ABSTRACT

QUDSIEH, RASHA ISSA. Growth Performance and Meat Quality of Broiler Chickens Fed Diets Supplemented with Different Levels of Trace Minerals. (Under the direction of Drs. Douglas P. Smith and John T. Brake).

Red discoloration of fully cooked poultry products has been a sporadic yet chronic problem. Zinc has been reported to replace iron in myoglobin in an irreversible reaction that has created a red pigment. Many poultry producers add Zn and Cu to feed, sometimes over supplementing 50-200% as these minerals have been perceived to be important for broiler performance. Therefore, this project evaluated whether Zn or Cu in broiler feed, at normal or high levels, caused an increased red discoloration in cooked product. These studies were conducted with broilers to determine: 1) the effect of feeding different levels of inorganic dietary zinc (Zn), copper (Cu), and Roxarsone (As) on growth performance, carcass and meat quality, and blood zinc protoporphyrin/heme ratio (ZPP/H); and 2) the effect of adding high levels of inorganic dietary zinc (Zn) on growth performance, carcass and meat quality, and blood ZPP/H ratio. In chapters 2 and 3, 1152 broilers were sexed and grown in 72 pens by sex and treatment (varied dietary levels of Zn, Cu, and As) to 56 d on litter. Results presented in chapters 2 and 3 showed that males in comparison to females had increased BW, BW gain, feed intake and mortality-adjusted feed conversion ratio (AdjFCR) but females had lower mortality. Supplementation of 240 mg/kg Zn improved AdjFCR in the starter period of males and grower period of females. Supplementation of 100 mg/kg Cu was beneficial for females in the finisher period. Roxarsone supplementation improved AdjFCR only in the finisher period. Minerals did not affect muscle redness. In chapters 4 and 5, 288 broilers were sexed

and raised in battery cages (to minimize availability of minerals from litter) to 42 d. Dietary Zn had no effect on feed intake of males, however, BW gain and AdjFCR measured from 1-42 d was improved in males fed diets supplemented with 120 mg Zn/kg. Dietary Zn had no effect on BW, feed intake, BW gain, or AdjFCR of females. Dietary Zn had no effect on carcass weight and parts yield other than breast fillet weights that were increased in males when Zn was added to the diets. Breast fillets cook yield and tenderness were not influenced by either sex or Zn. Raw breast fillets color was influenced by sex where males fillets were darker, more red, and less yellow color. Raw thigh color was also lighter and less yellow compared to females. Raw marrow color measured immediately after harvest was more red and yellow in females. Lightness (L^*) and redness (a^*) of raw and cooked bone marrow collected from males was influenced by Zn where L^* was greater at 120 mg Zn/kg for both raw and cooked marrow, while a^* of cooked marrow was greater at 240 mg Zn/kg. Dietary Zn had no effect on the measured ZPP/H ratio except at 28 d where it was numerically less at 120 mg Zn/kg. Results indicated that supplementing high levels of dietary Zn did not improve live performance of broiler females while 120 mg Zn/kg improved performance in males. Also, supplementing 120 and 240 mg Zn/diet improved breast tender size and increased marrow redness. Fe levels in the body, diet, and litter could have been sufficient to prevent replacement of Zn in the myoglobin and hemoglobin. It was also possible that levels of myoglobin and hemoglobin in chicken breast and thigh were not great enough to result in a clear effect. However, the increase in marrow redness due to Zn demonstrated the possibility that muscle in contact with bones could be affected with higher redness values.

Growth Performance and Meat Quality of Broiler Chickens Fed Diets Supplemented with Different Levels of Trace Minerals

by
Rasha Issa Qudsieh

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APPROVED BY:

Dr. Douglas P. Smith Committee Co-Chair	Dr. John T. Brake Committee Co-Chair
Dr. Julie Northcutt	Dr. Sophia Kathariou

DEDICATION

I dedicate this dissertation to my beloved husband Basheer, who supported and helped me in each step of the way. A special feeling of gratitude is extended to my loving family; my late father Issa, my mother Amal, my sisters Ruba and Basma, and my brothers Hassan and Muhannad.

BIOGRAPHY

Rasha Qudsieh is the first of the five siblings of Issa Qudsieh and Amal Sadieh. She was born in Saudi Arabia, and lived there for eight years before she moved to Jordan with her family where she continued her education. She obtained her Bachelor of Science degree in Animal Science from Jordan University of Science and Technology in 2001 where she ranked third place among the graduates. She worked as a research assistant at Jordan University of Science and Technology after earning her BSc. She received a Master's degree in Animal Science with Meat Science concentration in 2006 with her research mainly focused on carcass composition and meat quality of Awassi sheep as influenced by age. In 2008, she decided to shift gears and worked as a teacher of mental arithmetic. In 2010, she and her husband decided to move to the USA to pursue their PhD degrees from North Carolina State University where she joined Dr. Smith's lab and worked on the effect of trace mineral supplementation on broiler meat quality while earning a minor in food safety. She hopes to use her expertise to improve the safety and quality of meat and poultry products.

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LITERATURE REVIEW

1.1. Introduction

The poultry industry has witnessed significant improvements over the past several decades. During the past 60 years, the amount of time and quantity of feed per pound of meat required to reach broiler market weight had been reduced by 50% (Anthony, 1998) which has resulted from a combination of genetic improvement and progress in nutrition and poultry management. Furthermore, consumers have shifted from the consumption of whole chicken toward portioned (especially breast fillets) and further processed products (Mehaffey et al., 2006; Abdullah et al., 2010). The poultry industry has responded to these changing demands by further enhancing genetic selection for increased breast yield, faster growth rate, and improved feed efficiency. During the same period of time, feed cost has increased, and ethanol production has forced producers to use alternative feed ingredients such as the distiller's dried grains with solubles (DDGS) which are produced as byproducts of ethanol production. Coincidently, several studies have shown that enhancements achieved through genetic selection have resulted in a predisposition towards an increased incidence of abnormalities, mainly in breast muscles (Dransfield and Sosnicki, 1999; Mahon, 1999). In poultry meat, appearance and texture have been considered to be the two important attributes responsible for initial consumer meat evaluation as well as final product acceptance (Fletcher, 2002), so consumers were expected to reject meat with observed defects such as bruises and hemorrhages. Several color defects have been reported in the poultry industry such as pinking of raw and cooked meat, bone darkening, red/bloody discoloration, white striping, wooden breast, and pale, soft, exudative appearance of breast meat. Many of the underlying causes of color defects have not been fully explained.

It has been a common practice in the US poultry industry to add zinc and copper trace minerals to feed at levels 50-200% higher than recommended since they were expected to have beneficial effects other than meeting nutritional requirements (Pesti and Bakalli, 1996; Batal et al., 2001). On the other hand, zinc has been shown to cause irreversible red pigment in Italian (Parma) ham (Wakamatsu et al., 2004). During a long curing process, zinc present in the tissue replaced the iron in myoglobin and any hemoglobin from residual blood. The resulting zinc protoporphyrin molecule (ZPP) has been shown to be heat stable red pigment. Red/bloody discoloration of broiler raw and cooked white meat could be related to mineral supplementation and the formation of ZPP pigment. Therefore, the effects of trace mineral supplementation on broiler breast meat quality need to be investigated.

1.2. Poultry meat production and consumer trends in US

The United States was considered to be the world's largest poultry producer and the second-largest egg producer and exporter of poultry meat. According to the USDA National Agricultural Statistics Service (NASS), the poultry certified wholesome during July 2014 totaled 3.89 billion pounds based on ready to cook weight, which was 1 % greater than the amount certified in July 2013. The average live weight of young chicken slaughtered during July 2014 was 2 % greater than reported in July 2013 and averaged 5.95 pounds per bird, while turkeys slaughtered during July 2014 averaged 30.1 pounds per bird, up 1 percent from July 2013.

U.S. poultry meat production has been dominated by broiler meat. Most of the remainder was turkey meat, and a small fraction was other chicken meat. According the USDA Economic Research Service, poultry production based on ready to cook weight increased from 1,381 million pounds in 1950 to slightly over 38 million pounds in 2014 (Figure 1). In the early 1960s, over 80 percent of broiler production was marketed as whole birds, and only 2 percent was further processed products (Figure 2). However, this changed over time, and by 2011, only 12 percent of production was marketed as whole birds, as production shifted to cut-up parts and further processed products such as boneless chicken, breaded nuggets and tenders, and chicken sausages. Consumer preference has also shifted toward broiler breast meat compared to other cuts, which has led the poultry industry to produce larger, higher-yielding birds to meet these demands. In 2009, 88% of the broilers were marketed as cut-up parts and further processed. This percentage was expected to increase in the future (NCC, 2011b).

Poultry meat production and consumption have increased rapidly worldwide, and was expected to continue to grow (Cavani et al., 2009) due to its relatively low price compared to other meats, the absence of cultural or religious obstacles, and its dietary and nutritional properties as it has lower fat, cholesterol, and sodium content (Magdelaine et al., 2008).

This increased demand and the shift toward portioned and further processed products has been accompanied by genetic improvement and progress in nutrition and poultry management, where meat-type chickens have increased growth rates and improved feed efficiency. According to the National Chicken Council (NCC, 2011a) estimates of US broiler performance published in 2011, the feed to meat gain was 4.70 in 1925 and has decreased to 1.91 in 2011. However, since the selection of broiler chickens initially focused on increasing growth performance and improving body composition (Berri et al., 2001), this has led to indirect and often deleterious effects on meat quality traits, such as excessive deposition of abdominal fat, the formation of which represented inefficient use of feed (Abasht et al., 2006; Nadaf et al., 2007).

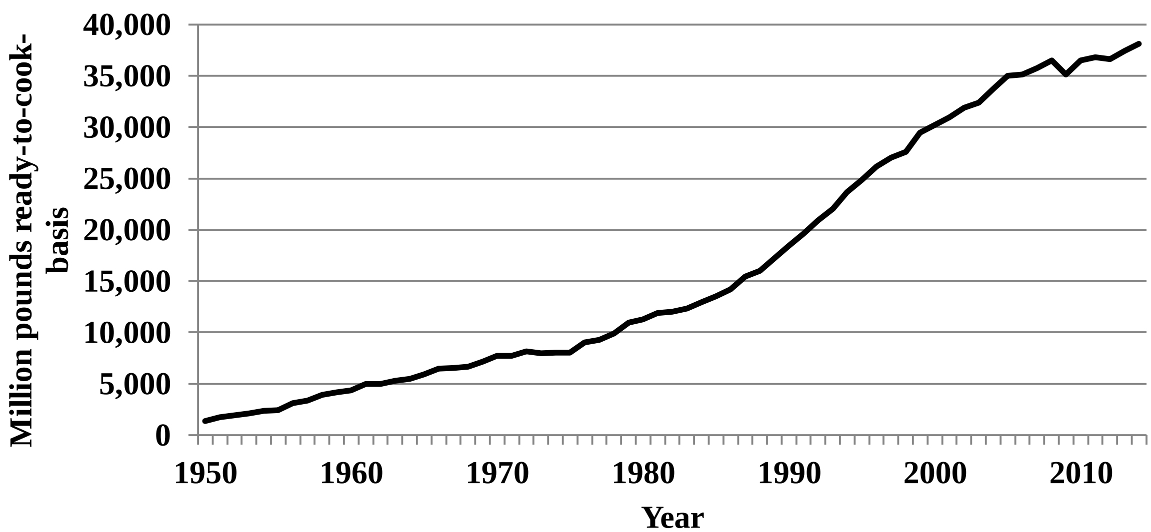

Figure I.1. US poultry meat production (USDA Economic Research Service)

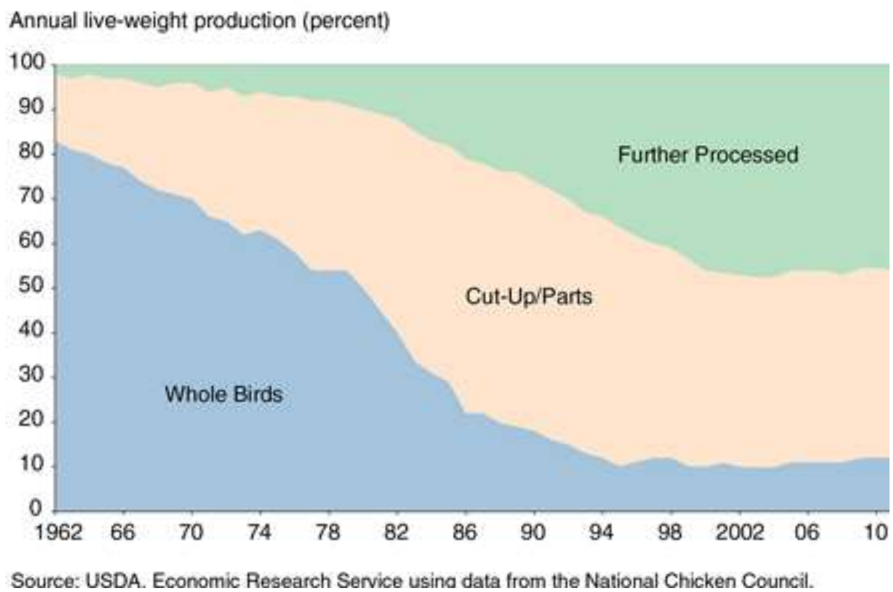

Figure I.2. Percentage of annual live weight poultry production represented according to final product marketed (Technology, Organization, and Financial Performance in U.S. Broiler Production, ERS report, EIB-126, June 2014)

1.3. Skeletal muscle structure

The basic structural unit of a muscle has been defined as the muscle fiber (Figure 3), which constituted of several myofibrils (contractile units). Each muscle fiber was surrounded by a connective tissue called the endomysium, muscle fibers were then grouped into fascicles and surrounded by another layer of connective tissue called the perimysium, then the whole muscle was made up of group of fascicles and was surrounded by epimysium that connected the muscle to bones. Collagen was the major constituent of these connective tissues. Muscle development and subsequent meat quality were influenced by these connective tissues. Skeletal muscles growth was achieved by increasing the size of preexisting muscle fibers (hypertrophy). Fiber number, size, and type varied with function and anatomical location of the muscle. Meat quality was also affected by these factors. A muscle that contained high proportion of oxidative fibers tended to have red color due to a greater amount of myoglobin (e.g. thigh muscles) as compared to glycolytic fibers, which tended to appear white in color that affected the appearance of muscle/meat (e.g. chicken breast muscle). Glycolytic fibers were larger and had a lower rate of protein turnover. Therefore, the white muscles were larger and more efficient. In poultry, genetic selection for increased breast yield resulted in pale breast meat color in broilers (Le Bihan-Duval et al., 1999), ducks (Baeza et al., 1997), and turkeys (Sante et al., 1991), which could result in poor meat quality.

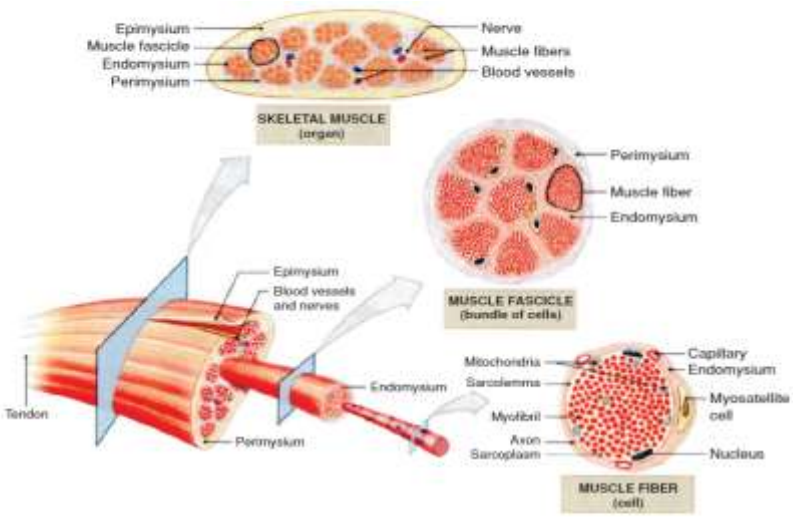

Figure I.3. Skeletal muscle organizational structure (Brandebourg, 2013; modified from Wood et al. (2010))

Collagen is the most abundant protein in the body and in connective tissues. The structure of collagen supported its function of providing strength to muscle and other tissues. Glycine constituted about one-third of all the amino acids found in collagen, while proline, which has been classified as an imino acid, and its analog hydroxyproline also constituted about one-third of all amino acids in collagen (Ramachandrana et al., 1973). Lysine has been considered to be another constituent of collagen where both proline and lysine were covalently modified to hydroxyproline and hydroxylysine, respectively. A collagen molecule (tropocollagen) was shown to be composed of three left-handed polypeptide helices coiled around each other to form a right-handed supercoil where glycine was found at every third residue. Strength of the collagen fibrils was due to the covalent bonds formed between and within tropocollagen triple helices, where collagen was cross-linked by lysine side chains that contributed to the strength of the collagen in meat that had an important role in development of meat tenderness (Alnaqeeb et al., 1984).

1.4. Conversion of muscle to meat

The process of converting muscle to meat has started with killing the bird. Upon exsanguination the blood/oxygen supply was removed, the muscle tried to maintain its functions even after oxygen depletion through anaerobic glycolysis of its glycogen reserves to produce ATP but with the absence of blood supply to remove the waste, heat and lactic acid accumulated in the muscle and decreased pH. Due to ATP depletion, the muscle remained contracted as a result of actin and myosin binding that resulted in muscle stiffness. This marked the onset of rigor mortis and conversion of muscle to meat where muscle proteins start to denature due to high temperature and low pH. Temperature and pH were the main postmortem factors influencing meat quality through affecting the onset and progression of rigor mortis and subsequent resolution (Wulf and Page, 2000; Cavitt and Sams, 2003; Jensen et al., 2004; White et al., 2006). During resolution, proteolysis of Z-disk proteins took place, and myofibrillar proteins were degraded into myofibrillar fragments by proteolytic enzymes that affect meat tenderness. In chickens, the process of converting muscle to meet has been found to start immediately after slaughter and be resolved within 2-4 hours. The extent of meat tenderization postmortem could be altered by the conditions under which the meat was processed. Factors have included temperature and duration of chilling, deboning time, postchill aging/holding duration, and marination.

1.5. Poultry meat quality

Meat quality has been a collective term used to describe indicators of a meat product wholesomeness and freshness, such as color, texture, flavor, pH, and juiciness. The two most important quality attributes for poultry meat were appearance and texture since they influenced initial consumer selection of the product as well as final satisfaction (Fletcher, 2002). Appearance quality attributes have included skin color, meat color, and appearance defects such as bruises and hemorrhages. Any deviation from a normal appearance has resulted in meat product rejection, subsequently leading to consumer complaints. Despite the importance of these quality attributes, the poultry grading system used was still based on aesthetic attributes such as conformation, presence or absence of carcass defects, bruises, missing parts, and skin tears, without taking into consideration the functional properties of meat (Barbut, 1996), which have been important for the further processing industry that was mainly interested in the functional properties of meat. Many factors have influenced poultry meat quality, including sex, strain, age, environmental factors, exercise, diet, and processing practices (i.e. chilling, deboning time, marination, and electrical stunning) (Lyon and Wilson, 1986; Braxton et al., 1996; Poole et al., 1999; Northcutt et al., 2001).

Poultry meat tenderness was one of the most important meat quality attributes related to consumer acceptance. Tenderness development was a function of myofibrillar proteins denaturation, connective tissue content, and juiciness. Deboning time, age, and strain were some of the major factors that affected poultry meat tenderness (Northcutt et al., 2001; Cavitt et al., 2004).

Lyon and Lyon (1990) reported that as time before deboning increased from 0 to 24 h postmortem, consumer acceptability of the meat texture increased, with fillets deboned at 0 and 2 h postmortem considered tough by a consumer panel, and samples deboned at 6 and 24 h postmortem considered slightly tender to moderately tender. Liu et al. (2004) reported decreased shear force of chicken breast as deboning time increased from 2 to 24 h postmortem. Similar results were also reported by Cavitt et al. (2005). Mehaffey et al. (2006) reported that fillets deboned 2 or 4 h postmortem from broilers raised to 7 wk were significantly more tough than those raised to 6 wk, indicating that age affected tenderness when deboning was performed shortly after harvest. Northcutt et al. (2001) reported that breast fillets harvested at less than 2 h postchill aging were more tender when taken from broilers slaughtered at 42 or 44 d of age compared to those harvested from birds 49 or 51 d of age, irrespective of any sex effect. On the other hand, Young et al. (2001) reported that females had greater fillet yields than males.

Connective tissue content has been reported to increase with age, collagen was the most abundant protein in the body and made up the majority of the connective tissue proteins (Hultin, 1985; Bechtel, 1986). In young broilers (6 to 8 wk), connective tissue did not affect tenderness since mature cross-links had not yet formed between tropocollagen molecules, which were the structural unit of collagen fibril. On the other hand, contraction of myofibrillar protein, which depended upon time and rate of rigor mortis development after the bird was killed, was related to processing rather than intrinsic factors (Fletcher, 2002).

