1996. Dietary lysine deficiency greatly affects muscle and liver protein turnover in growing chickens. Br. J. Nutr. 75:853-865.

U.S. Department of Agriculture Agricultural Marketing Service. 2019. Broiler market news report. 66:1-4.

Wang, H., C. Zhang, Y. Mi, and M. T. Kidd. 2014. Copper and lysine amino acid density responses in commercial broilers. J. Appl. Poult. Res. 23:470-477.

Wlostowski, T. 1993. Involvement of metallothionein and copper in cell proliferation. BioMetals. 6:71-76.

Xia, M. S., C. H. Hu, and Z. R. Xu. 2004. Effects of copper-bearing montmorillonite on growth performance, digestive enzyme activities, and intestinal microflora and morphology of male broilers. Poult. Sci. 83:1868-1875.

Zhou, W., E. T. Kornegay, H. Van Laar, J. W. G. M. Swinkels, E. A. Wong, and M. D. Lindemann. 1994. The role of feed consumption and feed efficiency in copper-stimulated growth. J. Anim. Sci. 72:2385-2394.

**Table 4.1.** Ingredient composition, calculated nutrient composition, and analyzed copper concentration of diets fed to male Yield Plus × Ross 708 from 1 to 53 d of age

| Ingredient, % | Starter (1 to 19 d of age) | Grower (20 to 28 d of age) | Finisher 1 (29 to 41 d of age) Cu Concentration (mg/kg) | | | Finisher 2 (42 to 53 d of age) Cu Concentration (mg/kg) | | |
|---|---|---|---|---|---|---|---|---|
| | | | 0 | 135 | 270 | 0 | 135 | 270 |
| Corn | 54.76 | 59.53 | 63.50 | 63.44 | 63.39 | 64.92 | 64.86 | 64.81 |
| Soybean meal[1] | 35.90 | 30.30 | 25.04 | 25.05 | 25.06 | 22.80 | 22.81 | 22.82 |
| DDGS[1] | 4.00 | 5.00 | 6.00 | 6.00 | 6.00 | 7.00 | 7.00 | 7.00 |
| Corn Oil | 1.83 | 2.02 | 2.72 | 2.74 | 2.76 | 2.73 | 2.75 | 2.77 |
| Calcium carbonate | 1.33 | 1.06 | 1.05 | 1.05 | 1.05 | 1.05 | 1.05 | 1.05 |
| Dicalcium phosphate | 1.32 | 0.91 | 0.64 | 0.64 | 0.64 | 0.53 | 0.53 | 0.53 |
| Sodium chloride | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.29 | 0.29 | 0.29 |
| DL-Methionine | 0.30 | 0.27 | 0.23 | 0.23 | 0.23 | 0.20 | 0.20 | 0.20 |
| L-Lysine | 0.20 | 0.21 | 0.20 | 0.20 | 0.20 | 0.19 | 0.19 | 0.19 |
| Mineral premix[2,3] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Vitamin premix[4] | 0.10 | 0.08 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| L-Threonine | 0.10 | 0.09 | 0.07 | 0.07 | 0.07 | 0.05 | 0.05 | 0.05 |
| Choline[5] | 0.07 | 0.08 | 0.08 | 0.08 | 0.08 | 0.07 | 0.07 | 0.07 |
| Intellibond Cu[6] | 0.03 | 0.03 | --- | 0.03 | 0.05 | --- | 0.03 | 0.05 |
| Phytase[7] | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Econase[8] | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Calculated Nutrient Content (% unless otherwise indicated) | | | | | | | | |
| Crude Protein | 22.47 | 20.45 | 18.61 | 18.61 | 18.61 | 17.93 | 17.93 | 17.93 |
| $\text{AME}_n$ (kcal/kg)[9] | 3,000 | 3,100 | 3,185 | 3,185 | 3,185 | 3,200 | 3,200 | 3,200 |
| Digestible Lys | 1.22 | 1.09 | 0.97 | 0.97 | 0.97 | 0.91 | 0.91 | 0.91 |
| Digestible Met | 0.60 | 0.55 | 0.50 | 0.50 | 0.50 | 0.46 | 0.46 | 0.46 |
| Digestible Cys | 0.30 | 0.28 | 0.26 | 0.26 | 0.26 | 0.25 | 0.25 | 0.25 |
| Digestible Thr | 0.82 | 0.73 | 0.65 | 0.65 | 0.65 | 0.61 | 0.61 | 0.61 |