Another important meat quality attribute was meat juiciness or water holding capacity, which refered to the ability of raw meat to retain its inherent water during force application and/or processing (Hamm, 1960). Water in muscle has been divided into three general types: bound, immobilized, and free. Bound water was held tightly via myofibrillar protein charges and represented 4 to 5 % of water in muscle (Hamm, 1960; Cooke and Wein, 1971), it was resistant to freezing, and could only be removed by severe drying processes not including conventional cooking (Fennema, 1996). Immobilized water was found within the muscle ultrastructure (within the space between actin and myosin), but was not bound to myofibrillar proteins as in the case of bound water. Immobilized water accounted for the largest portion of muscle bound water (88 to 95%). Free water was held within muscle by weak capillary force (Lawrie, 1991).

1.6. Poultry meat color

Poultry has been determined to be the only species known to have muscles/parts with clear differences in color as meat from poultry has been classified as either white or dark. In chicken, fresh raw breast meat has been expected to have a pale pink color, while raw thigh and leg meat were expected to be darker and redder. Meat color has played a major role in consumer purchase decisions (Mancini and Hunt, 2005; Mitsumoto et al., 2005; Ramirez and Cava, 2007). Consumers have tended to associate color with flavor, tenderness, safety, storage time, nutritional value and satisfaction level (Pedreschi et al., 2006) and as an indicator of freshness and wholesomeness.

Meat color was what the human eye see as light was reflected from the meat surface. Poultry meat absorbed most of blue and green color spectrum and reflected most of the yellow, orange, and red color spectrum, which was perceived by the human eye. The most commonly used colorimetric scale has been the CIE Lab (International Commission on Illumination, 1986), even though other color scales have been used, such as the Hunter L, a, b and YXZ space. However, accuracy of these instruments has depended upon thickness, background color, and illuminant wavelengths (Sandusky and Heath, 1996; Bianchi and Fletcher, 2002).

The CIE Lab system components measured include $L*$ that referred to lightness and had a range from 0 to 100 (black to white), component $a*$ had a range from -60 to +60 (green if negative to red if positive), and $b*$ has the same range as $a*$ (blue if negative to yellow if positive) (Papadakis et al., 2000; Yam and Papadakis, 2004). Another recent system used for color measurement was the computer vision system, which has been shown to give reproducible results with the ability to measure color of the entire sample instead of specific spots as has been the case with widely used colorimeters (Girolami et al., 2013).

Meat color was mainly due to its myoglobin pigment present in the muscle fibers. Myoglobin consisted of a protein (globin) and a non-protein heme ring which had an iron molecule in its center, iron has the ability to bind one of several ligands (e.g. oxygen, carbon monoxide, and nitric oxide) on its sixth coordination site. The form of myoglobin (deoxymyoglobin, oxymyoglobin, carboxymyoglobin, and metmyoglobin) differed depending upon the ligand bound to iron and on the redox state of the iron, thus, myoglobin and iron state were the two main ways through which meat color changes.

The flow diagram below (Figure 4) illustrates the colors of the different myoglobin states. Myoglobin (Deoxymyoglobin) had a red purple color in its nature when not bound to any ligands, the state of myoglobin changed to oxymyoglobin when oxygen was added and to carboxymyoglobin when carbon dioxide was added. In both forms the color was bright red (bloom) and iron was in the reduced ferrous form (Fe^{++}). Oxidation of myoglobin changed the form to metmyoglobin and the iron was in the oxidized ferric form (Fe^{+++}) which had a brown color, these myoglobin color changes were reversible, however, if heat-treated, metmyoglobin was denatured and color changed irreversibly to grayish-brown. Curing with nitrites/nitrates caused an irreversible color change to red color that upon heating converted to pink. Replacement of iron with zinc resulted in a stable red color of myoglobin due to the formation of Zn-protoporphyrin IX, which has been shown to give Parma ham its stable bright red color (Wakamatsu et al., 2004b). Within a chicken carcass, chicken breast muscles were mainly composed of white fibers (glycolytic) that had low myoglobin content, thus breast meat appeared white in color, while thigh muscles were composed of red fiber (oxidative) and appeared darker. Fleming et al. (1991) reported a myoglobin concentration of 0.16 and 0.30 mg/g in broiler breast and thigh muscles, respectively. Furthermore, Miller (1994) reported a lower myoglobin content of 0.01 and 0.40 mg/g in white and dark meat of 8 wk old broilers, respectively.

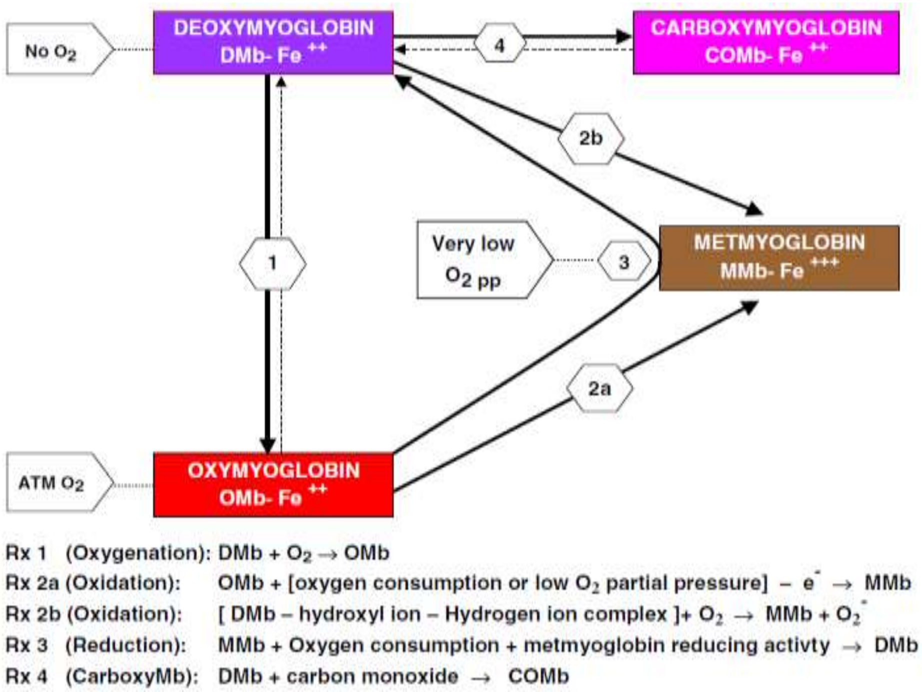

Figure I.4. Major pathways of interconversion between different forms of myoglobin in raw meat (Mancini and Hunt, 2005)

Froning (1995) classified the factors influencing meat color into three main categories (Table 1). Smith et al. (2002) investigated the effect of age, diet (carbohydrate source) and feed withdrawal on broiler meat color by slaughtering birds each day from 42 to 45 and 49 to 52 d of age with a carbohydrate source that was either corn, milo, or wheat, with feed withdrawal times of either 0 or 8 h. Color was not affected by age but feed withdrawal increased fillet lightness ($L*$) from an average of 46.1 to 48.9, decreased redness ($a*$) from 4.1 to 3.1, and increased yellowness ($b*$) from 2.8 to 3.7. Fillets from the birds fed the wheat diet were lighter than fillets from the corn or milo fed birds. The milo diet resulted in redder fillets than corn or wheat diets, while the corn diet produced more yellow fillets than milo or wheat diets.

Table I.1. Factors influencing poultry meat color (Froning, 1995)

Heme pigments	- Myoglobin, hemoglobin, cytochrome c, and their derivatives
	- Presence of ligands complexing with heme pigments
Pre-slaughter factors	- Genetics (fast growing strains)
	- Feed (e.g. moldy feed)
	- Feed withdrawal time
	- Hauling and handling stress
	- Heat and cold stress
	- Pre-slaughter gaseous environment of the bird
Slaughter, chilling, and further processing	- Stunning techniques
	- Presence of nitrates
	- Additives and pH (e.g. phosphates, salt)
	- End-point cooking temperature
	- Reducing conditions
	- Washing surimi-like processing of mechanically deboned poultry meat (MDPM)
	- Irradiation

In addition to meat color, skin color has been considered an important quality attribute mainly in whole carcass and skin-on cuts sale. Color of poultry skin has varied from cream-colored to yellow. Color varied mainly as a result of genetic variation and natural pigments in feed. Birds had differed in their ability to deposit the black melanin pigment in the epidermis and dermis layers of the skin and differed in their ability to deposit carotenoids from feed as combinations of different amounts of melanin and carotenoids produced different skin colors. However, in commercial strains, the ability to deposit melanin has been eliminated through genetic selection. Different skin colors as adopted from Fletcher (1999b) are illustrated in Table 2.

Table I.2. Combination of possible skin colors due to dietary xanthophyll deposition in epidermis or melanin production in either dermis or epidermis (Fletcher, 1999b)

Skin Color	Dermis	Epidermis
White	None	None
Black	Melanin	Melanin
Yellow	None	Xanthophyll
Green	Melanin	Xanthophyll
Blue (Slate)	Melanin	None

However, considerable variation in color and discoloration of poultry meat has occurred and remained of great concern for the industry. Discoloration may occur in the entire muscle or only in a portion of a muscle due to bruising or broken blood vessels (Froning, 1995). Possible poultry color defects are presented in Table 3.

Table I.3. Summary of poultry color defects (Fletcher, 1999b)

Defect	Description	Possible causes
Bruises and hemorrhages	Classic bruises, pin-point blood spots in meat, blood accumulation along bones and in joints	Physical trauma, nutrient deficiencies, mycotoxins, stunning
Over-scalding	Incomplete removal of epidermis, cooked discoloration on surface of meat	Too high scalding temperature, too long in scalder
Surface drying	Mottled appearance of skin or meat due to surface dehydration	Incomplete removal of epidermis, exposed meat, poor packaging, freezer burn
Heme reactions	Normal color ranges from raw pink meat, tan to brown raw meat, grey to brown cooked meat, pink cooked meat, cured meat color	Oxidative or redox state of the myoglobin, myoglobin complexing with nitrates/nitrites or other compounds such as carbon monoxide
Dark meat	Darker than normal appearing meat, possible mottling	High muscle pH due to ante-mortem depletion of muscle glycogen
Light meat	Pale breast meat	Low muscle pH (PSE-like condition)
Dark bones	Dark brown to black bones	Freezing, blood accumulation around bone

1.6.1. Poultry meat color defects

1.6.1.1. Pink discoloration of cooked white meat

Pinking of cooked white meat has been an undesirable color defect found in poultry, its occurrence was noticed sporadically and has negatively influenced consumer purchasing decisions (Maga, I994). According to Maga (1994), it was possible that pink color resulted from the presence of high levels of myoglobin that were not completely denatured during heat processing, incidental nitrate/nitrite contamination either in feed or water, or during processing. The presence of carbon monoxide and nitric oxide gases in oven gas while roasting has caused pink color on surface of turkey meat with carcasses from younger turkeys more susceptible than older ones (Pool, 1956). The proposed mechanism for pink color development of fully cooked while meat was related to the ligands to which the denatured myoglobin were bound, such as amino acids, denatured proteins, and nitrogen-containing compounds that form denatured hemochromes globin. Therefore, depending on the ligand to which the denatured heme will bind to, different shades of pink would result.

Binding of nitric oxide to myoglobin either from pre-slaughter contamination (feed and water, gases from the truck exhaust) or during/after processing (processing water, ice, spice mix, oven gas), has formed the pink nitric oxide myoglobin that upon cooking was converted to pink nitrosohemochrome. Furthermore, carbon monoxide binding to myoglobin has led to the formation of pink carboxymyoglobin that could form upon cooking in oven gases or during irradiation.

Cooking of meat harvested from birds prior to rigor mortis resolution could also cause pink color when meat was cooked when pH was higher than 6.0. At this high pH myoglobin was not denatured, and cytochrome C (electron transport protein) that was heat stable, was increased and contributed to the delayed denaturation of myoglobin since cytochrome C was still able to deliver electrons to myoglobin. Ahn and Maurer (1990) showed that a pH above 6.4 led to binding of myoglobin and hemoglobin with most naturally present ligands such as histidine, cysteine, methionine, nicotinamide, and solubilized proteins, which led to a pink color of the meat. At high pH, amino acids and protein ligands have donated electrons to the Fe resulting in formation of stable pink ferrohemochrome. High pH also reduced the susceptibility of meat pigments and lipids to oxidation resulting in a cooked pink color (Holownia et al., 2003).

1.6.1.2. Bone darkening or discoloration

Bone darkening has been described as a dark reddish brown or black discoloration on the surface of bone and muscle adjacent to the bone after cooking. Darkening was due to bone marrow passing from inside the bone onto the bone surface and adjacent tissue usually after freezing the meat (Lyon et al., 1976; Smith and Northcutt, 2004a) and after cooking of the frozen meat (Koonz and Ramsbottom, 1947). Lyon and Lyon (1986) described the variation in bone discoloration due to different preparation methods (precook, freeze, reheat) and found that freezing before cooking increased the severity of discoloration more than cooking followed by freezing and reheating. Lyon et al. (1976) demonstrated that meat and bone darkening of thigh pieces was related to pigment migration from the femur to muscle tissue. The commercial further processing industry has reported that redness was usually accompanied by blood in bone-in chicken carcasses, which could be rejected by consumers as the product appear to be undercooked and unsafe for consumption (Smith and Northcutt, 2003).

Migration of pigments from femur to muscle tissues has created darkening that was more prevalent in younger birds since their bones were less calcified, more porous, and had more red marrow as compared to older birds. The epiphysis of long bones in older birds was more calcified than young birds so it was more difficult for pigment to escape from bones onto surrounding tissue. The bone darkening only affects the appearance and not the organoleptic properties of the meat product (Koonz and Ramsbottom, 1947).

Smith and Northcutt (2003) studied discoloration prevalence in commercially fully cooked breasts, thighs, and drumsticks from various market sources. They speculated that about 11% of product could face consumer complaints or rejection since they were severely discolored. Furthermore, cooking chicken breast samples with bone marrow collected from femurs increased darkness and redness of both raw and cooked broiler meat (Smith and Northcutt, 2004b).

1.6.1.3. Red discoloration of white meat

Red and/or bloody discoloration of poultry meat, raw or cooked, has been a chronic yet sporadic problem for the poultry industry. Raw breast meat with red discoloration was objectionable to many customers, and cooked white or dark meat with the red defect was unacceptable to consumers due to the perception that it was undercooked. Red discoloration of white meat was closely related to bone darkening but with higher redness. Little research has been available concerning this red discoloration defect in poultry meat (Smith and Northcutt, 2003). According to Smith and Northcutt (2004a), bone marrow was an effective inducer of red, bloody discoloration in breast meat samples. In a previous investigation conducted concerning color of different parts of chicken, Lyon et al. (1976) reported that the initial color of breast was lighter and less red than thighs because breasts had a lower proportion of total bone area to muscle mass, fewer large calcified bones, a lower proportion of blood vessels per muscle mass (less hemoglobin), or lower myoglobin content than thighs or drums (Smith and Northcutt, 2004a).

The bright red color development has been investigated in Parma ham, where this north Italian traditional dry-cured ham ‘‘Prosciutto di Parma (Parma ham)’’ has been made from only the legs of fattened pigs and was salted with sea salt, dried, and matured over a period of 1 year (Wakamatsu et al., 2004a). It was initially postulated that sea salt used was contaminated with nitrate/nitrite, however, that was later investigated and results showed that this pigment was also formed in a nitrate/nitrite-free environment and that endogenous enzymes as well as microorganisms were involved in this pigment formation (Wakamatsu et al., 2004b). These results suggested that the bright red color in Parma ham was caused by Zn–protoporphyrin IX (ZPP) in which the iron in heme was substituted by zinc heme separated from the native heme-protein. Investigations on this lipophylic myoglobin derivative showed that it was a stable red pigment that increased with aging (Parolari et al., 2003). This process has now been patented for producing red pigments for food use that were heat stable (Numata and Wakamatsu, 2009). The addition of salt accelerated the reaction and increased redness (Adamsen et al., 2006). The process has also occurred in live animals, including humans, as lead poisoning and iron deficiency caused an increase of ZPP in blood as zinc replaced the iron in hemoglobin. The level of ZPP can be evaluated with a simple screening test using a hematofluorometer. Measurement of ZPP has only been used once with ducks to test for lead poisoning (Roscoe and Nielsen, 1979). An increased ZPP/heme ratio indicated that Zn has replaced Fe in the heme, thus changing the color of hemoglobin and myoglobin. Based on findings in Parma ham, it was possible that ZPP was responsible for the red discoloration in poultry meat, which either could be formed in myoglobin found in muscles or in hemoglobin stored in bone marrow. Thus, ZPP leaking out of the bones could cause the increased stable redness observed in white meat.

1.6.1.4. Green discoloration

Green discoloration of live muscles, raw meat, and cooked deli products can be produced by various mechanisms that lead to condemnation by the industry and consumers. In live muscles, Green muscle disease (deep muscle myopathy) was caused by lack of blood supply to the deep pectoral muscle that resulted in death of the muscle fibers thus giving the muscle a green appearance. Bruising of live birds has caused rupture of blood capillaries and blood accumulation under the skin or in the meat. The color of the bruise subsequently developed over time and turned either yellow or green depending upon heme degradation. Using lactic acid as a decontamination approach resulted in greening of chicken skin color (Deumier, 2004). Irradiation of fresh beef and pork meat has been thought to affect the stability of iron in the myoglobin and cause the breakdown of the porphyrin molecule, and/or formation of sulfmyoglobin that caused green pigments to appear (Brewer, 2004).

In cooked meat, contamination with microorganisms such as *Pseudomonas fluorescens* has produced a shiny transparent greenish exudate on the meat surface as a result of microbial degradation of the heme pigment. In sausage-type products, the presence of green rings was an indicator of microbial contamination where the microorganisms oxidized the heme pigment before the application of thermal treatment.

Iridescence, which was the appearance of a green-orange color on surface of meat products such as deli meat was mainly associated with the meat surface microstructure that could be interpreted as a color diffraction problem related to the ability of certain muscles to split the white light into its component. Thus, the reflection of the meat surface appeared in green-orange color. If a sharp knife was used to cut the meat, the smooth surface resulting from the cut caused this color diffraction but if a dull knife was used instead, this problem was eliminated.

1.6.2. Issues with trace minerals in poultry nutrition

The National Research Council (NRC) has played an important role in establishing suggested nutrient requirements for poultry and other animals. However, since the NRC for poultry has not been updated since 1994 (NRC, 1994), the accuracy of nutrient requirements has become a subject of debate. Genetic improvement has resulted in increased growth rate at a younger age thus broilers have exhibited higher growth rates and improved feed efficiency that probably required more available nutrients. Poultry producers have tended to supplement certain minerals (e.g. copper, zinc) in excess due to their growth promoting effect. Therefore, many trace minerals were included in the diet for purposes other than to meet traditional nutritional requirements and their levels of use were well beyond NRC recommendations. According to the NRC (1994), the copper requirement of broilers was 8 mg/kg but it was routinely supplemented at 125 to 250 mg/kg as copper sulfate into broiler and turkey diets to protect the health of birds and increase growth (Pesti and Bakalli, 1996). However, the oversupply of one mineral in the diet has caused inadequacy of another mineral due to interrelationships between minerals (Underwood and Suttle, 1999). It was also possible that these minerals formed chelates with phytate in the diet that reduced the efficacy of phytase (Maenz et al., 1999; Ondracek et al., 2002; Banks et al., 2004). Moreover, excess minerals in the manure have increased environmental burden (Blanco-Penedo et al., 2006).

There has been an increased regulatory pressure to minimize environmental contamination caused by animal production. The majority of trace minerals added to feed was excreted, and these extra trace minerals, especially copper and zinc, have created environmental pollution problem (Ferket et al., 2002). According to Nys et al. (2001), 95 to 99% of all trace minerals ingested appeared in the feces. Environmental concerns have been raised because excess minerals in feed polluted ground and surface water and soil. Manure from animals and poultry that was high in copper and zinc has led to accumulation of these minerals in soil because they remained bound to soil and did not migrate extensively to water (Ferket at al., 2002). Therefore, unless removed by plant uptake, these minerals have led eventually to crop toxicity. When zinc concentration in soil was over 200 ppm, the activity of soil microflora was reduced. Dozier et al. (2003) reported that decreasing zinc concentration in the diet of chicken from 120 to 40 mg/kg reduced zinc excretion by 50%, and decreasing copper supplementation from 12 to 4 mg/kg reduced copper excretion by 35%. These findings supported the use of exogenous enzymes that aid in liberating minerals and other chelated nutrients from feed ingredients, as well as the use of organic sources of minerals since they were added at lower concentrations to the feed.

1.7. Current issues with poultry meat quality

1.7.1. Breast muscle myopathy

Deep pectoral muscle myopathy, also known as green muscle disease and Oregon disease, was first identified in turkeys (Dickinson et al., 1968) and later in broiler breeders (Harper et al., 1971) and seven-wk-old broiler chickens (Richardson et al., 1980). This disease affected the wing elevating muscle (*M. supracoracoideus* or *pectoralis minor*) and was characterized by the death of the muscle (tenders) but did not cause death of the bird. Dead muscle decay while the bird was still alive resulted in the appearance of a yellowish-green color due to the breakdown of hemoglobin and myoglobin to bile salts, muscle myopathy could affect just one (unilateral) or both (bilateral) *pectoralis minor* muscles. Since affected tenders were located deep in the breast, this defect resulted in consumer complaints when the carcasses were sold as whole.

The *pectoralis minor* muscle was confined in a tight space between the sternum and the *pectoralis major* muscle (large breast fillet). It was also encased in a rigid fibrous sheath that restricted any increase in muscle volume in response to any physiological changes caused by muscle exercise such as wing flapping (Jordan and Pattison, 1998) that required increased blood flow to supply the oxygen and nutrients needed by the muscles. The incidence of green muscle disease has been also reported to be higher in high yielding crosses, especially the males.

On the other hand, the incidence of focal pectoral myopathy has increased recently, and it has been associated with increased growth rate and muscle size (Wilson et al., 1990;

Dransfield and Sosnicki, 1999). Further investigation will be required to determine the causes of this muscular defect since focal myopathy has even more detrimental effect on poultry industry as it has affected the *pectoralis major* muscle leading to consumer complaints and industry economic loss.

1.7.2. White striping in broiler breast muscles

White striping was a condition described in broiler chickens as characterized by the occurrence of white striations parallel to the direction of muscle fibers on both breast fillets and thighs of broilers. White striping was considered to be an emerging issue by the poultry meat industry that could be associated with enhanced growth rate and heavier BW in birds (Bauermeister et al., 2009; Kuttappan et al., 2012a,b), especially in the age of 6 to 8 wk (Bauermeister et al., 2009), and higher fat content in broiler breast fillets (Kuttappan et al., 2012a). The incidence of white striping was evaluated under commercial conditions, and the overall incidence in broiler breast meat was 12.0% of which 3.1% had severe striping (Petracci et al., 2013). It was possible that intense selection for rapid growth rate in birds could have accidentally been accompanied by the selection for inadequate capillary/fascial growth or muscle fiber defects leading to myopathic changes referred to as growth-induced myopathy (Mahon, 1999).

The precise etiology of white striping has not been defined yet (Kuttappan et al., 2013), however several speculations have been reported. In turkeys, Wilson et al. (1990) reported that rapid growth rate may have led to limited ability of muscle support systems leading to a condition called focal myopathy, which affected the major pectoral muscle.

Ischemia could also result from rapid growth rate and lead to muscular damage in turkeys (Sosnicki et al., 1991). It was also possible that reduced oxygen supply to breast muscle resulted from lower capillary density in fast-growing chickens (Hoving-Bolink et al., 2000). Higher growth rate could also lead to defective cation regulation in muscles leading to an increased level of sodium, potassium, magnesium, and calcium in muscle tissue (Sandercock et al., 2009). An increased level of calcium in muscle tissue could initiate several tissue changes including the activation of intracellular proteases or lipases resulting in myopathic changes (Jackson et al., 1984; Mahon, 1999; Mitchell and Sandercock, 2004; Millay et al., 2009). Kuttappan et al. (2013) reported that breast fillets showing severe white striping had reduced protein content and myopathic lesions, while Petracci et al. (2013) observed poor cohesion beneath the striation area.

1.7.3. Pale, soft, and exudative-like condition in poultry muscles

The incidence of pale, soft, and exudative (PSE) meat has been well-documented in swine, where meat had a very light gray color and was soft in texture and lacked the ability to hold water (Wismer-Pedersen, 1959; Cassens, 2000). This condition has been associated with heavy muscling (Judge et al., 1968). In poultry, similar PSE characteristics had been reported in turkey meat (Sosnicki et al., 1998; Owens et al., 2000), chickens (Swatland, 2008; Petracci et al., 2009), and ostriches (Van Schalkwyk et al., 2000). However, it was more difficult to distinguish and identify these characteristics in poultry meat compared to pork. This condition has been referred to as PSE since characteristics were similar to PSE in pork which is misleading since both conditions were not exactly the same. Poultry researchers have preferred to refer to the condition in poultry as “PSE-like” or “Pale poultry muscle syndrome” (Petracci et al., 2009; Smith and Northcutt, 2009). The PSE and PSE-like conditions were detrimental to the industry profit ability since it affected important meat quality attributes involved in the production of value-added products and further processed meat. Affected muscle has been reported to lose their rheological properties and become unable to hold water. For example, mortadella prepared with PSE-like chicken meat has reduced water holding capacity, altered texture, and diminished emulsion stability, and required additives to restore the functional properties of normal meat (Kissel et al., 2009). Also, poultry processors have been concerned with the appearance of PSE-like meat in fresh tray packs as the pale color affected color uniformity within the package and thus consumer acceptance. The occurrence of PSE-like in poultry meat has been believed to be the result of accelerated postmortem glycolysis (rapid pH decline) while the carcass was still warm (Pietrzak et al., 1997). In poultry, normal pH values at 15 min postmortem (pH_{15}) were around 6.2 to 6.5 (Kijowski and Niewiarowicz, 1978; Berri et al., 2005) whereas normal ultimate pH (pH_u) values were around 5.8 (Fletcher, 1999a; Van Laack et al., 2000). If the pH_{15} value was low (below 6.0) when the muscle was still warm, the proteins were subject to denaturation, which led to a decreased water-holding capacity and a lighter color of the meat.

The reasons for PSE-like condition have remained unclear, but up to 30% of broiler breast meat and up to 40% of turkey breast meat have shown this defect in commercial processing plants (Barbut, 1997a,b; Zhang and Barbut, 2005). Furthermore, it has been reported that the occurrence of PSE-like meat in birds may be affected by alteration to the intracellular calcium homeostasis caused by a mutation in the ryanodine receptor gene, which was different from the ryanodine receptor gene in swine, and also depended upon several aspects of pre-slaughter and post-slaughter management practices (Chiang et al., 2004; Oda et al., 2009). It was thought that the application of ‘‘snow chilling’’ with carbon dioxide intensified meat quality abnormalities (Wynveen et al., 1999). Also, other factors have been thought to contribute to this problem, such as heat stress during the finisher period or during the pre-slaughter period (Petracci et al., 2009), and stress and struggling prior to slaughter (Ma and Addis, 1973).

Differentiating PSE-like meat from normal meat has beens based on instrumental or visual assessment of color lightness ($L*$). However, the cut-off value for classifying meat as PSE-like has differed among researchers. Petracci et al. (2004) considered a $L*$ value of 56 as the cut-off, while Barbut (1993, 1996, and 1998) suggested to classify turkey breast meat as PSE-like when $L*$ values were greater than 52 at 24 h post-mortem. Fraqueza et al. (2006) classified breast meat as PSE-like when the $L*$ was greater than 50 and pH_u was less than 5.8 while Woelfel et al. (2002) used $L*$ values greater than 54 in broilers as their standards. Using $L*$ per se as an indicator of PSE-like condition has not been considered accurate and could be misleading because several factors have influenced poultry meat color. Feed ingredients used in poultry have been reported to change breast meat color (e.g. wheat-based vs corn-based diets). In addition, it has been shown that genetic selection for increased growth and breast meat yield resulted in a marked increase in muscle fiber size (Remignon et al., 1995; Guernec et al., 2003) with a shift towards a greater proportion of white fiber (glycolytic) and reduced dark fiber (oxidative), which produced meat that appeared pale but still had a high pH_u. Muscle thickness (Sandusky and Heath, 1996; Bianchi and Fletcher, 2002) and color measurement position on the fillet (Goshaw et al., 2000) also affected color measurement. Therefore, color, pH_u, and water holding capacity should all be considered when classifying poultry as PSE-like meat.

1.8. Poultry meat safety

Poultry meat quality and safety has been complementary. Poultry products should be of good quality and at the same time safe for human consumption. Contaminants or hazards in poultry products could be chemical, physical, or biological. Physical hazards have included extrinsic material such as metal, glass, wood, rock, and plastic, or intrinsic material such as bones. Chemical contamination such as with drug residues, pesticides, toxic metals, polychlorinated biphenyls, and dioxins has occurred through environmental pollution of the air, water, and soil. It has been difficult to attribute disease in humans caused by exposure to chemical hazards since the period of time between exposure to chemicals and their effect was usually long. On the other hand, biological hazards have been identified and attributed in a shorter period of time in comparison with chemical hazards.

Foodborne disease has been considered to be an important and growing public health and economic concern. The significance has arisen from its impact on health and well-being of the population, and the economic consequences for individuals and their families (Keene, 2006). Generally, food poisoning has occurred when the causative organisms, initially present in low numbers, were allowed to multiply during manufacture, distribution, preparation, or storage of foods. Several factors have been implicated in outbreaks of poultry-borne illness, including cross-contamination (Brown et al., 1988; Bryan and Doyle, 1995; MMWR, 1998; Jimenez et al., 2005; Moffatt et al., 2006), inadequate cooking (Bryan, 1980; Bean and Griffin, 1990; MMWR, 2008), improper handling of food (Altekruse et al., 1996; Shiferaw et al., 2000), and temperature abuse during transport, preparation, or storage (Worsfold and Griffith, 1997; Payne et al., 2008). However, to reduce the risk of foodborne illness, washing hands with warm soapy water has been ranked as one of the most important presentative consumer behaviors (Medeiros et al., 2001; Hillers et al., 2003).

Several microorganisms have been isolated from poultry and poultry products and have included *Campylobacter* (Dominguez et al., 2002; Jorgensen et al., 2002; Pezzotti et al., 2003), *Salmonella* (Schlosser et al., 2000; Dominguez et al., 2002; Jorgensen et al., 2002; Altekruse et al., 2006), *Listeria* (Genigeorgis et al., 1990), *E. coli* (Zhao et al., 2001; Northcutt et al., 2003), and spoilage organisms including *Pseudomonas* and *Lactobacillus*.

The three main foodborne pathogens associated with poultry (*Salmonella*, *Campylobacter*, and *Listeria spp*.) have been usually carried asymptomatically in the intestines of infected birds. Nontyphoidal *Salmonella* infections were considered an important public health problem worldwide (Zaidi et al., 2006; Kim et al., 2007). *Salmonella enterica* serovars Enteritidis and Heidelberg have been detected in chicken and were among the top five serovars associated with human infections (CDC, 2011). Therefore, the prevalence of similar serovars in both humans and poultry suggested a possible epidemiological connection, which resulted in classifying fresh and processed poultry as a leading cause of human salmonellosis. Recently, *S*. Kentucky became the most commonly detected serovar in chicken having multi drug resistance along with *S*. Typhimurium (FDA, 2010). In a recent CDC report (2013) on antibiotic resistance threats, approximately 5% of non-typhoidal *Salmonella* were resistant to five or more drugs. Scallan et al. (2011) estimated approximately 1.028 million cases, 19,000 hospitalizations, and 400 deaths in the United States were a result of foodborne illness caused by *S*. *enterica*. The significance of *Salmonella spp*. as an infecting agent was related to its ability to express shock proteins that enabled it to survive the gastrointestinal (GI) pH and exposure to short chain fatty acids in the poultry gut, as well as having the fimbria-associated proteins required to facilitate its adhesion to the intestinal surface of the host. In birds, *Salmonella* was able to multiply in the GI tract and has been shown to contaminate the environment through fecal excretion. It was also able to survive inside macrophages and cause systemic infection (Dunkley, 2009) while birds remained asymptomatic carriers.

Reports on the incident of *Salmonella* in live birds has not been well documented but environmental sampling of poultry houses including sampling water, feed, litter, and insects has been used to indicate *Salmonella* prevalence on live birds. Since *Salmonella* was ubiquitous in nature and considered to be a commensal microorganism, it was expected to be present in poultry flocks and the associated environment. Rodriguez et al. (2006) reported 16.2% *Salmonella* prevalence in poultry farms located in Tennessee, North Carolina, Alabama, California, and Washington over a 24-month period. *S.* Anatum was the main serovar isolated. Furthermore, *Salmonella* was more prevalent in the free-range (31%) and all-natural (25%) chickens surveyed than in chickens from the U.S. commercial poultry industry in 2003 (Bailey and Cosby, 2005).

Although many consumers commonly associate *Salmonella* with poultry products, the detection rates have been usually low. In a baseline study conducted prior to implementation of the Pathogen Reduction-Hazard Analysis and Critical Control Point (PR-HACCP) rule, Schlosser et al. (2000) reported an average rate of 15.9% on chicken carcasses and 11.9% on turkey carcasses for the four most frequently detected *Salmonella* species (Heidelberg, Kentucky, Hadar, and Typhimurium in chicken, and Hadar, Heidelber, Agona, and Senftenberg in turkeys). Only 10.9% of the 5697 rinse samples taken from broiler carcasses collected from 124 large processing plants during the first year of HACCP implementation were *Salmonella* positive (USDA-FSIS, 2000). Logue et al. (2003) reported an overall incidence rate of 16.7% in turkey carcasses collected monthly for one year from US Midwestern processing plants. During the first quarter of 2010, USDA-FSIS (2010) reported only 7% of broilers, 0% of turkeys, 13.1% of ground chicken, and 7.9% of ground turkey samples were positive for *Salmonella*. In their quarterly report, USDA-FSIS (2013) reported that only 2.6% out of 2,955 young chicken carcasses tested during the period from April through June 2013 were positive for *Salmonella*.

A recent study has been conducted by Oscar (2013) on the initial contamination of chicken parts with *Salmonella* at retail and potential cross-contamination of cooked chicken from raw chicken when cut on the same unwashed cutting board using the same utensils for the raw chicken parts to resemble a worst-case scenario for cross-contamination. Prevalence results of contamination on raw chicken was 3% (4 of 132), while the incidence of cross-contamination of the cooked chicken was 1.8% (1 of 57) and the maximum number of *Salmonella* cells on the contaminated parts was 3 CFU. This suggested that the poultry industry was providing consumers with chicken that had a low prevalence of *Salmonella* at retail. Despite the decreased prevalence of *Salmonella* on poultry carcass, human illness have not decreased. Given these facts, two possibilities have arisen. It was either that the human *Salmonella* illness and deaths caused by poultry were over-estimated, or *Salmonella* recovery methods in the poultry industry were not accurate enough to detect the actual incidence of contamination. The second possibility was that there was another food that contributed to salmonellosis other than poultry and this food had not been controlled yet.

Broiler chickens have been generally regarded as one of the main sources of campylobacteriosis (Harris et al., 1986; Kapperud et al., 1992; Van Gerwe et al., 2005; Cortez et al., 2006; Idris et al., 2006). Results of several studies showed that simultaneous increase both in the consumption of chicken meat and the numbers of reported campylobacteriosis cases suggested that poultry was one of the most important risk factors for human infections (Carrique-Mas et al., 2005; Wingstrand et al., 2006; Humphrey et al., 2007). The reduced consumption of chicken meat in Belgium during the dioxin crisis resulted in a 40% decrease in campylobacteriosis compared to previous years, which provided indirect evidence of the significance of chicken meat in human infections (Vellinga and Van Loock, 2002). Two major species of the genus *Campylobacter* that occur in the poultry industry were *C. jejuni* and *C. coli*. Relatively high numbers can be introduced into a processing plant on the skin and feathers and in the intestines of carrier birds.

Campylobacter was considered to be a commensal organism in many avian species, including those grown commercially, and has been found to spread rapidly (Keener et al., 2004). The frequent contamination of broiler chicken meat with *Campylobacter* was associated with the fact that *Campylobacter* existed widely in nature, and therefore the digestive tracts of broiler chickens were the principal reservoirs at farm level with the farm being the most important primary contamination site (Jacobs-Reitsma, 2000). Infection of commercial poultry with *Campylobacter spp*. was reported in broiler chickens (Prescott and Gelmer, 1984), ducks (Kasrazadeh and Genigeorgis, 1987), turkeys (Acuff et al., 1986), egg production flocks (Figueroa et al., 1983) and parent breeding stock (Shanker et al., 1986). It was suggested that the primary route of infection in broilers was through horizontal transmission from the environment (Jacobs-Reitsma et al., 1995). Potential sources and vectors for contamination have included infected livestock and wild-life animals (van de Giessen et al., 1996; Ellis-Iversen et al., 2009), rodents and flies (Hald et al., 2008), contaminated surface water (Shanker et al., 1990; Messens et al., 2009) especially if not chlorinated (Kapperud et al., 1993), personnel and farm equipment (Ramabu et al., 2004). Feed has not been regarded as a source of infection because of the low moisture content where *Campylobacter* cannot survive (Newell and Fearnley, 2003).

Most flocks have become infected at an age of two to four wk only (Jacobs-Reitsma et al., 1995; Evans and Sayers, 2000; van Gerwe et al., 2009) with the majority of birds in a flock being colonized within a few days after the first chick was infected (van Gerwe et al., 2009), and remain colonized until slaughter (Beery et al., 1988; Jacobs-Reitsma et al., 1995; Evans and Sayers, 2000).

Detection rates for *Campylobacter spp*. in poultry have been higher than those for *Salmonella* with greater than 50% of samples evaluated being frequently positive (Moore et al., 2002; Pezzotti et al., 2003; Cui et al., 2005; Rodrigo et al., 2005; Bohaychuk et al., 2006). Huat et al. (2010) reported *Campylobacter* contamination rates of 84% for chickens in conventional slaughterhouses, 94% in automated processing plants, and 92% in supermarket samples in Malaysia. Testing for generic *Escherichia coli* has been required by the PR-HACCP systems rule for all poultry slaughter plants as an indication of proper processing and sanitation procedures. Detection rates in poultry have varied with reported levels ranging from 11.9% to 71% in turkeys (Zhao et al., 2001; Kegode et al., 2008), and 38.7% to nearly 100% in chickens (Zhao et al., 2001; Northcutt et al., 2003; Forward et al., 2004; Kegode et al., 2008).

Spoilage of poultry was usually associated with psychrotrophic microorganisms. *Pseudomonas spp*. were the most common organisms associated with spoilage of refrigerated meats under aerobic conditions. *Lactobacillus spp*. were often associated with spoilage of refrigerated poultry stored under microaerophilic or anaerobic conditions. Yeast loads have been reported to increase in both chicken and turkey during cold storage (Ismail et al., 2000; Chouliara et al., 2008). Proteolytic and/or lipolytic yeasts such as *Candida zeylanoides* and

Yarrowia lipolytica were some of the most common species isolated from fresh and spoiled poultry (Diriye et al., 1993; Viljoen et al., 1998).

The most difficult problem to control in poultry processing was that of cross-contamination, which has arisen from aerosols, process water, and contact between carcasses and equipment or personnel. Also, line speeds did not provide sufficient time after one bird has been dealt with and before another was ready (Mead, 1993). Bacteria that were easily removed from a carcass posed a greater risk to public health (through cross-contamination of other foods) than bacteria that were present inside the carcass or firmly attached to the carcass so that they would be killed during cooking (Buhr et al., 2005). Scalding tank water was an important site for cross-contamination both within a flock and between flocks especially if a *Campylobacter*-negative flock was slaughtered after a *Campylobacter*-positive flock. The scalding water temperatures used has varied from 50 to 52°C (soft scald) to 56 to 68°C (hard scald). However, the various time–temperature combinations that can be used without denaturing the carcass skin do not decrease *Campylobacter* contamination level of carcasses (Nauta et al., 2007).

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CHAPTER II

Growth performance of male and female broilers fed diets supplemented with different levels of zinc, copper, and Roxarsone

2.1. ABSTRACT

This study was conducted to evaluate the effect of feeding different levels of inorganic dietary zinc (Zn), copper (Cu), and Roxarsone (Arsenic; As) on growth performance of male and female broilers raised to 56 d. A total of 1,152 day-old male and female Ross 344×708 broilers were raised sex-separate and randomly distributed among 9 dietary treatments with 8 replicate floor pens per treatment, and 16 birds per pen. Birds were fed diets either containing no mineral premix (negative control), or one of 8 factorial treatments supplemented with either 120 or 240 mg Zn/kg diet, 10 or 100 mg Cu/kg diet, and 0 or 0.5 mg As/kg diet. Birds received a crumble starter diet from 1-14 d, and pelleted grower and finisher diets from 15-56 d with feed and water provided *ad libitum*. Feed intake and BW were measured and BW gain and adjusted feed conversion ratio (AdjFCR) were calculated. Data was analyzed using GLM procedures of SAS to evaluate the effect of sex and mineral supplementation. Males were superior to females in BW, BW gain, feed intake, and AdjFCR, however, females exhibited lower mortality. Supplementation of 240 mg/kg Zn improved AdjFCR in the starter period in males and grower period in females. Supplementation of 100 mg/kg Cu was beneficial for both males and females in the grower and finisher periods. The AdjFCR for the period from 1-55 d in males was 1.92 and 1.90, and in females, AdjFCR was

2.05 and 2.00 for supplemented Cu at 10 and 100 mg/kg, respectively. Roxarsone supplementation improved AdjFCR only in the finisher period. Results indicated that Zn improved performance when added at 240 mg/kg only in the starter period, while Cu supplemented at 100 mg/kg improved performance when added in the grower and finisher periods.