|  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|
| Digestible Trp | 0.23 | 0.21 | 0.18 | 0.18 | 0.18 | 0.17 | 0.17 | 0.17 |
| Digestible Arg | 1.34 | 1.19 | 1.04 | 1.04 | 1.04 | 0.98 | 0.98 | 0.98 |
| Digestible Ile | 0.85 | 0.76 | 0.67 | 0.67 | 0.67 | 0.64 | 0.64 | 0.64 |
| Digestible Val | 0.91 | 0.83 | 0.77 | 0.77 | 0.77 | 0.74 | 0.74 | 0.74 |
| Digestible Met + Cys | 0.90 | 0.83 | 0.76 | 0.76 | 0.76 | 0.71 | 0.71 | 0.71 |
| Calcium | 0.96 | 0.87 | 0.79 | 0.79 | 0.79 | 0.76 | 0.76 | 0.76 |
| Non Phytate P | 0.48 | 0.44 | 0.38 | 0.38 | 0.38 | 0.36 | 0.36 | 0.36 |
| Sodium | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 |
| Choline (mg/kg) | 1,700 | 1,600 | 1,500 | 1,500 | 1,500 | 1,400 | 1,400 | 1,400 |
| Copper (mg/kg)[10] | 149 | 148 | 12 | 147 | 282 | 12 | 147 | 282 |
|  |  |  |  |  |  |  |  |  |
| Analyzed Cu (mg/kg) | 139 | 146 | 14 | 149 | 292 | 18 | 145 | 280 |

---

[1] Soybean meal contained 47.5% crude protein. Dried distillers grains with solubles contained 5% ether extract.

[2] Mineral premix included per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (ethylenediaminedihydroxide), 1.4mg; Se (sodium selenite), 0.3 mg.

[3] Mineral premix and feed ingredients provided 14, 13, 12, and 12 mg/kg of Cu in the starter, grower finisher 1, and finisher 2 phases, respectively.

[4] Vitamin premix included per kg of diet: Vitamin A (Vitamin A acetate), 9,370 IU; Vitamin D (cholecalciferol), 3,300 IU; Vitamin E (DL-alpha tocopheryl acetate), 33 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.3 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 11 mg; niacin (niacinamide), 44 mg; thiamin (thiamin mononitrate), 2.7 mg; D-biotin (biotin), 0.09 mg; and pyridoxine (pyridoxine hydrochloride), 3.8 mg.

[5] Choline chloride-60 (Balchem Corporation, New Hamptopn, NY).

[6] IntelliBond (Micronutrients, Indianapolis, IN) was the source of basic copper chloride that contained 59.21% Cu.

[7] Quantum ® Blue 5G (AB Vista Feed Ingredients, Marlborough, UK) provides 1,000 FTU/kg phytase activity / kg diet.

[8] Econase® XT (AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 16,000 BXU of xylanase activity / 100 gram per ton inclusion.

[9] $AME_n$ = nitrogen-corrected apparent metabolizable energy.

[10] In the common starter and grower diets, 135 mg/kg of supplemental Cu from tribasic Cu chloride was added for total Cu concentrations of 149 and 148 mg/kg in the starter and grower phases, respectively. In the finisher 1 and 2 diets, supplemental Cu from tribasic Cu chloride provided a total of 147 and 282 mg/kg of Cu in the 135 and 270 mg/kg diets, respectively.

**Table 4.2**. Growth performance of Yield Plus × Ross 708 male broilers fed diets varying in supplemental copper concentration from 29 to 41 d of age[1]

| | Body weight, kg | Body weight gain, kg | Feed intake, kg | FCR[2], kg:kg | Mortality[3], % |
|---|---|---|---|---|---|
| Supplemental Cu concentration (mg/kg)[4] | | | | | |
| 0 | 3.288 | 1.468 | 2.459 | 1.710 | 2.3 |
| 135 | 3.308 | 1.468 | 2.453 | 1.655 | 1.7 |
| 270 | 3.328 | 1.492 | 2.464 | 1.663 | 1.7 |
| SEM[5] | 0.021 | 0.016 | 0.027 | 0.027 | 0.8 |
| | *Probabilities* | | | | |
| Supplemental Cu concentration | 0.24 | 0.16 | 0.96 | 0.25 | 0.63 |

[1] Values are least-square means of 12 replicate pens for treatments receiving 0 mg/kg of supplemental Cu, and 24 replicate pens for treatments receiving 135 or 270 mg/kg of supplemental Cu, with each pen having 21 chicks at placement. Birds were fed common starter (1 to 19 d of age) and grower (20 to 28 d of age) diets containing 135 mg/kg of Cu. Birds performed similarly ($P > 0.05$) from 1 to 28 d of age.

[2] Feed conversion ratio was corrected for mortality.

[3] Mortality values were arcsine transformed.

[4] All diets contained between 12 to 14 mg/kg of Cu from the trace mineral premix and feed ingredients.

[5] Pooled standard error.

**Table 4.3.** Growth performance of Yield Plus × Ross 708 male broilers fed diets varying in supplemental copper concentration from 42 to 53 days of age[1]

| | Body weight, kg | Body weight gain, kg | Feed intake, kg | FCR[2], kg:kg | Mortality[3], % |
|---|---|---|---|---|---|
| Supplemental Cu concentration (mg/kg)[4] | | | | | |
| 0-0 | 4.828 | $1.552^{ab}$ | 3.203 | $2.068^{ab}$ | 3.3 |
| 135-0 | 4.812 | $1.508^{b}$ | 3.179 | $2.117^{a}$ | 3.9 |
| 270-0 | 4.851 | $1.536^{ab}$ | 3.191 | $2.127^{a}$ | 3.6 |
| 135-135 | 4.922 | $1.631^{a}$ | 3.238 | $1.988^{b}$ | 4.0 |
| 270-270 | 4.889 | $1.552^{ab}$ | 3.106 | $2.011^{ab}$ | 4.3 |
| SEM[5] | 0.035 | 0.027 | 0.046 | 0.032 | 1.3 |
| | *Probabilities* | | | | |
| Supplemental Cu concentration | 0.12 | 0.030 | 0.35 | 0.010 | 0.71 |

[1] Values are least-square means of 12 replicate pens for treatments receiving 0 mg/kg of supplemental Cu, and 24 replicate pens for treatments receiving 135 or 270 mg/kg of supplemental Cu, with each pen having 21 chicks at placement. Birds were fed common starter (1 to 19 d of age) and grower (20 to 28 d of age) diets containing 135 mg/kg of Cu. Birds performed similarly ($P > 0.05$) from 1 to 28 d of age.

[2] Feed conversion ratio was corrected for mortality.

[3] Mortality values were arcsine transformed.

[4] Supplemental Cu above the Cu in the trace mineral premix and feed ingredients, represented as Finisher 1-Finisher 2 Cu mg/kg. All diets contained between 12 to 14 mg/kg of Cu from the trace mineral premix and feed ingredients.

[5] Pooled standard error.

**Table 4.4.** Growth performance of Yield Plus × Ross 708 male broilers fed diets varying in supplemental copper concentration from 29 to 53 days of age[1]

| | Body weight, kg | Body weight gain, kg | Feed intake, kg | FCR[2], kg:kg | Mortality[3], % |
|---|---|---|---|---|---|
| Supplemental Cu concentration (mg/kg)[4] | | | | | |
| 0-0 | 4.828 | 2.996 | 5.690 | 1.890 | 4.5 |
| 135-0 | 4.812 | 2.989 | 5.604 | 1.877 | 5.7 |
| 270-0 | 4.851 | 3.009 | 5.698 | 1.892 | 5.4 |
| 135-135 | 4.922 | 3.089 | 5.696 | 1.845 | 5.8 |
| 270-270 | 4.889 | 3.059 | 5.589 | 1.834 | 5.7 |
| SEM[5] | 0.035 | 0.032 | 0.074 | 0.019 | 1.3 |
| | | | *Probabilities* | | |
| Supplemental Cu concentration | 0.12 | 0.09 | 0.66 | 0.08 | 0.95 |

[1] Values are least-square means of 12 replicate pens for treatments receiving 0 mg/kg of supplemental Cu, and 24 replicate pens for treatments receiving 135 or 270 mg/kg of supplemental Cu, with each pen having 21 chicks at placement. Birds were fed common starter (1 to 19 d of age) and grower (20 to 28 d of age) diets containing 135 mg/kg of Cu. Birds performed similarly ($P > 0.05$) from 1 to 28 d of age.