Key words: Broiler, zinc, copper, Roxarsone, arsenic, live performance

2.2. INTRODUCTION

Trace minerals has been considered to be an essential component of poultry nutrition as they have important roles in vital body functions. Zinc (Zn) has been required for growth, bone development, feathering, wound healing, and enzyme structure and function (Linder, 1991; Batal et al., 2001). The primary function of Zn was a cofactor for enzyme activation and functions and affected many physiological and metabolic activities (Vallee, 1983; Gillespie, 1987; Smith, 1988). It has been a common practice in the broiler industry to formulate diets to contain 100–120 mg supplemental Zn/kg. Burrell et al. (2004) reported improved performance when broilers consumed diets formulated to contain 110 mg Zn/kg even though the NRC (1994) minimum requirement of Zn for broiler chickens was only 40 mg/kg, which was based on purified or semi-purified diets with growth as the requirement criterion (Wedekind and Baker, 1990; Emmert and Baker, 1995; Batal et al., 2001). This standard may not be applicable to corn-soybean diets because of the absence of phytate and fiber. Copper (Cu) has also played an important role in enzymatic functions related to iron

(Fe) metabolism, elastin and collagen formation, and was important for feather development and feather color. It was also important because of its antimicrobial properties that improve performance in broilers when fed above the minimum requirement (Smith, 1969; Jenkins et al., 1970). The minimum nutritional requirement for Cu in broilers was suggested to be approximately 4 to 5 mg/kg (Franchini and Bertuzzi, 1991; NRC, 1994). In the United States, Cu was normally added as a growth promoter; however, commercial producers often add levels higher than required. Iron was also an essential trace mineral that played an important role in cellular respiration, metabolism, activation of oxygen during electron transport, and the immune system. The minimum nutritional requirements of Fe was between 45 mg/kg (Franchini and Bertuzzi, 1991) and 80 mg/kg in broiler diets (NRC, 1994). Despite the fact that most practical diets contained adequate Fe and Cu to satisfy nutritional requirements for broilers (Scott et al., 1982), both minerals were still generally supplemented in order to avoid possible deficiencies.

Zinc was known to antagonize Cu (Van Campen and Scaife, 1967) as high Zn intake inhibited intestinal absorption and hepatic accumulation of Cu and induced clinical signs of Cu deficiency (Bremner and Beattie, 1995; Gonzalez et al., 2005). Similarly, high Cu intake depressed Zn absorption (Van Campen, 1969; Evans et al., 1974). The competition for mineral-binding ligands and mineral uptake sites in the mucosa of the gut (Santon et al., 2002) was proposed as the main factor causing an antagonism between Zn and Cu.

Roxarsone (3-Nitro-4-Hydroxyphenylarsonic Acid) has moderate anticoccidial effect and has been shown to improve growth and feed utilization (Kowalski and Reid, 1972;

Damron et al., 1975; Waldroup et al., 1984; Izquierdo et al., 1987). Chapman and Johnson (2002) estimated that from 1995 to 2000, Roxarsone was used in 69.8 and 73.9% of the broiler starter and broiler grower feeds in the United States. However, there has been environmental concerns regarding the amount of arsenic (As) present in the excreta and litter of broilers fed Roxarsone.

Because inorganic trace minerals were inexpensive, there was a tendency to over-formulate to provide an inexpensive safety factor. Excess minerals and other unabsorbed nutrients have been reported in the litter, which may be spread onto fields as a soil amendment and fertilizer and result in an accumulation of these minerals in the soil. Also, adding trace minerals at higher levels could negatively influence the metabolism of each other. Therefore, this research was conducted to evaluate different levels of dietary inorganic Zn and Cu on live performance of broilers.

2.3. MATERIALS AND METHODS

Birds and diets

This study was conducted from January 18 to March 14, 2012 at North Carolina State University, Raleigh, NC, USA. A total of 1152 day-old male and female broiler chicks of Ross 344×708 strain were hatched from eggs collected from breeders at the site, which were 40 wk old. Birds were feather-sexed and randomly distributed among 4 blocks with a total of 72 pens. Birds were raised sex-separate with 16 birds per pen (173×113 cm for length×width) on used litter. Birds were exposed to continuous light for the first 2 days, then to 23 h light and 1 h dark until 7 d of age. Day length was then gradually reduced to reach 12 h at 28 d where it was maintained until 56 d. The initial house temperature was set at 32°C and gradually reduced to 25°C. Feed and water were provided for *ad libitum* consumption. Feed was mixed 2 times daily to stimulate uniform feed consumption. The nine experimental diets were formulated from the same basal diet, which did not contain any mineral premix, to ensure that birds had access to the same nutrients. Diets were formulated to contain corn, soybean meal (SBM), distilled dried grain with solubles (DDGS), and poultry by-product meal (PBM) as the major ingredients. Table 1 illustrates the experimental diets. The coccidiostat coban was used from 1-14 d and Roxarsone as an arsenic source as well after 14 d. Diets were formulated to either meet or exceed the NRC (1994) requirements for broilers except for Zn and Cu. Table 2 shows the basal diet formulation and proximate composition. The negative control diet did not contain any mineral premix. To formulate the remaining 8 experimental diets, inorganic zinc sulfate ($ZnSO_4$) and copper sulfate ($CuSO_4$), and Roxarsone (As) were used and added at the same level in starter, grower, and finisher diets for each treatment. Zinc sulfate (Zinc Nacional, S.A., Mexico) was added to achieve a Zn concentration in the experimental diets of either 120 or 240 mg Zn/kg diet. Copper sulfate was added to achieve a concentration of either 10 or 100 mg Cu/kg diet. Roxarsone was either not added, or added at a level of 45.4 mg/kg to achieve an As concentration of 0.5 mg/kg diet. Birds were fed crumbled starter feed from 1-14 d and pelleted grower and finisher feeds from 15-56 d. All methods regarding bird care in this experiment were approved by the NCSU Institutional Animal Care and Use Committee.

*Diets and litter mineral concentratio*n

Concentration of Cu, Zn, Fe, and As was measured in the diets, major ingredients, and litter before placement of the birds using inductively coupled plasma optical emission spectrometer (Perkin Elmer ICP-OES 2000 DV).

Performance

Birds and feed were weighed for each pen at 1, 14, 35, and 56 d to determine BW gain, feed consumption, and feed conversion ratio (FCR). Mortality was monitored and recorded daily, and FCR was adjusted for mortality (AdjFCR).

Statistical analysis

Data were analyzed as a randomized complete block design using the GLM procedure of SAS (SAS version 9.4, SAS Institute, Cary, NC, USA, 2014). Data was analyzed to assess the effect of sex, and then males and females data were analyzed separately to assess the effect of dietary treatments within each sex. Differences between means were separated using the LS means method and significance was reported at a probability level of $P \leq 0.05$ unless otherwise indicated. The experimental unit for statistical analysis of measured parameters was the pen.

2.4. RESULTS AND DISCUSSION

Diets and litter mineral concentration

The analyzed mineral content of the litter was as follows: Cu, 38 mg/kg; Zn, 224 mg/kg; Fe, 4680 mg/kg; and As, 0.5 mg/kg. Mineral content of DDGS was as follows: Cu, 6 mg/kg; Zn, 69 mg/kg; Fe, 180 mg/kg; and As was not detected. For PBM, mineral content was: Cu, 12 mg/kg; Zn, 106 mg/kg; Fe, 208 mg/kg; and As was not detected. Results for the experimental diets mineral analyses are shown in Table 3. The analyzed total Cu and Zn content were higher than the theoretically calculated levels which could have come from minerals present in the feed ingredients but the intended differences between the treatments were still distinct. The mineral content of the used litter was higher than some of the treatments, in addition, the Fe level was extremely high, which might have affected the performance results since minerals could affect the metabolism of each other either positively or negatively. Reported Zn and Cu concentrations in poultry litter from several investigations were 631 and 1196 mg/kg (van der Watt et al., 1994), 647 and 55 mg/kg (Edwards et al., 1997), 718 and 748 mg/kg (Moore et al., 1998), and 378 and 97 mg/kg (Nicholson et al., 1999), respectively. Arsenic concentration was reported to be from 20 to 37 mg/kg (van der Watt et al., 1994), and 43 mg/kg (Moore et al., 1998). Most As passes through the bird unchanged and remains in the litter (Garbarino et al., 2003). In another study, Edwards and Daniel (1992) reported the following concentrations in broiler litter: Zn, 188; Cu, 56; Fe, 842; and As, 22 mg/kg.

Sex effect on growth performance

Results of live performance of birds as affected by sex are shown in Table 4. Males were heavier at hatch (P = 0.003) and remained heavier (P = 0.0001) throughout the experimental period. Hatch BW was 48 and 47 g, while BW at 55 d was 4821 and 3808 g for males and females, respectively. Feed intake was measured for starter (1-14 d), grower (15-35 d), finisher (36-55 d) periods, and cumulatively. Males had greater feed intake (P =

0.0001) in all of the measured periods. Cumulative feed intake from 1-55 d being 9046 and 7625 g for males and females, respectively. The BW gain was also higher in males (*P* = 0.0001), with cumulative body weight gain of 4773 g in males and 3761 g in females from 1-55 d. The AdjFCR was comparable (*P* = 0.43) among males and females during the starter period (1-14 d) but males had a better AdjFCR during grower, finisher, and cumulative periods (*P* = 0.0001). For the grower period, AdjFCR was 1.55 in males and 1.70 in females, while AdjFCR in the finisher period was 2.34 in males and 2.50 in females. From 1-55 d, AdjFCR was 1.90 in males and 2.02 in females. Mortality was greater (*P* = 0.0001) in males with cumulative mortality of 7.4% compared to 1.2% in females. It has been reported that males have 1% higher BW at hatch compared to females and this difference increased with age to reach 17% at age of harvest (North and Bell, 1990). Several investigators have reported that males were heavier than females (Miles et al., 1998; Young et al., 2001; Kidd et al., 2005; Lopez et al., 2011; Salim et al., 2012).

Effect of supplemented minerals on growth performance of males and females

Least-squares means for the main effects of supplemented minerals on live performance of broiler males and females are shown in Tables 5 and 6, respectively, while results of the interaction among minerals in males and females are represented in Tables 7 and 8, respectively. Roxarsone (As) was added after 14 d, therefore results for parameters measured after 14 d only are available for As. Means of the negative control treatments are presented for comparison. Dietary Zn had no effect on BW and BW gain of males and females. The BW measured at 55 d was 4856 and 4786 g in males and 3824 and 3792 g in females for dietary Zn of 120 and 240 mg/kg, respectively. Male BW was not also affected by dietary Cu or As treatments, however, 55 d BW and 36-55 d BW gain of females were improved (P = 0.01) by dietary Cu supplementation (55 d body weight of 3768 and 3848 g for 10 and 100 mg Cu/kg, respectively). Burrell et al. (2004) reported improved BW gain when male broilers were raised to 45 d and consumed diets containing 110 mg Zn/kg without affecting FCR. However, in the present study we were able to detect feed intake and AdjFCR effect with no effect on BW gain in females only. Gajula et al. (2011) did not find significant differences in BW gain and FCR when broilers were fed dietary inorganic Zn (40-160 mg/kg) from 6 to 35 d of age. Similarly, Mehring et al. (1956) reported comparable BW and feed efficiency of broilers fed different levels of Zn (0-778 mg/kg) supplemented as zinc oxide up to 9 wk of age. On the other hand, Gheisari et al. (2011) reported improved FCR in the starter period when mixed-sex broilers were fed diets supplemented with inorganic Zn, Mn, and Cu (100, 100, and 10 mg/kg, respectively). Feed intake was not affected by dietary Zn treatment in males but dietary Cu supplemented at 100 mg/kg increased (P = 0.001) feed intake from 1-14 d (668 and 696 g for 10 and 100 mg Cu/kg, respectively). However, dietary Cu decreased feed intake from 36-55 d (P = 0.04) and 1-55 d (P = 0.02) when supplemented at 100 mg/kg. Feed intake from 1-55 d was 9137 and 8954 g for dietary Cu of 10 and 100 mg/kg, respectively. Supplementing dietary As decreased (P = 0.04) feed intake from 36-55 d where non-supplemented birds had a feed intake of 5359 g compared to 5252 g in birds receiving 0.5 mg As/kg. Feed intake and BW gain in males were also affected by an interaction between Cu and As. Supplementing 100 mg Cu/kg in combination with 0.5 mg

As/kg increased (P = 0.02) feed intake from 15-35 d but decreased it from 36-55 d (P = 0.01). On the other hand, BW gain was the lowest (P = 0.04) when Cu was supplemented at 10 mg/kg with no As supplementation from 15-35 d but then was increased (P = 0.03) from 36-55 d. For females, dietary Zn significantly decreased feed intake when supplemented at 240 mg/kg during the experimental period; 1-14 d feed intake was 647 and 626 g (P = 0.05), 15-35 d was 2831 and 2771 g (P = 0.05), and 36-55 d was 4237 and 4139 g (P = 0.04) for dietary Zn at 120 and 240 mg/kg, respectively. Kim and Patterson (2004) reported that feed intake, BW, and feed efficiency were reduced when dietary inorganic Zn (500, 1000, and 1500 mg/kg) was supplemented to male broilers raised to 21 d of age. The AdjFCR was affected only by supplemented minerals from 1-14 d by both Zn (P = 0.01) and Cu (P = 0.001) in males, values were 1.47 and 1.43 for Zn supplemented at 120 and 240 mg/kg, while for Cu, values were 1.41 and 1.49 for 10 and 100 mg Cu/kg. In females, Zn supplementation improved AdjFCR from 1-35 d (P = 0.01), 1-55 d (P = 0.05), and 15-35 d (P = 0.01). Values from 1-55 d were 2.04 and 2.01 for Zn supplemented at 120 and 240 mg/kg. Supplementing Cu also had beneficial effect on AdjFCR of females from 36-55 d where birds supplemented with 10 mg Cu/kg exhibited an AdjFCR of 2.56 compared to 2.44 when Cu was supplemented at 100 mg/kg. There was an interaction effect between Zn and Cu on AdjFCR from 1-14 d in both males and females (P = 0.03), where in both males and females supplementing 240 mg Zn/kg in combination with 10 mg Cu/kg resulted in the best AdjFCR (1.37 in males and 1.38 in females). There was also a Cu and As interaction in males only where AdjFCR from 15-35 d was improved (P = 0.01) when Cu was supplemented at 100 mg/kg with no As, however, this was reversed (P = 0.0001) from 36-55 d. In broilers raised to 42 d and fed either 0, 200, 400, or 600 mg/kg of copper sulfate, BW and AdjFCR did not differ in birds fed up to 400 mg Cu /kg, however birds fed 600 mg Cu /kg had lower feed intake as well as poorer growth and poorer feed conversion (Miles et al., 1998). Banks et al. (2004) noted that Cu supplementation with 250 mg/kg from copper sulfate decreased BW gain, feed consumption, and feed efficiency. On the other hand, Thomas and Goatcher (1976) fed dietary Cu at 480 mg/kg as copper sulfate to laying hens without any adverse effect on egg production. When 800 mg Cu /kg was fed as copper sulfate to turkeys in a practical diet, no adverse effects were also reported (Jensen, 1975).

According to Bao et al. (2010), Zn was considered to be the first limiting trace mineral for broilers when compared to Cu, Mn, and Fe. This could have explained the improved AdjFCR from 1-14 d when Zn was supplemented at 240 mg/kg compared to 120 mg/kg. Liu et al. (2011) reported that supplementation of 60 mg Zn/kg was sufficient to reach an optimal growth performance in broilers raised to 42 d of age regardless of the Zn sources (organic or inorganic). Mortality was not affected by mineral supplementation in males and females. These results indicated that higher dose of Cu in males negatively influenced feed intake and AdjFCR during the starter period (1-14 d) but was beneficial in the finisher period (36-55 d) for both males and females, which suggested that a high Cu treatment can be implemented after the starter phase. Using an arsenical coccidiostat after 14 d decreased the feed intake but did not produce any statistical significance effects on BW gain or AdjFCR in both males and females. However, the interaction results suggested that supplementing 100 mg Cu/kg in combination with 0.5 mg As/kg improved AdjFCR from 36-55 d only. Dozier et al. (2003) reported that inorganic dietary Zn (40-120 mg/kg) and Cu (4-12 mg/kg) had no effect on BW, FCR, or incidence of mortality when supplemented to broilers from 1-17 d of age.

2.5. CONCLUSION

Results of the current study emphasized that males and females differed in their live performance with males being superior to females for growth. Dietary Zn supplementation improved performance when added at 240 mg/kg only in the starter period, while Cu supplemented at 100 mg/kg improved live performance when added in the grower and finisher periods. Mineral accumulation in the litter could interfere with dietary minerals since birds can consume the extra nutrients from the litter through coprophagy.

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Table II.1. Experimental diets

Diet	**General Description of Starter Diets + Coban in all diets**	**Zn, mg/kg**	**Cu, mg/kg**	**Roxarsone, mg/kg**	**Coban**
1	Corn-soy-DDGS-PBM basal diet with no supplementation of (mineral premix), Zn or Cu (basal, negative control)	0	0	0	+
2/4	Basal+1816 g/ton mineral premix	120	10	0	+
3/5	Basal+1816 mg/kg mineral premix+0 mg of Zn as sulfate/kg of diet+324.283 mg Cu as sulfate/kg of diet	120	100	0	+
6/8	Basal+1816 mg/kg mineral premix+306.93 mg of Zn as sulfate/kg of diet+0 mg Cu as sulfate/kg of diet	240	10	0	+
7/9	Basal+1816 mg/kg mineral premix+306.93 mg of Zn as sulfate/kg of diet+324.283 mg Cu as sulfate/kg of diet	240	100	0	+
Diet	**General description of grower and finisher diets**	**Zn, mg/kg**	**Cu, mg/kg**	**Roxarsone, mg/kg**	**Coban**
1	Corn-soy-DDGS-PBM basal diet with no supplementation of (mineral premix), Zn, Cu, or Roxarsone (basal, Roxarsone negative control) (+Coban)	0	0	0	+
2	Basal+1816 mg/kg mineral premix+9.072 kg of Roxarsone/ton of diet (Roxarsone positive control)	120	10	45.4	0
3	Basal+1816 mg/kg mineral premix+0 mg of Zn as sulfate/kg of diet+324.283 mg Cu as sulfate/kg of diet +9.072 kg of Roxarsone /ton of diet	120	100	45.4	0
4	Basal+1816 mg/kg mineral premix+0 mg of Zn as sulfate/kg of diet+0 mg of Cu as sulfate/kg of diet+0 g of Roxarsone /ton of diet (+Coban)	120	10	0	+
5	Basal+1816 mg/kg mineral premix+0 mg of Zn as sulfate/kg of diet+324.283 mg Cu as sulfate/kg of diet+0 g of Roxarsone /ton of diet (+Coban)	120	100	0	+
6	Basal+1816 mg/kg mineral premix+306.93 mg of Zn as sulfate/kg of diet+0 mg Cu as sulfate/kg of diet +9.072 kg of Roxarsone /ton of diet	240	10	45.4	0
7	Basal+1816 mg/kg mineral premix+306.93 mg of Zn as sulfate/kg of diet+324.283 mg Cu as sulfate/kg of diet +9.072 kg of Roxarsone /ton of diet	240	100	45.4	0
8	Basal+1816 mg/kg mineral premix+306.93 mg of Zn as sulfate/kg of diet+0 mg Cu as sulfate/kg of diet +0 g of Roxarsone /ton of diet (+Coban)	240	10	0	+
9	Basal+1816 mg/kg mineral premix+306.93 mg of Zn as sulfate/kg of diet+324.283 mg Cu as sulfate/kg of diet +0 g of Roxarsone /ton of diet (+Coban)	240	100	0	+

Table II.2. Composition and nutrient content (as-fed basis) of the experimental basal diets

Item	Starter (1-14 d)	Grower (15-35 d)	Finisher (36-55 d)
Ingredients	(%)		
Corn	54.38	53.65	61.38
Soybean meal, 48%	31.21	24.91	17.70
DDGS	5.00	10.00	10.00
Poultry fat	2.84	4.60	4.58
Poultry by-product meal	2.50	2.10	1.60
Dicalcium phosphate	1.57	1.31	1.13
Limestone	0.87	0.84	1.06
Salt (NaCl)	0.50	0.50	0.50
Vermiculite (inert filler)[1]	0.35	1.30	1.31
DL-Methionine	0.22	0.18	0.11
L-Lysine	0.15	0.20	0.25
L-Threonine	0.11	0.11	0.08
Choline chloride	0.10	0.10	0.10
Selenium premix[2]	0.10	0.10	0.10
Vitamin premix[3]	0.05	0.05	0.05
Coccidiostat[4]	0.05	0.00	0.00
Calculated nutrient content			
Metabolizable energy (kcal/g)	2.85	2.95	3.02
Crude protein (%)	22.00	20.00	17.00
Calcium (%)	0.90	0.80	0.80
Available Phosphorus (%)	0.45	0.40	0.35
Lysine (%)	1.26	1.14	0.97
Methionine (%)	0.57	0.52	0.42
Analyzed nutrient content, as fed			
Crude fat (%)	5.41	7.06	7.16
Crude protein (%)	21.43	20.34	18.52
Crude fiber (%)	2.50	2.50	2.70
Ash (%)	6.10	6.06	5.88
Total Phosphorus (%)	0.68	0.70	0.65
Iron (mg/kg)	328	491	374
Copper (mg/kg)	8	7	5
Zinc (mg/kg)	46	47	42

[1]Vermiculite was used as an inert filler to be replaced by inorganic zinc sulfate ($ZnSO_4$), copper sulfate, and coccidiostat in the experimental diets.
[2]Selenium premix provided 0.2 mg Se (as Na_2SeO_3).
[3]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 1,984 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.
[4]Coban was used as the coccidiostat in the starter (0.05%). Either Roxarsone (0.005%) or Coban (0.05%) was used in the grower. Coban supplied monensin sodium at 90 mg/kg of feed. Roxarsone supplied arsenic at 0.5 mg/kg of feed.