[2] Feed conversion ratio was corrected for mortality.

[3] Mortality values were arcsine transformed.

[4] Supplemental Cu above the Cu in the trace mineral premix and feed ingredients, represented as Finisher 1-Finisher 2 Cu mg/kg. All diets contained between 12 to 14 mg/kg of Cu from the trace mineral premix and feed ingredients.

[5] Pooled standard error.

**Table 4.5.** Growth performance of Yield Plus × Ross 708 male broilers fed diets varying in supplemental copper concentration from 1 to 53 days of age[1]

| | Body weight, kg | Body weight gain, kg | Feed intake, kg | FCR[2], kg:kg | Mortality[3], % |
|---|---|---|---|---|---|
| Supplemental Cu concentration (mg/kg)[4] | | | | | |
| 0-0 | 4.828 | 4.786 | 8.074 | 1.683 | 9.8 |
| 135-0 | 4.812 | 4.769 | 7.994 | 1.676 | 9.8 |
| 270-0 | 4.851 | 4.808 | 8.145 | 1.682 | 9.5 |
| 135-135 | 4.922 | 4.879 | 8.109 | 1.662 | 9.9 |
| 270-270 | 4.889 | 4.846 | 7.991 | 1.658 | 10.2 |
| SEM[5] | 0.035 | 0.035 | 0.076 | 0.011 | 1.4 |
| | | | *Probabilities* | | |
| Supplemental Cu concentration | 0.12 | 0.12 | 0.50 | 0.34 | 0.99 |

[1] Values are least-square means of 12 replicate pens for treatments receiving 0 mg/kg of supplemental Cu, and 24 replicate pens for treatments receiving 135 or 270 mg/kg of supplemental Cu, with each pen having 21 chicks at placement. Birds were fed common starter (1 to 19 d of age) and grower (20 to 28 d of age) diets containing 135 mg/kg of Cu. Birds performed similarly ($P > 0.05$) from 1 to 28 d of age.

[2] Feed conversion ratio was corrected for mortality.

[3] Mortality values were arcsine transformed.

[4] Supplemental Cu above the Cu in the trace mineral premix and feed ingredients, represented as Finisher 1-Finisher 2 Cu mg/kg. All diets contained between 12 to 14 mg/kg of Cu from the trace mineral premix and feed ingredients.

[5] Pooled standard error.

**Table 4.6.** Carcass characteristics of Yield Plus × Ross 708 male broilers fed diets varying in supplemental copper concentration from 29 to 53 days of age[1]

| | Carcass | Carcass yield[2] | Ab. Fat[3] | Ab. Fat percentage[2,3] | Fillet | Fillet yield[2] | Tenders | Tender yield[2] | Total Breast[4] | Total breast yield[2] | Back half[5] | Wings[2] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Kg | % | kg | % | kg | % | kg | % | kg | % | kg | kg |
| Supplemental Cu concentration (mg/kg)[6] | | | | | | | | | | | | |
| 0-0 | 3.773 | 76.7$^{b}$ | 0.046$^{a}$ | 0.92$^{a}$ | 1.154$^{b}$ | 23.2$^{b}$ | 0.215 | 4.350 | 1.372$^{ab}$ | 27.6$^{b}$ | 1.445 | 0.373 |
| 135-0 | 3.767 | 76.8$^{ab}$ | 0.041$^{b}$ | 0.83$^{b}$ | 1.144$^{b}$ | 23.5$^{b}$ | 0.212 | 4.353 | 1.355$^{b}$ | 27.7$^{ab}$ | 1.420 | 0.369 |
| 270-0 | 3.794 | 76.9$^{ab}$ | 0.043$^{ab}$ | 0.88$^{ab}$ | 1.158$^{ab}$ | 23.6$^{ab}$ | 0.217 | 4.399 | 1.376$^{ab}$ | 28.0$^{ab}$ | 1.429 | 0.370 |
| 135-135 | 3.799 | 77.1$^{ab}$ | 0.044$^{ab}$ | 0.89$^{ab}$ | 1.164$^{ab}$ | 23.7$^{ab}$ | 0.217 | 4.321 | 1.384$^{ab}$ | 28.1$^{ab}$ | 1.424 | 0.371 |
| 270-270 | 3.831 | 77.3$^{a}$ | 0.044$^{ab}$ | 0.90$^{ab}$ | 1.186$^{a}$ | 23.9$^{a}$ | 0.217 | 4.367 | 1.401$^{a}$ | 28.1$^{a}$ | 1.432 | 0.374 |
| SEM[7] | 0.024 | 0.133 | 0.001 | 0.022 | 0.008 | 0.129 | 0.003 | 0.042 | 0.010 | 0.151 | 0.009 | 0.004 |
| | | | | | | *Probabilities* | | | | | | |
| Supplemental Cu concentration | 0.07 | 0.001 | 0.004 | 0.016 | 0.003 | 0.005 | 0.34 | 0.91 | 0.010 | 0.017 | 0.23 | 0.69 |