Table II.3. Analyzed mineral concentrations in the experimental diets (as fed)

	Mineral concentration (mg/kg)			
Starter diets[1]	**Cu**	**Zn**	**Fe**	**As**
1	8	46	328	0
2/4	15	137	278	0
3/5	236	145	265	0
6/8	13	302	335	0
7/9	191	326	365	0
Grower diets				
1	7	47	491	0
2	16	143	272	2.7
3	205	138	255	3
4	17	137	488	0
5	179	136	461	0
6	11	363	251	2.6
7	229	350	237	3.3
8	15	315	415	0
9	225	363	478	0
Finisher diets				
1	5	42	374	0
2	13	143	222	3.6
3	205	138	205	3.2
4	12	140	506	0
5	192	139	434	0
6	23	329	217	4
7	199	301	209	3
8	20	323	316	0
9	209	332	385	0

[1]Diet numbers are described in Table 1.

Table II.4. Least-squares means for the main effect of sex on growth performance of broilers raised to 56 d of age

Age	Sex: Male	Female	SE[1]	P-value
(d)	**Body weight (g)**			
1	48^{A}	47^{B}	0.1	0.003
14	521^{A}	492^{B}	2.3	0.0001
35	2517^{A}	2139^{B}	13.1	0.0001
55	4821^{A}	3808^{B}	19.4	0.0001
	Feed intake (g)			
1-14	682^{A}	636^{B}	5.6	0.0001
1-35	3740^{A}	3437^{B}	23.6	0.0001
1-55	9046^{A}	7625^{B}	37.9	0.0001
15-35	3058^{A}	2801^{B}	23.6	0.0001
36-55	5305^{A}	4188^{B}	39.7	0.0001
	Body weight gain (g)			
1-14	473^{A}	445^{B}	2.3	0.0001
1-35	2469^{A}	2092^{B}	13.1	0.0001
1-55	4773^{A}	3761^{B}	19.4	0.0001
15-35	1996^{A}	1648^{B}	12.7	0.0001
36-55	2304^{A}	1669^{B}	21.7	0.0001
	Adjusted feed conversion ratio (g:g)			
1-14	1.45	1.43	0.01	0.43
1-35	1.52^{B}	1.64^{A}	0.01	0.0001
1-55	1.90^{B}	2.02^{A}	0.01	0.0001
15-35	1.55^{B}	1.70^{A}	0.02	0.0001
36-55	2.34^{B}	2.50^{A}	0.03	0.0001
	Mortality (%)			
1-55	7.4^{A}	1.2^{B}	0.9	0.0001

^{A,B}Means in a row that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Standard error (SE) for n=36 pens of 16 birds each.

Table II.5. Least-squares means for the main effect of minerals on live performance of broiler males raised to 56 d of age

	Dietary treatments[1]												
	Negative control	**Zn (mg/kg)**		**SE[2]**	**Cu (mg/kg)**		**SE[2]**	**As (mg/kg)**		**SE[2]**	**P-value**		
Age		**120**	**240**		**10**	**100**		**0**	**0.5**		**Zn**	**Cu**	**As**
(d)	**Body weight (g)**												
1	48	48	48	0.2	48	48	0.2	-	-	-	0.73	0.21	-
14	515	518	523	3.1	524	518	3.1	-	-	-	0.32	0.19	-
35	2516	2515	2518	20.2	2524	2509	20.2	2503	2530	20.2	0.89	0.60	0.35
55	4798	4856	4786	29.2	4845	4796	29.2	4823	4819	29.2	0.10	0.25	0.93
	Feed intake (g)												
1-14	674	688	676	5.0	668^{B}	696^{A}	5.0	-	-	-	0.10	0.001	-
1-35	3731	3706	3775	36.9	3742	3739	36.9	-	-	-	0.20	0.96	-
1-55	9204	9067	9024	49.4	9137^{a}	8954^{b}	49.4	-	-	-	0.54	0.02	-
15-35	3057	3018	3099	36.0	3074	3042	36.0	3022	3095	36.0	0.13	0.54	0.17
36-55	5473	5361	5250	55.4	5395^{a}	5216^{b}	55.4	5359^{a}	5252^{b}	55.4	0.17	0.04	0.04
	Body weight gain (g)												
1-14	466	471	475	3.1	476	470	3.1	-	-	-	0.30	0.22	-
1-35	2468	2467	2471	20.3	2476	2462	20.3	-	-	-	0.89	0.61	-
1-55	4750	4808	4738	29.2	4797	4749	29.2	-	-	-	0.10	0.25	-
15-35	2002	1996	1996	20.1	2001	1991	20.1	1982	2010	20.1	0.98	0.75	0.32
36-55	2282	2341	2267	34.2	2321	2287	34.2	2320	2289	34.2	0.14	0.49	0.52
	Adjusted feed conversion ratio (g:g)												
1-14	1.45	1.47^{A}	1.43^{B}	0.01	1.41^{B}	1.49^{A}	0.01	-	-	-	0.01	0.001	-
1-35	1.53	1.51	1.54	0.02	1.52	1.53	0.02	-	-	-	0.43	0.57	-
1-55	1.95	1.90	1.91	0.01	1.92	1.90	0.01	-	-	-	0.57	0.23	-
15-35	1.55	1.53	1.57	0.03	1.55	1.55	0.03	1.55	1.55	0.03	0.27	0.95	0.95
36-55	2.42	2.35	2.33	0.03	2.37	2.32	0.03	2.37	2.32	0.03	0.72	0.30	0.74
	Mortality (%)												
1-55	7.8	9.4	5.5	1.7	6.3	8.6	1.7	-	-	-	0.12	0.35	-

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Negative control diets did not contain any added minerals. For the experimental diets, zinc (Zn) and copper (Cu) were included in the diets in the form of zinc sulfate and copper sulfate, respectively. Arsenic was added as Roxarsone.
[2]Standard error (SE) for n=16 pens of 16 birds each.

Table II.6. Least-squares means for the main effect of minerals on live performance of broiler females raised to 56 d of age

	Dietary treatments[1]												
	Negative control	Zn (mg/kg)		SE[2]	Cu (mg/kg)		SE[2]	As (mg/kg)		SE[2]	P-value		
Age		120	240		10	100		0	0.5		Zn	Cu	As
(d)					**Body weight (g)**								
1	47	47	47	0.2	47	47	0.2	-	-	-	0.84	0.83	-
14	482	495	488	3.4	490	493	3.4	-	-	-	0.13	0.61	-
35	2090	2135	2143	16.7	2137	2141	16.7	2136	2142	16.7	0.73	0.87	0.79
55	3753	3824	3792	16.3	3768^{B}	3848^{A}	16.3	3793	3823	16.3	0.19	0.01	0.20
					Feed intake (g)								
1-14	617	647^{a}	626^{b}	6.7	628	645	6.7	-	-	-	0.05	0.10	-
1-35	3370	3478^{a}	3397^{b}	24.0	3441	3434	24.0	-	-	-	0.03	0.83	-
1-55	7511	7714^{A}	7536^{B}	44.1	7650	7600	44.1	-	-	-	0.01	0.43	-
15-35	2753	2831^{a}	2771^{b}	19.7	2813	2789	19.7	2819	2783	19.7	0.05	0.41	0.21
36-55	4140	4237^{a}	4139^{b}	30.6	4209	4166	30.6	4221	4154	30.6	0.04	0.33	0.14
					Body weight gain (g)								
1-14	435	448	441	3.4	443	446	3.4	-	-	-	0.12	0.62	-
1-35	2043	2088	2096	16.7	2090	2094	16.7	-	-	-	0.73	0.87	-
1-55	3707	3777	3745	16.2	3721^{b}	3801^{a}	16.2	-	-	-	0.19	0.01	-
15-35	1608	1640	1655	15.1	1647	1648	15.1	1647	1648	15.1	0.47	0.94	0.99
36-55	1664	1689	1649	18.1	1631^{b}	1707^{a}	18.1	1657	1681	18.1	0.14	0.01	0.35
					Adjusted feed conversion ratio (g:g)								
1-14	1.42	1.44	1.42	0.02	1.42	1.45	0.02	-	-	-	0.36	0.20	-
1-35	1.65	1.66^{A}	1.62^{B}	0.01	1.64	1.64	0.01	-	-	-	0.01	0.78	-
1-55	2.02	2.04^{a}	2.01^{b}	0.01	2.05^{A}	2.00^{B}	0.01	-	-	-	0.05	0.01	-
15-35	1.72	1.72^{A}	1.67^{B}	0.01	1.71	1.69	0.01	1.71	1.69	0.01	0.01	0.45	0.21
36-55	2.47	2.51	2.49	0.03	2.56^{a}	2.44^{b}	0.03	2.53	2.48	0.03	0.59	0.03	0.18
					Mortality (%)								
1-55	3.1	1.2	1.2	0.5	1.2	1.2	0.6	-	-	-	1.00	1.00	-

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Negative control diets did not contain any added minerals. For the experimental diets, zinc (Zn) and copper (Cu) were included in the diets in the form of zinc sulfate and copper sulfate, respectively. Arsenic was added as Roxarsone.
[2]Standard error (SE) for n=16 pens of 16 birds each.

Table II.7. Least-squares means for the 2-way interaction effects of minerals on live performance of broiler males raised to 56 d of age

	Dietary treatments[1]																	
	Zn (mg/kg)				SE[2]	Zn (mg/kg)				SE[2]	Cu (mg/kg)				SE[2]	P-value		
	120		240			120		240			10		100			Zn* Cu	Zn*As	Cu* As
	Cu (mg/kg)					As (mg/kg)					As (mg/kg)							
Age	10	100	10	100		0	0.5	0	0.5		0	0.5	0	0.5				
(d)								**Body weight (g)**										
1	48	48	48	47	0.3	-	-	-	-	-	-	-	-	-	-	0.49	-	-
14	518	519	529	516	4.4	-	-	-	-	-	-	-	-	-	-	0.11	-	-
35	2544	2486	2505	2532	28.6	2522	2507	2483	2554	28.6	2479^{b}	2570^{a}	2527^{b}	2491^{b}	28.6	0.15	0.15	0.04
55	4917	4796	4775	4797	41.3	4885	4828	4761	4810	41.3	4873	4817	4772	4820	41.3	0.10	0.21	0.22
								Feed intake (g)										
1-14	678	699	658	694	7.1	-	-	-	-	-	-	-	-	-	-	0.32	-	-
1-35	3740	3672	3743	3806	52.2	-	-	-	-	-	-	-	-	-	-	0.22	-	-
1-55	9195	8940	9080	8968	69.9	-	-	-	-	-	-	-	-	-	-	0.32	-	-
15-35	3062	2973	3085	3112	50.9	2993	3042	3050	3147	50.9	3108^{a}	3039^{ab}	2935b	3150^{a}	50.9	0.26	0.64	0.02
36-55	5454	5269	5337	5163	78.4	5457	5266	5261	5239	78.4	5324^{A}	5467^{B}	5394^{B}	5037^{B}	78.4	0.94	0.29	0.01
								Body weight gain (g)										
1-14	470	471	482	469	4.3	-	-	-	-	-	-	-	-	-	-	0.12	-	-
1-35	2496	2438	2457	2485	28.7	-	-	-	-	-	-	-	-	-	-	0.15	-	-
1-55	4869	4748	4726	4750	41.2	-	-	-	-	-	-	-	-	-	-	0.10	-	-
15-35	2026	1967	1975	2016	28.4	2005	1988	1958	2033	28.4	1955^{b}	2046^{a}	2009^{ab}	1974^{ab}	28.4	0.10	0.12	0.04
36-55	2373	2310	2270	2265	48.3	2362	2321	2278	2256	48.3	2395^{a}	2248^{b}	2245^{b}	2330^{ab}	48.3	0.56	0.84	0.03
								Adjusted feed conversion ratio (g:g)										
1-14	1.45^{b}	1.49^{a}	1.37^{c}	1.48^{ab}	0.01	-	-	-	-	-	-	-	-	-	-	0.03	-	-
1-35	1.50	1.53	1.53	1.54	0.03	-	-	-	-	-	-	-	-	-	-	0.81	-	-
1-55	1.91	1.90	1.92	1.90	0.02	-	-	-	-	-	-	-	-	-	-	0.69	-	-
15-35	1.51	1.54	1.58	1.56	0.04	1.50	1.55	1.57	1.57	0.04	1.59^{AB}	1.50^{B}	1.48^{C}	1.62^{A}	0.04	0.56	0.52	0.01
36-55	2.38	2.33	2.36	2.31	0.05	2.37	2.34	2.33	2.33	0.05	2.26^{B}	2.47^{A}	2.44^{A}	2.20^{B}	0.05	1.000	0.76	0.0001
								Mortality (%)										
1-55	8.6	10.2	3.9	7.0	2.4	-	-	-	-	-	-	-	-	-	-	0.75	-	-

[a,b,c]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B,C]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]For the dietary treatments, zinc (Zn) and copper (Cu) were included in the diets in the form of zinc sulfate and copper sulfate, respectively. Arsenic was added as Roxarsone.
[2]Standard error (SE) for n=8 pens of 16 birds each.

Table II.8. Least-squares means for the 2-way interaction effects of minerals on live performance of broiler females raised to 56 d of age

	Dietary treatments[1]																	
	Zn (mg/kg)					**Zn (mg/kg)**					**Cu (mg/kg)**					**P-value**		
	120		**240**		**SE[2]**	**120**		**240**		**SE[2]**	**10**		**100**		**SE[2]**	**Zn*Cu**	**Zn*As**	**Cu*As**
	Cu (mg/kg)					**As (mg/kg)**					**As (mg/kg)**							
Age (d)	**10**	**100**	**10**	**100**		**0**	**0.5**	**0**	**0.5**		**0**	**0.5**	**0**	**0.5**				
	Body weight (g)																	
Day 1	47	47	47	47	0.3	-	-	-	-	-	-	-	-	-	-	0.73	-	-
Day 14	493	498	487	488	4.8	-	-	-	-	-	-	-	-	-	-	0.71	-	-
Day 35	2139	2131	2135	2152	23.7	2141	2129	2130	2156	23.7	2131	2143	2141	2142	23.7	0.60	0.43	0.83
Day 55	3801	3846	3735	3850	23.0	3814	3833	3772	3813	23.0	3758	3778	3828	3868	23.0	0.14	0.64	0.67
	Feed intake (g)																	
Day 1-14	648	645	609	644	9.4	-	-	-	-	-	-	-	-	-	-	0.06	-	-
Day 1-35	3502	3453	3380	3414	34.0	-	-	-	-	-	-	-	-	-	-	0.24	-	-
Day 1-55	7756	7672	7544	7528	62.4	-	-	-	-	-	-	-	-	-	-	0.59	-	-
Day 15-35	1646	1633	1647	1663	21.4	1645	1634	1650	1661	21.4	1644	1650	1651	1646	21.4	0.51	0.61	0.79
Day 36-55	1662	1715	1600	1699	25.6	1673	1705	1641	1657	25.6	1626	1636	1687	1727	25.6	0.38	0.76	0.56
	Body weight gain (g)																	
Day 1-14	446	451	440	441	4.8	-	-	-	-	-	-	-	-	-	-	0.72	-	-
Day 1-35	2092	2084	2088	2105	23.6	-	-	-	-	-	-	-	-	-	-	0.60	-	-
Day 1-55	3754	3799	3688	3803	23.0	-	-	-	-	-	-	-	-	-	-	0.14	-	-
Day 15-35	2854	2808	2772	2770	27.9	2859	2803	2779	2763	27.9	2827	2798	2811	2768	27.9	0.44	0.48	0.82
Day 36-55	4255	4218	4164	4114	43.3	4256	4217	4186	4092	43.2	4213	4206	4229	4103	43.3	0.87	0.53	0.19
	Adjusted feed conversion ratio (g:g)																	
Day 1-14	1.46[a]	1.43[ab]	1.38[b]	1.46[a]	0.02	-	-	-	-	-	-	-	-	-	-	0.03	-	-
Day 1-35	1.67	1.66	1.62	1.62	0.01	-	-	-	-	-	-	-	-	-	-	0.93	-	-
Day 1-55	2.06	2.02	2.04	1.97	0.02	-	-	-	-	-	-	-	-	-	-	0.52	-	-
Day 15-35	1.72	1.72	1.69	1.66	0.02	1.73	1.72	1.69	1.66	0.02	1.71	1.70	1.71	1.68	0.02	0.45	0.58	0.80
Day 36-55	2.56	2.47	2.57	2.42	0.04	2.54	2.48	2.51	2.47	0.04	2.56	2.57	2.50	2.38	0.04	0.44	0.73	0.10
	Mortality (%)																	
Day 1-55	1.6	0.8	0.8	1.6	0.9	-	-	-	-	-	-	-	-	-	-	0.40	-	-

[a,b,c]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).

[1]For the dietary treatments, zinc (Zn) and copper (Cu) were included in the diets in the form of zinc sulfate and copper sulfate, respectively. Arsenic was added as Roxarsone.

[2]Standard error (SE) for n=8 pens of 16 birds each.

CHAPTER III

Carcass and meat quality of male and female broilers fed diets supplemented with different levels of zinc, copper, and Roxarsone

3.1. ABSTRACT

This study was conducted to examine the effect of different dietary levels of inorganic dietary zinc (Zn), copper (Cu), and arsenic (As) as Roxarsone on carcass yield, breast meat quality, bone marrow color, and blood zinc protoporphyrin to heme ratio (ZPP/H) of male and female Ross 344 × 708 broilers raised to 56 d. Experimental diets consisted of a negative control and 8 diets formulated with either: 120 or 240 mg Zn/kg diet, 10 or 100 mg Cu/kg diet, and 0 or 0.5 mg As/kg diet. At 56 d, 2 birds per pen were slaughtered. Carcasses were dressed and breast fillets and femurs were collected for meat quality assessment. Deboned breast fillets were used to measure color, tenderness, and cook yield. Bone marrow was collected from the interior epiphyseal end caps of femurs for color measurement. Data was analyzed using GLM procedures of SAS to evaluate the effects of minerals and sex. Carcass and parts weights were heavier in males, however, females had greater yield of breast tenders. Cook yield and shear force values were greater in males than in females. Breast fillets from females had lighter and more yellow color compared to color of breast fillets from males, and lightness remained after cooking. Males had higher Zn concentration in muscles compared to females. Supplementing 120 mg Zn and 10 mg Cu/kg produced heavier carcasses in both males and females, while supplementing Zn at 240 mg/kg

improved breast fillet tenderness in males but had no effect on breast fillets in females. Supplementing Zn and Cu at 120 and 100 mg/kg, respectively, produced more red color of bone marrow in both males and females. Copper supplemented at 100 mg/kg increased Cu concentration in muscles of both males and females, while As supplementation reduced Fe concentration in muscles of males and bones of females. Based on results from this experiment, males and females differed in their response to mineral supplementation with regard to carcass and meat quality, bone marrow color, and tissue mineral concentrations. Supplementing basal broiler diets with trace minerals affected breast meat color and shear force.

Key words: Broiler, zinc, copper, Roxarsone, carcass quality, arsenic

3.2. INTRODUCTION

Zinc (Zn) and copper (Cu) have been identified as essential trace minerals that have important roles in digestive, physiological, and biosynthetic processes within the body (Vallee and Falchuk, 1993; Salim et al., 2008). In the United States, the broiler industry has used a large safety margin in feed formulation because of the low cost inorganic trace mineral sources despite the fact that most practical diets contain adequate iron (Fe) and Cu to satisfy nutritional requirements (Scott et al., 1982).

Roxarsone has been approved for use in broilers for increased growth rates and improved feed utilization. It has been also shown to be effective against intestinal parasites, particularly coccidiosis. Therefore, improved intestinal health could be the apparent reason for improved growth and feed utilization (Alpharma, 1999). In addition, recent field experience has revealed that Roxarsone may be effective at suppressing *Salmonella* and possibly other enteric pathogens that can cause food safety hazards for consumers (Jones, 2007).