[1] Values are least-square means of 12 replicate pens for each treatment, with each pen having 22 chicks at placement. Birds were fed common starter (1 to 19 d of age) and grower (20 to 28 d of age) diets containing 135 mg/kg of Cu.

[2] Carcass yield was calculated as the percentage of live weight at 53 days of age. All other yields and percentages were calculated as percentages of the carcass weight.

[3] Ab. Fat = abdominal fat pad weight and percentage.

[4] Total breast = breast + tenders

[5] Back half consisted of the two thigh quarters

[6] Supplemental Cu above the Cu in the trace mineral premix and feed ingredients, represented as Finisher 1-Finisher 2 Cu mg/kg. All diets contained between 12 to 14 mg/kg of Cu from the trace mineral premix and feed ingredients.

[7] Pooled standard error.

**Table 4.7.** Breast fillet quality defect scores in Yield Plus × Ross 708 male broilers fed diets varying in supplemental copper concentration from 29 to 53 days of age[1,2]

| | Striping Proportion[3,4] | | | | Hardness Proportion[3,4] | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 |
| Supplemental Cu concentration (mg/kg)[5] | | | | | | | | |
| 0-0 | 0.00 | 22.88 | 52.03 | 25.09 | 8.13 | 37.11 | 37.18 | 20.26 |
| 135-0 | 0.69 | 16.32 | 54.08 | 29.37 | 7.26 | 34.66 | 36.46 | 23.37 |
| 270-0 | 0.64 | 25.00 | 46.28 | 26.41 | 6.09 | 43.82 | 35.64 | 16.41 |
| 135-135 | 0.00 | 21.89 | 48.76 | 29.35 | 5.25 | 35.15 | 35.18 | 22.24 |
| 270-270 | 0.00 | 19.46 | 52.24 | 27.70 | 7.61 | 29.75 | 38.62 | 24.49 |
| SEM[6] | 0.42 | 3.87 | 4.55 | 3.05 | 2.24 | 3.49 | 4.12 | 3.07 |
| | *Probabilities* | | | | | | | |
| Supplemental Cu concentration | 0.42 | 0.18 | 0.67 | 0.81 | 0.08 | 0.95 | 0.96 | 0.25 |

[1] Values are least-square means of 12 replicate pens for each treatment, with each pen having 22 chicks at placement. Birds were fed common starter (1 to 19 d of age) and grower (20 to 28 d of age) diets containing 135 mg/kg of Cu.

[2] Broilers were processed at 54 d of age.

[3] Proportions were arcsine transformed prior to analysis.

[4] Striping and Hardness were measured on a scale of 0 to 3, where 3 was the most severe.

[5] Supplemental Cu above the Cu in the trace mineral premix and feed ingredients, represented as Finisher 1-Finisher 2 Cu mg/kg. All diets contained between 12 to 14 mg/kg of Cu from the trace mineral premix and feed ingredients.

[6] Pooled standard error.

# V. CONCLUSIONS

The food service industry is increasing demand for food production animals raised without the use of antibiotics. This has led to reduced use of subtherapeutic antibiotics as growth promotors. Thus, the broiler industry is evaluating alternative strategies to maintain growth efficiency afforded by subtherapeutic antibiotics. Previous research has shown that feeding high concentrations of dietary Cu may be effective in enhancing the growth of broilers; however, knowledge gaps exist on employing this strategy.