Zinc and Cu have been used as antimicrobial agents and growth promoters, respectively (NRC, 1994) where Zn was usually added in response to footpad lesions and skin scratches. In commercial broiler production, birds have been raised on recycled litter, which will contain various levels of Zn, Cu, and other nutrients, thus providing an additional source of nutrients other than feed. Also, these minerals and other unabsorbed nutrients have accumulated in the litter that was intended to be used as a soil amendment and fertilizer, which might result in an unnecessary accumulation of these minerals in soil. Moreover, the excess of one supplemented mineral has been demonstrated to negatively influence metabolism of another. The Zn was known to antagonize Cu (Van Campen and Scaife, 1967) possibly due to the fact that ions of these elements had valence shells that were isoelectric, had a coordination number of four, and formed tetrahedral complexes (Hill et al., 1963).

The competition for mineral-binding ligands and mineral uptake sites in the mucosa of the gut (Santon et al., 2002) was proposed to be the main factor causing antagonism between Zn and Cu. Some studies have also showed that high Zn intake induced a high level of metallothionein in the intestinal mucosa that had high binding affinity for Cu (Fischer et al., 1983; Santon et al., 2002).

Zinc has been reported to compete with and replace Fe for binding to myoglobin in muscles and hemoglobin in blood. The resulting zinc protoporphyrin (ZPP) has been shown to cause red color in raw meat, and the redness did not decrease even at elevated cooking temperatures (Wakamatsu et al., 2004). Cooked poultry meat with a red discoloration negatively affected consumer acceptance and sales. Therefore, this research was conducted to evaluate the influence of adequate and excessive levels of Zn and Cu and their interaction in the presence and absence of an arsenical coccidiostat (Roxarsone) on carcass and meat quality of broiler males and females, and whether excessive Zn would promote red discoloration of both raw and cooked poultry meat.

3.3. MATERIALS AND METHODS

Birds and diets

A total of 1152 day-old male and female broiler chicks of Ross 344×708 strain were feather sexed and randomly distributed among 4 blocks with a total of 72 floor pens furnished with recycled litter. Birds were raised sex-separate with 16 birds per pen. Diets were formulated to either meet or exceed the NRC (1994) requirements for broilers except for Zn and Cu. Nine experimental diets were formulated from the same basal diet to ensure that birds had access to similar nutrients. The negative control diet did not contain any mineral premix. The remaining 8 experimental diets were formulated by adding inorganic zinc sulfate ($ZnSO_4$), copper sulfate ($CuSO_4$), and Roxarsone. Zinc sulfate (Zinc Nacional, S.A., Mexico) was added to achieve a Zn concentration in the experimental diets of either 120 or 240 mg Zn/kg diet. Copper sulfate was added to achieve a concentration of either 10 or 100 mg Cu/kg diet. Roxarsone was either not added, or added at a level of 45.4 mg/kg to achieve an As concentration of 0.5 mg/kg diet. The Fe concentration in all diets was fixed and determined to be 328, 491, and 374 mg/kg in the starter, grower, and finisher diets, respectively. All methods regarding bird care in this experiment were approved by the NCSU Institutional Animal Care and Use Committee.

Carcass and parts yield

At 56 d of age, birds were fasted and 2 birds per pen (a total of 144) were randomly selected and slaughtered at the NCSU pilot processing plant. Birds were weighed (fasted live weight), electrically stunned using 25 milliamps for 11 s, killed by exsanguination, and allowed to bleed for 90 s. Birds were then scalded at 55°C for 90 s in a rotary scalder, picked for 30 s in a drum picker, and manually eviscerated. Carcasses were dressed by removing giblets (liver, gizzard, and heart), oil gland, crop, proventriculus, neck, head, feet, lungs, and viscera to determine the hot WOG weight (carcass weight without giblets). Carcasses were then chilled overnight by immersion in ice chilled water at 0 to 4°C, and manually deboned on stationary cones the following day. Parts of the legs, thighs, breast fillets (*Pectoralis major*), breast tenders (*Pectoralis minor*), wings, and ribs, back, and skin were weighed. The carcass yield was calculated for both the hot and cold WOG as a percentage of the fasted live weight. Edible carcass yield was calculated by including the weight of giblets and neck. Parts yield was calculated as a percentage of the chilled WOG weight. Femur bones were collected from the thighs for bone marrow harvest and determination of bone minerals concentration.

Cook yield

Breast fillets (*Pectoralis major*) were weighed, placed on aluminum pans, and cooked in a forced air oven at 204°C (SilverStar Southbend, Model SLES/10sc, gas type, NC, USA). Fillets were cooked to an internal temperature of 75°C (approximately 25 min), as measured by a Therma Plus thermocouple with a 10-cm needle temperature probe (ThermoWorks Model 221-071, UT, USA). The cooked fillets were cooled to room temperature and re-weighed to determine cook yield as a percentage of the cooked weight relative to the raw weight.

Shear force

Cooked breast fillets samples were tested for texture using a Warner-Bratzler shear device (Warner-Bratzler meat shear, Bodine Electric Company, Chicago, USA). Two samples per breast fillets (2×2×2 cm) were sheared in a direction perpendicular to the muscle fiber. Crosshead speed was 4 mm/sec. The maximum force measured when cutting the samples was expressed in kg.

Color

Skin was removed from breast fillets and color was measured on the medial side of both raw and cooked breast fillets. Color was measured by the CIE L*a*b* system using a Minolta Chroma Meter CR-400 (Konica Minolta Sensing, Inc., Japan). A measuring area of 10 mm and illuminant D65 and 2° standard observer were used. The colorimeter was calibrated using a white tile (reference number 13033071; Y = 93.9, x = 0.3156, y = 0.3318).

Triplicate measurements were taken for each sample. CIE lightness (L^*), redness (a^*), and yellowness (b^*) were measured.

Red discoloration induction

This test was conducted according to the procedure described by Smith and Northcutt (2004a) which aimed to resemble the effect of bone marrow leakage to adjacent meat. Collected femurs were pooled by treatment and sex. Bone marrow was collected by removing the exterior bone from the epiphyseal end caps, and the porous hard bone marrow was collected, manually minced using mortar and pestle until it turned into a thick granular paste. Boneless, skinless broiler breast fillets were obtained fresh (refrigerated) from a local retail store. The breast meat contained up to 5% retained water (Nature's Place breast meat, USA). External fat was trimmed and connective tissue removed. Cleaned meat was chopped manually and blended for 30 s in a food processor (KitchenAid Model KFP600WH, MI, USA). Glass tubes open at both ends were used (OD = 20 mm, ID = 17 mm × 200 mm length). For each tube, 10 g of minced meat was divided into 2 equal parts. One part meat was put into the tube at one end, followed by 1 g of minced bone marrow, then the second part of the meat and each tube was sealed with a #2 rubber stopper. Two replicate tubes were prepared for each treatment per sex. Tubes were placed in a circulating water bath (Model MW-1120A-1, Blue M Electric Company, IL, USA) set at 95°C. Tubes were removed immediately when temperature reached 75°C (approximately 2 min) as measured by a Therma Plus thermocouple with a 125-cm hard-wired temperature probe (Probe Type K, ThermoWorks Model 221-071, UT, USA). Tubes were placed in an ice water bath until the temperature reached 4°C (approximately 45 min). Cooked plugs were then pushed out of the tubes (plug diameter = 16 mm) and separated manually into 2 parts at the contact surfaces with bone marrow; the excess marrow was cleaned off the meat surface. CIE lightness (L^*), redness (a^*), and yellowness (b^*) were measured at the contact surface of the meat with bone marrow in replicate.

Minerals concentration in tissues

Concentrations of Zn, Cu, and Fe were determined in collected breast muscles and bones using an inductively coupled plasma optical emission spectrometer (Perkin Elmer ICP-OES 2000 DV). Breast meat samples were prepared by mincing the meat and drying at 70°C for 72 h before each was finely ground. Bone samples were prepared by cleaning femurs of any exterior adhering tissues and then drying at 105°C. Bones were then soaked in ethyl ether (Ethyl Oxide, Diethyl Ether $C_4H_{10}O$) for 48 h to extract the fat before analyzing for mineral concentration.

Blood ZPP/H ratio

Blood ZPP/H ratio was determined on only 3 treatments at 4, 6, and 8 wk of age. The treatments were the negative control (Diet 1), normal dietary Zn (Diet 2), and high dietary Zn (Diet 6). Blood was collected from a total of 8 birds per treatment and sex interaction (2 birds/pen) at each collection period. Blood samples were collected from the wing via the brachial vein. The ZPP/H concentration in whole blood was measured immediately using a hematofluorometer (Aviv hematofluorometer Model 206D, Aviv Biomedical, NJ, USA). The instrument measured the ratio of ZPP fluorescence to heme absorption. A drop of whole blood was placed on a microscope cover glass (Aviv Biomedical, NJ, USA) for insertion into the ZPP hematofluorometer and fluorescence was measured in µmol/mol heme with an assumed hematocrit of 42%.

Statistical analysis

Data were analyzed as a randomized complete block design using the GLM procedure of SAS (SAS version 9.4, SAS Institute, Cary, NC, USA, 2014). Data was analyzed to assess the effect of sex, and then data from males and females were analyzed separately to assess the effect of dietary treatments within each sex. Differences between means were separated using the LS means method and significance was reported at a probability level of $P \leq 0.05$. Data from the negative control treatment were not included in the analysis but means were provided for inspection. The experimental unit for statistical analysis of measured parameters was the pen.

3.4. RESULTS AND DISCUSSION

Sex effect on carcass and parts weights and yield

Results for the effect of sex on carcass and parts weights and yield are presented in Table 1. Males exhibited greater ($P = 0.0001$) fasted live BW, hot and chilled carcass weight and edible carcass weight compared to females but percentage carcass yields as a percent of live weight were comparable. These findings differ from those of Kidd et al. (2005) who found that male broilers had lower dressing percentage when compared with females. Weights of carcass parts were also heavier ($P = 0.0001$) from males than from females. Percentage breast tenders yield was greater ($P = 0.0001$) in females than males while yield for legs and thighs were greater in males than females. Yield of breast tenders was 5.1 and 5.7% for males and females, respectively. Legs yield was 12.2 and 11.5% while thighs yields were 16.9 and 16.1% for males and females, respectively. Abdullah et al. (2010) reported similar results when investigating carcass yield from males and females from different broiler strains. According to their findings, males had higher BW and percentage leg, while females had greater percentage breast. Singh and Essary (1974) reported similar dressing yield between male and female broilers slaughtered at the same age. Lopez et al. (2011) reported that male broilers had greater carcass and breast weights compared to females when slaughtered at 42 d. Similar findings were also reported by several authors (Ojedapo et al., 2008; Raji et al., 2010; Muthukumar et al., 2011) where males exhibited heavier carcass weight compared to females. In a study conducted by Musa et al. (2006a) concerning carcass characteristics of chicken breeds, males had heavier carcass and breast muscle weights compared to females slaughtered at 12 wk of age.

Effect of supplemented minerals on carcass and parts weights and yield of males and females

Tables 2 and 3 show results for the main effect of minerals on carcass and parts weights and yields of males and females, respectively. Supplemented minerals had no effect on carcass weights and yields of males but adding Roxarsone (As) to feed produced heavier fasted live BW, heavier hot and chilled carcass weight, and edible carcass compared to non-supplemented females. Breast fillets of males were heavier (P = 0.04) and had greater percentage yield (P = 0.01) when Cu was supplemented at 10 mg/kg, while thighs were also heavier (P = 0.04) when Zn was supplemented at 120 mg/kg. In females, ribs, back, and skin weight was increased (P = 0.05) when diets were supplemented with Roxarsone. The yield of thighs in males was increased when Cu was supplemented at 100 mg/kg (P = 0.01) and in the absence of Roxarsone (P = 0.01). Parts yield of females were not affected by mineral supplementation. Results for the two-way interactions among minerals are shown in Tables 4 and 5 for males and females, respectively. Chilled carcass weight was influenced by Zn and Cu interaction in both males (P = 0.04) and females (P = 0.01). Supplementing 120 mg Zn and 10 mg Cu/kg produced heavier carcasses in both males and females. In females, supplementing 240 mg Zn and 100 mg Cu/kg produced higher fasted live BW (P = 0.01), chilled carcass weight (P = 0.01), breast fillets, (P = 0.01), breast tenders (P = 0.05), and thighs (P = 0.02) weights. Breast fillets yield was affected by the Cu and As interaction in females where increased percentage breast fillets yield was obtained when supplementing 100 mg Cu and 0 mg As/kg. Mondal et al. (2010) investigated feeding diets supplemented with either inorganic or organic trace minerals (Zn, Fe, Cu, and Mn) to broilers and reported that the inorganic mix produced carcasses with heavier breasts and legs parts while carcass weight and yield were comparable to the organic mix. Collins and Moran (1999) indicated that supplemental Mn and Zn did not alter processed carcass weights and abdominal fat percentage of diverse broiler strains, while Rossi et al. (2007) stated that carcass and cut-up (drumstick, thigh, and breast) yield were not influenced by the addition of increasing levels of dietary organic zinc in broilers.

Sex effect on meat quality

Cook yield of breast fillets was increased (P = 0.05) in males and raw and cooked breast fillets were also heavier in males than females (Table 6). Lopez et al. (2011) did not report any significant differences in breast fillets cook yield between male and female broilers slaughtered at 42 d. Shear force values, which was a measure of meat tenderness, were higher in males (P = 0.05) indicating that females possessed more tender meat compared to males. This could be explained by increased activity in males, it could also be the cause of their larger breast fillet size. Results with regards to breast fillets shear force as affected by sex have been variable. Some other investigators reported that males had meat that was more tender than females (Simpson and Goodwin, 1975; Salakova et al., 2009; Abdullah and Matarneh, 2010) while others have reported the opposite (Lyon et al., 1992; Musa et al., 2006b). Furthermore, bird sex had no effect on breast fillets tenderness in other studies (Ngoka et al., 1982; Lyon and Wilson, 1986; Poole et al., 1999; and Northcutt et al., 2001; Lopez et al., 2011).

Results for the color of breast fillets and bone marrow as affected by sex are presented in Table 11. Raw breast fillets of females had lighter (P = 0.0001) and yellower (P = 0.0001) color compared to males. The lightness (L^*) remained after cooking (P = 0.04). Cooked breast meat with bone marrow and raw bone marrow also was lighter in color in females than males. Color has been an important consideration for consumer satisfaction, and previous reports have indicated that color can be affected by strain, age, and sex (Froning, 1995). Fillets of processed females were all lighter in color than fillets of males, which was consistent with the findings of Brewer et al. (2012). However; Salakova et al. (2009) did not find any color differences between males and females with regards to raw or cooked meat color.

Effect of supplemented minerals on meat quality

Supplementing Zn at 240 mg/kg improved breast fillet tenderness in males (P = 0.02) but had no effect in females (Tables 7 and 8, respectively). On the other hand, the presence of As in diets had negative effect on tenderness in both males and females. Male breast fillet shear force values increased from 3.6 to 4.6 in the As supplemented birds, while in females values increased from 3.3 to 3.9 in As supplemented birds. An interaction effect of Zn and Cu was observed in females only (Table 10) where raw and cooked breast weight were increased when Zn and Cu were supplemented at 240 and 100 mg/kg.

Raw breast fillets yellowness (b^*) was decreased (P = 0.05) when dietary Zn was supplemented at 240 mg/kg in males only. Yellowness was increased (P = 0.01) in breast fillets by supplementing As in both males and females (Tables 12 and 13, respectively). However, the effect of As remained after cooking only in males (Table 12). Lightness of cooked breast meat with bone marrow was decreased by supplementing Zn at 240 mg/kg in both males (P = 0.01) and females (P = 0.01), while As increased (P = 0.02) L^* of cooked breast meat with marrow in males only.

Raw marrow color was influenced by mineral supplementation in both males and females. Redness and yellowness were decreased in males and females when Zn was supplemented at 240 mg/kg. Supplementing Cu at 100 mg/kg increased (P = 0.002) redness

(a^*) in females only, and yellowness in both sexes. Supplementing As also increased (P = 0.0001) a^* in breast fillets from females only. Based on the interaction results, supplementing Zn and Cu at 120 and 100 mg/kg, respectively, produced more red color of bone marrow in both males and females (Tables 14 and 15, respectively). Therefore, using high levels of Cu as a way to improve growth accompanied with a normal level of Zn could produce red marrow that might leach into the meat and cause a red discoloration in chicken meat. Cooked white meat with red color defect was considered unacceptable by consumers since it was an indicator that meat was undercooked and thus unsafe to eat. Red discoloration of cooked white meat was not a safety concern yet it has resulted in substantial losses to the poultry industry due to rejection, rework, and condemnation. Studies on carcass and meat quality of poultry as influenced by supplemented minerals have been limited. Hess et al. (2001) reported that Zn supplementation (40 mg/kg, either as Zn-Met or Zn-Lys) reduced skin tearing of broiler carcasses. Rossi et al. (2007) also reported that organic Zn (45 mg/kg, added to a basal diet containing 60 mg/kg $ZnSO_4$) minimized skin tearing and improved carcass appearance of broilers. Sahin et al. (2005) found that Zn supplementation (either as $ZnSO_4$ or Zn picolinate) improved carcass weight of Japanese quail. Tronina et al. (2007) reported that organic Zn (Zn-Gly) increased the proportion of breast and leg muscles, dressing percentage, and fat content in the breast and leg muscles of broilers. Liu et al. (2011) demonstrated that Zn supplementation up to 180 mg/kg from either organic or inorganic sources increased a^* and b^* values and decreased drip loss of breast muscle.

Sex effect on tissue mineral concentration

Concentrations of Cu, Fe, and Zn in breast muscles and femurs are presented in Table 16. Males and females had comparable tissue mineral concentrations except that males had more muscle Zn (P = 0.03) compared to females. Values were 25.1 and 23.6 mg/kg DM for males and females, respectively. Mohanna and Nys (1998) did not observe consistent sex differences of whole body mineral concentration (Zn, Cu, Fe, and Mn) of broilers measured at multiple ages. Saiz et al. (1990) also observed little effect of sex on liver, spleen, and kidney Fe concentrations in 4 and 8-wk-old broilers.

Effect of supplemented minerals on tissue mineral concentration

Cu supplemented at 100 mg/kg increased (P = 0.05) Cu concentration in muscles of both males and females (Tables 17 and 18, respectively), while As supplementation reduced Fe concentration in muscles of males (P = 0.05) and bones of females (P = 0.05). Mineral supplementation had no effect on Zn concentration in muscles and bones in either males or females. However, there was an interaction effect concerning Zn concentration in bones of males where supplementing Zn and As at 120 and 0.5 mg/kg, respectively, increased (P = 0.01) Zn concentration in bones of males with no effect on females. Total tibia Zn content was increased as dietary supplementation of either Zn sulfate (inorganic) or Zn Bioplex (organic) was increased (Ao et al., 2006; 2007; 2011). Also, feeding different levels of organically complexed Cu, Fe, Zn, and Mn increased tibia concentration of Zn, Cu, and Mn but had no effect on Fe tibia concentration in broilers raised in cages (Bao et al., 2007). Bafundo et al. (1984) also showed that tibia concentration of Zn increased with an increased dietary concentration of Zn up to 85 mg/kg diet. In another study, Zn supplementation at 200 mg/kg feed did not affect the content of this mineral in mixed dark and white raw broiler meat when slaughtered at 42 d (Bou et al., 2004). Leonhardt and Wenk (1997) studied the content of various trace elements and vitamins in several meat parts (pork, beef, veal, and chicken) and observed that Zn content in chicken breast and thigh had the lowest coefficient of variation. Therefore, they attributed this result to the fact that the content of this mineral in meat has been mainly genetically determined and has been only slightly affected by feed composition. Antagonisms between Zn and Cu have been well documented (Fischer et al., 1983; Santon et al., 2002) but we were unable to detect any antagonism based on mineral concentration in muscles and bones. On the other hand, results of Hamilton et al. (1979) indicated that there was evidence of antagonism of Cu with low levels of Zn in diets of young Japanese quails. Pimental et al. (1991) concluded that the source of Zn did not affect tibio tarsus and liver Zn, Cu, and Fe concentrations or immune function. Measured As was below detection levels in both muscles and bones so results were not reported. Kerr et al. (1969) reported that accumulation, plateau, and depletion of tissue (muscle, skin, liver, and kidney) residues of an organic arsenical (Roxarsone) administered in the feed to growing chickens was comparable to total arsenic residue in chickens receiving non-medicated feed.

Sex effect on blood ZPP/H ratio

Blood ZPP/H ratio was measured in males and females from only 3 out of the 9 experimental diets. These diets were negative control (no mineral supplementation), Diet 2 (120 Zn, 10 Cu, and 0.5 As mg/kg), and diet 6 (240 Zn, 10 Cu, and 0.5 As mg/kg). These values were all greater (P = 0.0001) in females. Values in males and females were 40.3 and 51.6 µmol/mol, 35.1 and 48.2 µmol/mol, and 39.6 and 54.3 µmol/mol, measured at 4, 6, and 8 wk of age, respectively. There was no diet effect on ZPP/H ratio of females measured at 4, 6, and 8 wk. However, in males, ZPP/H ratio measured at 4 wk was the lowest (P = 0.02) in birds fed diet 2 with values being 48.0, 39.1, and 43.3 µmol/mol for the negative control, diet 2, and diet 6, respectively. It was known that ZPP was a normal metabolite of heme metabolism but in states of Fe inadequacy, ZPP formation was enhanced, and thus the ZPP/H ratio was elevated during erythropoiesis (Labbe et al., 1999). Such elevation could be caused by Fe and Zn interaction as ferrochelatase substrates, with Zn utilization being increased when Fe supply was diminished. There was no reported research that has investigated ZPP/H as affected by diet or sex so these results cannot be compared with other findings.