Experiment 1 evaluated the interactive effects of dietary amino acid (**AA**) density and different feeding schedules of high concentrations of Cu on growth performance, processing characteristics, and ileal microflora composition in broilers from 1 to 32 d of age. Cumulative feed conversion ratio was decreased 2.3 points through a reduction in feed intake in broilers fed 270 mg/kg of Cu from 1 to 25 d of age and 135 mg/kg from 26 to 32 d of age compared with those fed 135 mg/kg supplemental Cu from 1 to 32 d of age. Broilers fed 270 mg/kg of Cu from 1 to 25 d of age and 135 mg/kg from 26 to 32 d of age had lower carcass weights and yields as well as decreased drum and wing weight compared with those fed 135 mg/kg of Cu from 1 to 32 d of age. This resulted in a 91 g decrease of feed intake in broilers fed diets containing 270 mg/kg of Cu from 1 to 25 d of age and 135 mg/kg from 26 to 32 d of age compared with birds provided diets with 135 mg/kg Cu from 1 to 32 d of age, leading to a decrease in digestible AA intake. Few interactions were observed between AA density and Cu concentration for either growth performance or carcass characteristics. Broilers fed high AA density diets had consistently higher processing weights and yields compared with broilers fed low AA density diets. The decrease in feed conversion ratio caused by Cu supplementation is often attributed to the bacteriostatic properties of Cu. In this study, biological differences in the proportions of bacteria in the ileum were observed, but no statistical differences were detected.

Experiment 2 assessed the effects of dietary Cu concentration and AA density on AA digestibility of broilers from 1 to 14 d of age. Interactions were observed where broilers fed moderate AA density diets had increased digestibility of Lys, Val, His, and Arg when supplemented with 270 mg/kg of Cu compared with 135 mg/kg of Cu, while AA digestibility of broilers fed low AA density diets was not affected by Cu concentration. This could be due to Cu causing a shift in the microbial use of AA, or through increased dietary fat supplementation between the moderate and low AA diets causing a change in the rate of feed passage, which would affect digestibility. Copper inclusion at either 135 or 270 mg/kg caused a decrease in Cys digestibility compared with the negative control. The decrease in Cys digestibility is likely due to its role as a component of metallothionein, a protein utilized in the metabolism of heavy metals. Metallothionein is rich in Cys, so Cys is excreted during Cu metabolism, reducing apparent digestibility.

Experiment 3 ascertained the effects of feeding broilers high concentrations of Cu from 29 to 53 d of age on growth performance and processing characteristics. Broilers were provided with diets formulated to contain 95% of the AA specifications from the primary breeder guidelines throughout the experiment and 135 mg/kg of Cu from 1 to 28 d of age. Carcass and total breast meat yield were increased in broilers fed diets containing 270 mg/kg of Cu from 29 to 53 d of age compared with those fed no supplemental Cu. Furthermore, total breast meat weight was increased 46 g in broilers fed 270 mg/kg of Cu from 29 to 53 d of age compared with those fed 135 mg/kg from 29 to 41 d of age and 0 mg/kg supplemental Cu from 42 to 53 d of age. This may have been due to altered AA digestibility or induced through the effects of metallothionein. Metallothionein has been shown to be present in higher concentrations in cells going through rapid growth in development. Thus, it may play a role in increasing muscle accretion.

Overall, these findings support the utilization of pharmacological concentrations of Cu beyond the starter period for broilers fed to either 32 or 53 d of age. This is contrary to common practice in industry where broilers are often fed high concentrations of Cu during the starter phase only, due to the cost of Cu supplementation. Despite the different responses in broilers raised to 32 or 53 d of age, profits on birds raised to approximately 2.5 or 4.0 kg may be increased through including high concentration of dietary Cu throughout the grow-out period. While the mechanism of action of pharmacological Cu is still unknown, these studies indicate that it may be due to an alteration of AA digestibility, or that it could be mediated through the production of metallothionein. Additional research is warranted to better elucidate whether the mechanism of action of Cu is through increasing digestibility, altering microflora, or through another mechanism. The information gained from these experiments can help the poultry industry maintain growth efficiency to meet performance objectives.