3.5. CONCLUSION

From the present study it was concluded that even though males had a heavier absolute parts weights, females had higher percentage yield of breast tenders. Color of raw breast fillet harvested from females was lighter and more yellow than the color of fillets harvested from males. Supplementing Zn and Cu at 120 and 100 mg/kg, respectively, produced more red color of bone marrow in both males and females, which indicated that using high levels of Cu as a way to improve growth accompanied by a normal level of Zn could produce red marrow that might leach into the meat and cause a red discoloration in chicken meat.

Based on results from this experiment, males and females differed in their response to mineral supplementation with regard to carcass and meat quality, bone marrow color, and tissue mineral concentrations. Supplementing basal broiler diets with trace minerals affected breast meat color and shear force.

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Table III.1. Least-squares means for the main effect of sex on carcass and parts weights and yield of broilers raised to 56 d of age

	Sex		**SE[1]**	**P-value**
	Male	**Female**		
Carcass weight (g) and yield (%)				
Fasted live weight	4875^{A}	3866^{B}	31.0	0.0001
Hot WOG[2] weight	3636^{A}	2889^{B}	31.8	0.0001
Chilled WOG weight	3807^{A}	3017^{B}	26.0	0.0001
Giblets and neck weight	287^{A}	240^{B}	4.1	0.0001
Edible[3] carcass weight	3924^{A}	3129^{B}	33.3	0.0001
Hot carcass yield[4]	74.6	74.7	0.5	0.85
Chilled carcass yield	78.1	78.1	0.2	0.92
Edible carcass yield	80.5	80.9	0.5	0.52
Parts weight (g)				
Breast fillets	963^{A}	770^{B}	11.0	0.0001
Breast tenders	193^{A}	171^{B}	3.4	0.0001
Wings	370^{A}	294^{B}	2.8	0.0001
Legs	463^{A}	346^{B}	3.6	0.0001
Thighs	642^{A}	487^{B}	7.2	0.0001
Ribs, back, and skin	1167^{A}	910^{B}	21.9	0.0001
Parts yield[5] (%)				
Breast fillets	25.3	25.5	0.2	0.39
Breast tenders	5.1^{B}	5.7^{A}	0.1	0.0001
Wings	9.7	9.7	0.1	0.96
Legs	12.2^{A}	11.5^{B}	0.1	0.0001
Thighs	16.9^{A}	16.1^{B}	0.2	0.01
Ribs, back, and skin	30.7	30.1	0.6	0.48

^{A,B}Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Standard error (SE) for n=128 birds.
[2]WOG weight is carcass weight without giblets (heart, liver, gizzard, and neck).
[3]Edible carcass weight includes the weight of giblets and neck.
[4]Hot carcass yield calculated based on hot WOG weight as a percentage of fasted live weight, chilled carcass yield calculated based on chilled WOG weight as a percentage of fasted live weight, and edible carcass yield calculated based on edible carcass weight as a percentage of fasted live weight.
[5]Yield of parts calculated based on chilled WOG weight.

Table III.2. Least-squares means for the main effect of minerals on carcass and parts weights and yield of broiler males raised to 56 d of age

	Dietary treatments[1]												
	Negative control	Zn (mg/kg)		SE[2]	Cu (mg/kg)		SE[2]	As (mg/kg)		SE[2]	P-value		
		120	240		10	100		0	0.5		Zn	Cu	As
Carcass weight (g) and yield (%)													
Fasted live weight	4842	4895	4855	47.7	4871	4879	47.7	4822	4928	47.7	0.55	0.91	0.12
Hot WOG[3] weight	3639	3671	3602	45.5	3662	3611	45.5	3594	3679	45.5	0.28	0.44	0.19
Chilled WOG weight	3784	3839	3776	41.3	3818	3797	41.3	3773	3841	41.3	0.29	0.72	0.25
Giblets and neck weight	283	283	292	5.5	289	286	5.5	285	290	5.5	0.26	0.70	0.52
Edible[4] carcass weight	3921	3954	3893	46.7	3950	3897	46.7	3879	3968	46.7	0.36	0.42	0.18
Hot carcass yield[5]	75.1	75.0	74.2	0.5	81.8	79.9	0.5	80.4	80.5	0.5	0.28	0.12	0.88
Chilled carcass yield	78.1	78.4	77.8	0.3	78.4	77.8	0.3	74.5	74.6	0.5	0.17	0.20	0.50
Edible carcass yield	81.0	80.8	80.2	0.5	81.8	79.9	0.5	80.4	80.5	0.5	0.43	0.09	0.89
Parts weight (g)													
Breast fillets	948	977	949	18.6	991^{a}	935^{b}	18.6	957	969	18.6	0.30	0.04	0.65
Breast tenders	208	196	191	5.2	198	190	5.2	196	191	5.2	0.43	0.38	0.55
Wings	369	370	371	4.5	369	372	4.5	369	372	4.5	0.86	0.60	0.67
Legs	463	466	460	6.3	456	470	6.3	468	459	6.3	0.48	0.14	0.29
Thighs	643	657^{a}	626^{b}	10.2	651	637	10.2	629	655	10.2	0.04	0.21	0.08
Ribs, back, and skin	1102	1130	1204	38.7	1114	1220	38.7	1178	1157	38.7	0.18	0.06	0.71
Parts yield[6] (%)													
Breast fillets	25.1	25.4	25.1	0.3	25.9^{A}	24.6^{B}	0.3	25.3	25.2	0.3	0.51	0.01	0.77
Breast tenders	5.5	5.1	5.0	0.1	5.1	5.0	0.1	5.2	5.0	0.1	0.71	0.49	0.24
Wings	9.7	9.6	9.8	0.1	9.7	9.8	0.1	9.8	9.7	0.1	0.15	0.31	0.40
Legs	12.2	12.2	12.2	0.1	12.0^{B}	12.4^{A}	0.1	12.4^{A}	11.9^{B}	0.1	0.86	0.01	0.01
Thighs	17.0	17.1	16.6	0.2	17.0	16.7	0.2	16.7	17.1	0.2	0.11	0.29	0.25
Ribs, back, and skin	29.1	29.5	32.0	1.1	29.2	32.2	1.1	31.3	30.2	1.1	0.10	0.06	0.47

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Negative control diets did not contain any added minerals. For the experimental diets, zinc (Zn) and copper (Cu) were included in the diets in the form of zinc sulfate and copper sulfate, respectively. Arsenic was added as Roxarsone.
[2]Standard error (SE) for n=64 birds.
[3]WOG weight is carcass weight without giblets (heart, liver, gizzard, and neck).
[4]Edible carcass weight includes the weight of giblets and neck.
[5]Hot carcass yield calculated based on hot WOG weight as a percentage of fasted live weight, chilled carcass yield calculated based on chilled WOG weight as a percentage of fasted live weight, and edible carcass yield calculated based on edible carcass weight as a percentage of fasted live weight.
[6]Yield of parts calculated based on chilled WOG weight.

Table III.3. Least-squares means for the main effect of minerals on carcass and parts weights and yield of broiler females raised to 56 d of age

	Dietary treatments[1]												
	Negative control	Zn (mg/kg)		SE[2]	Cu (mg/kg)		SE[2]	As (mg/kg)		SE[2]	P-value		
		120	240		10	100		0	0.5		Zn	Cu	As
Carcass weight (g) and yield (%)													
Fasted live weight	3705	3888	3844	36.1	3835	3897	36.1	3797[B]	3935[A]	36.1	0.40	0.23	0.01
Hot WOG[3] weight	2734	2896	2882	42.6	2836	2942	42.6	2807[B]	2971[A]	42.6	0.81	0.09	0.01
Chilled WOG weight	2942	3021	3014	30.2	2998	3037	30.2	2959[B]	3076[A]	30.2	0.86	0.36	0.01
Giblets and neck weight	235	238	242	6.2	241	239	6.2	232	249	6.2	0.69	0.87	0.08
Edible[4] carcass weight	2968	3135	3124	45.8	3077	3182	45.8	3039[B]	3220[A]	45.8	0.87	0.11	0.01
Hot carcass yield[5]	74.7	74.5	75.0	0.8	74.0	75.5	0.8	73.9	75.5	0.8	0.65	0.17	0.16
Chilled carcass yield	80.4	77.7	78.4	0.3	78.2	77.9	0.3	77.9	78.2	0.3	0.08	0.50	0.52
Edible carcass yield	81.1	80.6	81.3	0.8	80.2	81.6	0.8	80.0	81.8	0.8	0.57	0.25	0.14
Parts weight (g)													
Breast fillets	752	763	778	11.1	764	777	11.1	762	779	11.1	0.33	0.42	0.29
Breast tenders	169	170	172	4.3	168	173	4.3	172	170	4.3	0.71	0.41	0.83
Wings	283	291	296	3.4	295	293	3.4	289	298	3.4	0.34	0.71	0.08
Legs	339	347	345	3.9	344	348	3.9	343	348	3.9	0.73	0.51	0.32
Thighs	462	490	483	9.1	478	495	9.1	475	499	9.1	0.60	0.18	0.07
Ribs, back, and skin	875	921	898	17.3	909	911	17.3	885[b]	935[a]	17.3	0.35	0.94	0.05
Parts yield[6] (%)													
Breast fillets	25.6	25.2	25.8	0.2	25.5	25.6	0.2	25.7	25.3	0.2	0.10	0.73	0.22
Breast tenders	5.8	5.6	5.7	0.1	5.6	5.7	0.1	5.8	5.5	0.1	0.74	0.60	0.18
Wings	9.6	9.7	9.8	0.1	9.8	9.7	0.1	9.8	9.7	0.1	0.16	0.14	0.45
Legs	11.5	11.5	11.5	0.1	11.5	11.5	0.1	11.6	11.3	0.1	0.81	0.84	0.11
Thighs	15.7	16.2	16.0	0.3	15.9	16.3	0.3	16.1	16.2	0.3	0.65	0.34	0.68
Ribs, back, and skin	29.7	30.5	29.8	0.4	30.3	30.0	0.4	29.9	30.4	0.4	0.29	0.59	0.41

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Negative control diets did not contain any added minerals. For the experimental diets, zinc (Zn) and copper (Cu) were included in the diets in the form of zinc sulfate and copper sulfate, respectively. Arsenic was added as Roxarsone.
[2]Standard error (SE) for n=64 birds.
[3]WOG weight is carcass weight without giblets (heart, liver, gizzard, and neck).
[4]Edible carcass weight includes the weight of giblets and neck.
[5]Hot carcass yield calculated based on hot WOG weight as a percentage of fasted live weight, chilled carcass yield calculated based on chilled WOG weight as a percentage of fasted live weight, and edible carcass yield calculated based on edible carcass weight as a percentage of fasted live weight.
[6]Yield of parts calculated based on chilled WOG weight.

Table III.4. Least-squares means for the 2-way interaction effects of minerals on carcass and parts weights and yield of broiler males raised to 56 d of age

	Dietary treatments[1]																	
	Zn (mg/kg)					**Zn (mg/kg)**					**Cu (mg/kg)**					**P-value**		
	120		**240**		**SE[2]**	**120**		**240**		**SE[2]**	**10**		**100**		**SE[2]**	**Zn*Cu**	**Zn*As**	**Cu*As**
	Cu (mg/kg)					**As (mg/kg)**					**As (mg/kg)**							
	10	**100**	**10**	**100**		**0**	**0.5**	**0**	**0.5**		**0**	**0.5**	**0**	**0.5**				
Carcass weight (g) and yield (%)																		
Fasted live weight	4959	4832	4784	4926	67.5	4851	4940	4794	4916	67.5	4818	4925	4827	4931	67.4	0.06	0.81	0.99
Hot WOG[3] weight	3730	3613	3593	3610	64.3	3649	3693	3539	3664	64.3	3649	3675	3540	3683	64.3	0.31	0.53	0.37
Chilled WOG weight	3911[a]	3760[ab]	3725[b]	3827[ab]	58.4	3807	3870	3740	3812	58.4	3797	3839	3750	3843	58.4	0.04	0.93	0.66
Giblets and neck weight	290	275	287	296	7.8	277	289	292	291	7.8	285	293	284	287	7.8	0.15	0.40	0.73
Edible[4] carcass weight	4020	3888	3881	3906	66.1	3926	3982	3831	3955	66.1	3933	3967	3824	3970	66.1	0.24	0.61	0.40
Hot carcass yield[5]	75.2	74.7	75.1	73.3	0.7	75.2	74.7	73.8	74.5	0.7	75.7	74.6	73.3	74.7	0.7	0.37	0.45	0.10
Chilled carcass yield	78.9	77.9	77.9	77.7	0.5	78.5	78.3	78.0	77.5	0.5	78.8	77.9	77.7	77.9	0.5	0.36	0.68	0.21
Edible carcass yield	81.1	80.4	81.1	79.3	0.7	80.9	80.6	79.9	80.5	0.7	81.6	80.6	79.2	80.5	0.7	0.39	0.57	0.11
Parts weight (g)																		
Breast fillets	1018	936	964	934	26.3	976	978	938	960	26.3	992	989	921	949	28.3	0.33	0.70	0.57
Breast tenders	206	187	187	194	7.3	201	192	191	190	7.3	200	194	192	189	7.3	0.08	0.58	0.85
Wings	374	365	363	379	6.3	366	374	372	370	6.3	370	367	368	376	6.3	0.06	0.39	0.42
Legs	463	470	450	470	8.9	472	461	464	456	8.9	461	452	475	465	8.9	0.47	0.81	0.88
Thighs	673	642	629	624	14.5	641	674	617	636	14.5	642	660	615	650	14.5	0.38	0.63	0.55
Ribs, back, and skin	1129	1132	1100	1309	54.7	1108	1153	1248	1161	54.7	1100	1129	1255	1186	54.7	0.07	0.23	0.37
Parts yield[6] (%)																		
Breast fillets	26.0	24.8	25.8	24.4	0.5	25.6	25.2	25.1	25.1	0.5	26.1	25.7	24.6	24.7	0.5	0.80	0.66	0.60
Breast tenders	5.3	5.0	5.0	5.1	0.2	5.3	5.0	5.1	5.0	0.2	5.3	5.0	5.1	4.9	0.2	0.32	0.59	0.99
Wings	9.6	9.7	9.8	9.9	0.1	9.6	9.7	10.0	9.7	0.1	9.8	9.6	9.8	9.8	0.1	0.99	0.23	0.68
Legs	11.8	12.5	12.1	12.3	0.1	12.4	11.9	12.4	12.0	0.1	12.1	11.8	12.7	12.1	0.1	0.14	0.84	0.44
Thighs	17.2	17.0	16.9	16.3	0.3	16.9	17.4	16.5	16.7	0.3	16.9	17.2	16.5	16.9	0.3	0.58	0.58	0.80
Ribs, back, and skin	28.9	30.1	29.6	34.4	1.5	29.1	29.8	33.5	30.5	1.5	29.0	29.5	33.6	30.9	1.5	0.25	0.23	0.29

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[1]For the dietary treatments, zinc (Zn) and copper (Cu) were included in the diets in the form of zinc sulfate and copper sulfate, respectively. Arsenic was added as Roxarsone.
[2]Standard error (SE) for n=32 birds.
[3]WOG weight is carcass weight without giblets (heart, liver, gizzard, and neck).
[4]Edible carcass weight includes the weight of giblets and neck.
[5]Hot carcass yield calculated based on hot WOG weight as a percentage of fasted live weight, chilled carcass yield calculated based on chilled WOG weight as a percentage of fasted live weight, and edible carcass yield calculated based on edible carcass weight as a percentage of fasted live weight.
[6]Yield of parts calculated based on chilled WOG weight.

Table III.5. Least-squares means for the 2-way interaction effects of minerals on carcass and parts weights and yield of broiler females raised to 56 d of age

	Dietary treatments[1]																	
	Zn (mg/kg)				**SE[2]**	**Zn (mg/kg)**				**SE[2]**	**Cu (mg/kg)**				**SE[2]**	**P-value**		
	120		**240**			**120**		**240**			**10**		**100**			**Zn*Cu**	**Zn*As**	**Cu*As**
	Cu (mg/kg)					**As (mg/kg)**					**As (mg/kg)**							
	10	**100**	**10**	**100**		**0**	**0.5**	**0**	**0.5**		**0**	**0.5**	**0**	**0.5**				
Carcass weight (g) and yield (%)																		
Fasted live weight	3941^{A}	3834^{AB}	3729^{B}	3959^{A}	51.0	3812	3963	3782	3907	51.0	3757	3914	3837	3957	51.0	0.01	0.80	0.71
Hot WOG[3] weight	2903	2890	2769	2995	60.3	2807	2986	2807	2957	60.3	2786	2886	2828	3057	60.3	0.06	0.80	0.28
Chilled WOG weight	3061^{A}	2982^{AB}	2935^{B}	3092^{A}	42.7	2956	3086	2962	3066	42.7	2947	3049	2971	3103	42.7	0.01	0.76	0.73
Giblets and neck weight	241	235	240	244	8.8	236	240	227	257	8.8	234	248	230	249	8.8	0.59	0.15	0.75
Edible[4] carcass weight	3144	3125	3009	3238	64.7	3043	3227	3034	3213	64.7	3020	3133	3057	3306	64.7	0.07	0.97	0.30
Hot carcass yield[5]	73.6	75.3	74.3	75.7	1.1	73.6	75.3	74.2	75.7	1.1	74.2	73.7	73.7	77.2	1.1	0.91	0.93	0.09
Chilled carcass yield	77.6	77.8	78.7	78.1	0.4	77.5	77.9	78.3	78.5	0.4	78.4	77.9	77.4	78.4	0.4	0.33	0.78	0.07
Edible carcass yield	79.8	81.4	80.7	81.8	1.2	79.8	81.3	80.2	82.3	1.2	80.4	80.1	79.7	83.5	1.2	0.82	0.83	0.09
Parts weight (g)																		
Breast fillets	779^{AB}	746^{B}	749^{B}	808^{A}	15.7	755	770	769	788	15.7	742	786	782	771	15.7	0.01	0.90	0.09
Breast tenders	173^{ab}	166^{ab}	163^{b}	181^{a}	6.1	175	165	169	175	6.1	166	171	177	170	6.1	0.05	0.18	0.35
Wings	297	286	293	299	4.8	286	297	293	299	4.8	291	298	288	298	4.8	0.08	0.70	0.70
Legs	349	345	339	350	5.5	341	353	345	344	5.5	344	343	342	353	5.5	0.17	0.26	0.26
Thighs	497^{a}	483^{ab}	459^{b}	508^{a}	12.9	475	505	474	492	12.9	470	486	479	512	12.9	0.02	0.62	0.55
Ribs, back, and skin	928	914	890	907	24.5	888	955	882	915	24.5	892	925	878	944	24.5	0.53	0.49	0.50
Parts yield[6] (%)																		
Breast fillets	25.4	25.0	25.5	26.1	0.3	25.5	25.0	25.9	25.7	0.3	25.1^{B}	25.8^{AB}	26.3^{A}	24.8^{B}	0.3	0.14	0.65	0.01
Breast tenders	5.7	5.6	5.6	5.9	0.2	5.9	5.3	5.7	5.7	0.2	5.6	5.6	6.0	5.5	0.2	0.40	0.14	0.28
Wings	9.7	9.6	10.0	9.7	0.1	9.7	9.6	9.9	9.8	0.1	9.9	9.8	9.7	9.6	0.1	0.43	0.85	0.77
Legs	11.4	11.6	11.6	11.3	0.2	11.6	11.4	11.7	11.2	0.2	11.7	11.3	11.5	11.4	0.2	0.27	0.37	0.37
Thighs	16.3	16.2	15.6	16.4	0.4	16.1	16.4	16.0	16.1	0.4	16.0	15.9	16.1	16.5	0.4	0.28	0.77	0.56
Ribs, back, and skin	30.3	30.6	30.3	29.3	0.6	30.0	30.9	29.7	29.8	0.6	30.2	30.4	29.5	30.4	0.6	0.29	0.50	0.52

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]For the dietary treatments, zinc (Zn) and copper (Cu) were included in the diets in the form of zinc sulfate and copper sulfate, respectively. Arsenic was added as Roxarsone.
[2]Standard error (SE) for n=32 birds.
[3]WOG weight is carcass weight without giblets (heart, liver, gizzard, and neck).
[4]Edible carcass weight includes the weight of giblets and neck.
[5]Hot carcass yield calculated based on hot WOG weight as a percentage of fasted live weight, chilled carcass yield calculated based on chilled WOG weight as a percentage of fasted live weight, and edible carcass yield calculated based on edible carcass weight as a percentage of fasted live weight.
[6]Yield of parts calculated based on chilled WOG weight.

Table III.6. Least-squares means for the main effect of sex on cook yield and texture of breast fillets harvested from broilers raised to 56 d of age

	Sex		SE[1]	P-value
	Male	**Female**		
Raw breast weight (g)	479^{A}	386^{B}	6.0	0.0001
Cooked breast weight (g)	334^{A}	262^{B}	4.7	0.0001
Cook loss (%)	30.4^{b}	32.3^{a}	0.6	0.03
Cook yield (%)	69.6^{a}	67.7^{b}	0.6	0.05
Shear force (kg)	4.1^{a}	3.6^{b}	0.2	0.05

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Standard error (SE) for n=128 birds.

Table III.7. Least-squares means for the main effect of minerals on cook yield and texture of breast fillets harvested from broiler males raised to 56 d of age

	Dietary treatments[1]												
	Negative control	Zn (mg/kg)		SE[2]	Cu (mg/kg)		SE[2]	As (mg/kg)		SE[2]	P-value		
		120	240		10	100		0	0.5		Zn	Cu	As
Raw breast weight (g)	477	486	473	9.8	494[a]	465[b]	9.8	479	479	9.8	0.38	0.04	0.98
Cooked breast weight (g)	324	335	332	8.2	344	323	8.2	335	332	8.2	0.81	0.07	0.80
Cook loss (%)	32.2	31.0	29.8	0.8	30.2	30.6	0.8	30.0	30.8	0.8	0.33	0.76	0.50
Cook yield (%)	67.8	69.0	70.2	0.8	69.8	69.4	0.8	70.0	69.2	0.8	0.33	0.76	0.50
Shear force (kg)	4.0	4.5[a]	3.7[b]	0.2	3.8	4.4	0.2	3.6[B]	4.6[A]	0.2	0.02	0.09	0.01

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Negative control diets did not contain any added minerals. For the experimental diets, zinc (Zn) and copper (Cu) were included in the diets in the form of zinc sulfate and copper sulfate, respectively. Arsenic was added as Roxarsone.
[2]Standard error (SE) for n=64 birds.

Table III.8. Least-squares means for the main effect of minerals on cook yield and texture of breast fillets harvested from broiler females raised to 56 d of age

	Dietary treatments[1]												
	Negative control	**Zn (mg/kg)**		**SE[2]**	**Cu (mg/kg)**		**SE[2]**	**As (mg/kg)**		**SE[2]**	**P-value**		
		120	**240**		**10**	**100**		**0**	**0.5**		**Zn**	**Cu**	**As**
Raw breast weight (g)	383	382	390	6.6	380	392	6.6	381	391	0.6	0.42	0.22	0.27
Cooked breast weight (g)	253	259	264	4.8	260	263	4.8	257	267	4.8	0.49	0.69	0.15
Cook loss (%)	34.0	32.4	32.1	1.0	31.5	33.0	1.0	32.9	31.7	1.0	0.83	0.28	0.37
Cook yield (%)	66.0	67.6	67.9	1.0	68.5	67.0	1.0	67.1	68.3	1.0	0.94	0.42	0.54
Shear force (kg)	4.0	3.8	3.5	0.2	3.8	3.4	0.2	3.3[b]	3.9[a]	0.2	0.25	0.22	0.05

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).

[1]Negative control diets did not contain any added minerals. For the experimental diets, zinc (Zn) and copper (Cu) were included in the diets in the form of zinc sulfate and copper sulfate, respectively. Arsenic was added as Roxarsone.

[2]Standard error (SE) for n=64 birds.

Table III.9. Least-squares means for the 2-way interaction effects of minerals on cook yield and texture of breast fillets harvested from broiler males raised to 56 d of age

	Dietary treatments[1]																		
	Zn (mg/kg)				SE[2]	Zn (mg/kg)				SE[2]	Cu (mg/kg)				SE[2]	P-value		
	120		240			120		240			10		100			Zn*Cu	Zn*As	Cu*As
	Cu (mg/kg)					As (mg/kg)					As (mg/kg)							
	10	100	10	100		0	0.5	0	0.5		0	0.5	0	0.5				
Raw breast weight (g)	508	463	480	466	13.9	488	482	470	476	13.9	497	491	462	468	13.9	0.28	0.65	0.41
Cooked breast weight (g)	349	321	340	325	11.6	336	334	334	331	11.6	347	342	323	323	11.6	0.53	0.95	0.81
Cook loss (%)	31.1	30.8	29.3	30.3	1.2	31.1	30.8	28.9	30.8	1.2	29.9	30.5	30.1	31.0	1.2	0.60	0.33	0.91
Cook yield (%)	68.9	69.2	70.7	69.7	1.2	68.9	69.2	71.1	69.2	1.2	70.1	69.5	69.9	69.0	1.2	0.60	0.33	0.91
Shear force (kg)	4.3	4.7	3.4	4.1	0.3	4.0	5.0	3.2	4.2	0.3	3.5	4.2	3.7	5.0	0.3	0.60	0.87	0.30

[1]For the dietary treatments, zinc (Zn) and copper (Cu) were included in the diets in the form of zinc sulfate and copper sulfate, respectively. Arsenic was added as Roxarsone.

[2]Standard error (SE) for n=32 birds.

Table III.10. Least-squares means for the 2-way interaction effects of minerals on cook yield and texture of breast fillets harvested from broiler females raised to 56 d of age

	Dietary treatments[1]																
	Zn (mg/kg)					**Zn (mg/kg)**					**Cu (mg/kg)**					**P-value**		
	120		**240**		**SE[2]**	**120**		**240**		**SE[2]**	**10**		**100**		**SE[2]**	**Zn* Cu**	**Zn* As**	**Cu* As**
	Cu (mg/kg)					**As (mg/kg)**					**As (mg/kg)**							
	10	**100**	**10**	**100**		**0**	**0.5**	**0**	**0.5**		**0**	**0.5**	**0**	**0.5**				
Raw breast weight (g)	389[AB]	376[B]	372[B]	408[A]	9.3	382	383	380	400	9.3	370	390	392	392	9.3	0.01	0.31	0.30
Cooked breast weight (g)	265[ab]	254[b]	256[b]	273[a]	6.8	256	263	258	271	6.8	251	269	262	264	6.8	0.04	0.66	0.26
Cook loss (%)	31.7	33.0	31.3	32.9	1.4	33.6	31.2	32.2	32.1	1.4	32.0	31.1	33.8	32.2	1.4	0.93	0.40	0.80
Cook yield (%)	68.3	67.0	68.7	67.1	1.4	66.4	68.8	67.8	67.9	1.4	68.0	68.9	66.2	67.8	1.4	0.71	0.58	0.97
Shear force (kg)	4.0	3.6	3.6	3.3	0.3	3.4	4.2	3.3	3.6	0.3	3.6	4.1	3.1	3.8	0.3	0.96	0.44	0.64

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]For the dietary treatments, zinc (Zn) and copper (Cu) were included in the diets in the form of zinc sulfate and copper sulfate, respectively. Arsenic was added as Roxarsone.
[2]Standard error (SE) for n=32 birds.

Table III.11. Least-squares means for the main effect of sex on color of breast fillets and bone marrow harvested from broilers raised to 56 d of age

	Sex		SE[1]	P-value
	Male	**Female**		
Raw breast fillets color				
*L**	49.98^{B}	52.54^{A}	0.3	0.0001
*a**	1.95	1.64	0.1	0.14
*b**	5.97^{B}	7.12^{A}	0.2	0.0001
Cooked breast fillets color				
*L**	82.98^{b}	83.82^{a}	0.3	0.04
*a**	2.87	2.83	0.1	0.83
*b**	15.90	16.02	0.2	0.83
Cooked breast meat with marrow				
*L**	51.47^{B}	54.07^{A}	0.6	0.01
*a**	14.81	13.66	0.7	0.24
*b**	13.50	14.72	0.5	0.09
Raw marrow color				
*L**	38.72^{b}	40.11^{a}	0.4	0.02
*a**	31.57	33.69	1.0	0.13
*b**	13.62^{b}	15.34^{a}	0.5	0.03

^{a,b}Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
^{A,B}Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Standard error (SE) for n=128 birds for raw and cooked breast fillets, n=64 samples for cooked breast meat with marrow, and n=32 samples for raw marrow color.

Table III.12. Least-squares means for the main effect of minerals on color of breast fillets and bone marrow harvested from broiler males raised to 56 d of age

	Dietary treatments[1]												
	Negative control	Zn (mg/kg)		SE[2]	Cu (mg/kg)		SE[2]	As (mg/kg)		SE[2]	P-value		
		120	240		10	100		0	0.5		Zn	Cu	As
Raw breast fillets color													
*L**	51.75	50.12	49.83	0.5	50.24	49.71	0.5	50.19	49.76	0.5	0.68	0.45	0.54
*a**	1.72	1.71	2.19	0.2	2.17	1.73	0.2	1.89	2.01	0.2	0.07	0.10	0.63
*b**	5.95	6.34[a]	5.60[b]	0.3	5.89	6.04	0.3	5.48[B]	6.46[A]	0.3	0.05	0.70	0.01
Cooked breast fillets color													
*L**	83.69	82.84	83.13	0.4	82.98	82.99	0.4	82.95	83.02	0.4	0.63	0.99	0.90
*a**	2.92	2.76	2.98	0.2	2.95	2.79	0.2	3.02	2.73	0.2	0.46	0.60	0.34
*b**	16.08	16.23	15.56	0.3	15.57	16.23	0.3	15.29[B]	16.51[A]	0.3	0.10	0.10	0.01
Cooked breast meat with marrow													
*L**	55.32	52.57[A]	50.37[B]	0.5	51.21	51.73	0.5	50.46[b]	52.47[a]	0.5	0.01	0.49	0.02
*a**	15.83	15.38	14.24	1.1	15.14	14.48	1.1	15.28	14.34	1.1	0.47	0.68	0.55
*b**	14.28	13.55	13.44	0.8	13.59	13.41	0.8	13.45	13.54	0.8	0.92	0.87	0.94
Raw marrow color													
*L**	35.67	37.47[B]	39.96[A]	0.1	37.09[B]	39.34[A]	0.1	38.45[b]	38.98[a]	0.1	0.0001	0.001	0.03
*a**	24.48	34.09[A]	29.05[B]	0.3	31.08	32.07	0.3	31.90	31.23	0.3	0.0001	0.08	0.19
*b**	9.39	14.95[A]	12.28[B]	0.1	13.13[B]	14.11[A]	0.1	13.89[a]	13.34[b]	0.1	0.0001	0.001	0.03

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Negative control diets did not contain any added minerals. For the experimental diets, zinc (Zn) and copper (Cu) were included in the diets in the form of zinc sulfate and copper sulfate, respectively. Arsenic was added as Roxarsone.
[2]Standard error (SE) for n=64 birds for raw and cooked breast fillets, n=32 samples for cooked breast meat with marrow, and n=16 samples for raw marrow color.

Table III.13. Least-squares means for the main effect of minerals on color of breast fillets and bone marrow harvested from broiler females raised to 56 d of age

	Dietary treatments[1]											
	Negative control	Zn (mg/kg)		SE[2]	Cu (mg/kg)		SE[2]	As (mg/kg)		SE[2]	P-value		
		120	240		10	100		0	0.5		Zn	Cu	As
Raw breast fillets color													
*L**	51.88	53.07	52.00	0.4	52.41	52.67	0.4	52.64	52.43	0.4	0.08	0.66	0.73
*a**	1.44	1.75	1.54	0.2	1.78	1.50	0.2	1.55	1.73	0.2	0.51	0.39	0.56
*b**	6.32	7.03	7.20	0.3	6.83	7.42	0.3	6.73^{b}	7.52^{a}	0.3	0.64	0.14	0.05
Cooked breast fillets color													
*L**	82.21	83.75	83.89	0.4	83.99	83.64	0.4	84.00	83.63	0.4	0.79	0.51	0.48
*a**	3.39	2.85	2.81	0.2	2.80	2.86	0.2	2.78	2.87	0.2	0.88	0.83	0.77
*b**	17.08	15.80	16.24	0.4	15.82	16.21	0.4	15.89	16.15	0.4	0.41	0.45	0.62
Cooked breast meat with marrow													
*L**	53.28	55.88^{A}	52.25^{B}	0.7	54.34	53.80	0.7	53.38	54.75	0.7	0.01	0.61	0.20
*a**	13.29	13.65	13.68	1.0	13.67	13.65	1.0	14.18	13.15	1.0	0.99	0.99	0.46
*b**	12.63	15.00	14.44	0.7	14.95	14.48	0.7	14.34	15.10	0.7	0.56	0.63	0.44
Raw marrow color													
*L**	39.69	40.36	39.86	0.2	40.13	40.09	0.2	40.68^{A}	39.55^{B}	0.2	0.18	0.92	0.01
*a**	37.17	36.14^{A}	31.25^{B}	0.1	33.19^{B}	34.19^{A}	0.1	31.85^{B}	35.54^{A}	0.1	0.0001	0.002	0.0001
*b**	17.64	16.39^{A}	14.29^{B}	0.1	15.08^{b}	15.59^{a}	0.1	14.49^{B}	16.19^{A}	0.1	0.0001	0.04	0.0001

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Negative control diets did not contain any added minerals. For the experimental diets, zinc (Zn) and copper (Cu) were included in the diets in the form of zinc sulfate and copper sulfate, respectively. Arsenic was added as Roxarsone.
[2]Standard error (SE) for n=64 birds for raw and cooked breast fillets, n=32 samples for cooked breast meat with marrow, and n=16 samples for raw marrow color.

Table III.14. Least-squares means for the 2-way interaction effects of minerals on color of breast fillets and bone marrow harvested from broiler males raised to 56 d of age

	Dietary treatments[1]															P-value		
	Zn (mg/kg)				SE[2]	Zn (mg/kg)				SE[2]	Cu (mg/kg)				SE[2]	Zn*Cu	Zn*As	Cu*As
	120		240			120		240			10		100					
	Cu (mg/kg)					As (mg/kg)					As (mg/kg)							
	10	100	10	100		0	0.5	0	0.5		0	0.5	0	0.5				
Raw breast fillets color																		
*L**	50.67	49.57	49.81	49.85	0.7	50.92	49.32	49.45	50.21	0.7	50.20	50.29	50.18	49.24	0.7	0.41	0.10	0.46
*a**	1.98	1.43	2.35	2.02	0.3	1.55	1.87	2.23	2.15	0.3	1.74^{B}	2.60^{A}	2.09^{AB}	1.42^{B}	0.3	0.67	0.44	0.01
*b**	6.41	6.26	5.38	5.82	0.4	5.83	6.84	5.13	6.07	0.4	5.49	6.30	5.47	6.61	0.4	0.44	0.93	0.65
Cooked breast fillets color																		
*L**	82.79	82.88	83.17	83.10	0.6	82.46	83.21	83.43	82.84	0.6	83.82^{A}	82.14^{B}	82.08^{B}	83.90^{A}	0.6	0.90	0.28	0.01
*a**	2.90	2.63	3.00	2.96	0.3	2.98	2.54	3.05	2.92	0.3	2.68^{AB}	3.22^{A}	3.35^{A}	2.24^{B}	0.3	0.70	0.60	0.01
*b**	15.80	16.66	15.34	15.79	0.4	15.84	16.62	14.73	16.39	0.4	14.92	16.21	15.65	16.80	0.4	0.60	0.27	0.86
Cooked breast meat with marrow																		
*L**	51.40^{b}	53.74^{a}	51.02^{b}	49.71^{b}	0.7	52.80^{A}	52.34^{A}	48.13^{B}	52.60^{A}	0.7	50.76	51.66	50.17	53.28	0.7	0.03	0.01	0.15
*a**	15.70	15.07	14.57	13.90	1.6	16.81	13.96	13.75	14.72	1.6	15.44	14.83	15.12	13.85	1.6	0.99	0.23	0.83
*b**	13.10	14.00	14.08	12.81	1.1	14.22	12.89	12.69	14.19	1.1	13.68	13.50	13.23	13.58	1.1	0.34	0.21	0.81
Raw marrow color																		
*L**	37.27^{C}	37.68^{C}	38.92^{B}	41.00^{A}	0.2	37.34	37.61	39.57	40.36	0.2	37.84	38.34	39.06	39.63	0.2	0.01	0.23	0.88
*a**	32.83^{B}	35.35^{A}	29.33^{C}	28.78^{C}	0.5	35.71^{A}	32.47^{B}	28.10^{D}	30.00^{C}	0.5	30.64^{B}	31.51^{B}	33.17^{A}	30.97^{B}	0.5	0.01	0.001	0.01
*b**	13.82^{bB}	16.09^{A}	12.44^{C}	12.13^{C}	0.2	16.16^{A}	13.75^{B}	11.62^{D}	12.94^{C}	0.2	12.95^{B}	13.31^{B}	14.84^{A}	13.38^{B}	0.2	0.001	0.0001	0.002

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A-D]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]For the dietary treatments, zinc (Zn) and copper (Cu) were included in the diets in the form of zinc sulfate and copper sulfate, respectively. Arsenic was added as Roxarsone.
[2]Standard error (SE) for n=32 birds for raw and cooked breast fillets, n=16 samples for cooked breast meat with marrow, and n=8 samples for raw marrow color.

Table III.15. Least-squares means for the 2-way interaction effects of minerals on color of breast fillets and bone marrow harvested from broiler females raised to 56 d of age

	Dietary treatments[1]																	
	Zn (mg/kg)					Zn (mg/kg)					Cu (mg/kg)					P-value		
	120		240		SE[2]	120		240		SE[2]	10		100		SE[2]	Zn*Cu	Zn*As	Cu*As
	Cu (mg/kg)					As (mg/kg)					As (mg/kg)							
	10	100	10	100		0	0.5	0	0.5		0	0.5	0	0.5				
Raw breast fillets color																		
*L**	52.75	53.40	52.07	51.94	0.6	53.39	52.76	51.89	52.11	0.6	52.09	52.72	53.20	52.15	0.6	0.52	0.48	0.17
*a**	1.96	1.53	1.60	1.47	0.3	1.72	1.77	1.37	1.70	0.3	1.63	1.92	1.46	1.55	0.3	0.64	0.65	0.75
*b**	6.57	7.49	7.09	7.34	0.4	6.61	7.45	6.84	7.58	0.4	6.90^{b}	6.75^{b}	6.55^{b}	8.28^{a}	0.4	0.40	0.90	0.02
Cooked breast fillets color																		
*L**	83.81	83.69	84.18	83.59	0.5	84.07	83.42	83.94	83.84	0.5	84.00	83.98	84.00	83.28	0.5	0.65	0.60	0.52
*a**	2.90	2.79	2.69	2.92	0.3	2.75	2.94	2.82	2.80	0.3	2.74	2.86	2.83	2.88	0.3	0.56	0.72	0.91
*b**	15.45	16.15	16.19	16.28	0.5	15.80	15.80	15.98	16.49	0.5	15.98	15.67	15.80	16.63	0.5	0.56	0.63	0.28
Cooked breast meat with marrow																		
*L**	56.27	55.49	52.40	52.11	1.1	55.88	55.87	50.87	53.63	1.1	52.89	55.78	53.86	53.73	1.1	0.81	0.20	0.17
*a**	14.24	13.06	13.11	14.25	1.4	14.60	14.71	13.77	13.58	1.4	14.31	13.03	14.05	13.26	1.4	0.41	0.54	0.86
*b**	15.02	14.98	14.88	13.99	1.0	14.71	15.30	13.97	14.90	1.0	14.12	15.78	14.55	14.42	1.0	0.66	0.86	0.36
Raw marrow color																		
*L**	41.25^{A}	39.47^{B}	39.00^{B}	40.72^{A}	0.3	40.83	39.89	40.53	39.20	0.3	40.95	39.31	40.41	39.78	0.3	0.001	0.58	0.18
*a**	36.03^{A}	36.25^{A}	30.36^{C}	32.14^{B}	0.2	35.81^{A}	36.46^{A}	27.89^{C}	34.61^{B}	0.2	32.40^{C}	33.99^{B}	31.30^{D}	37.09^{A}	0.2	0.01	0.0001	0.0001
*b**	16.65^{A}	16.12^{A}	13.52^{cC}	15.06^{B}	0.2	16.45^{A}	16.33^{A}	12.53^{B}	16.05^{A}	0.2	14.93^{B}	15.24^{B}	14.04^{C}	17.14^{A}	0.2	0.001	0.0001	0.0001

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A-D]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]For the dietary treatments, zinc (Zn) and copper (Cu) were included in the diets in the form of zinc sulfate and copper sulfate, respectively. Arsenic was added as Roxarsone.
[2]Standard error (SE) for n=32 birds for raw and cooked breast fillets, n=16 samples for cooked breast meat with marrow, and n=8 samples for raw marrow color.

Table III.16. Least-squares means for the main effect of sex on tissue mineral concentration of broilers raised to 56 d of age

	Sex		SE[1]	P-value
	Male	Female		
Muscles (mg/kg DM)				
Copper	1.9	1.8	0.1	0.50
Iron	16.9	17.1	1.3	0.89
Zinc	25.1[a]	23.6[b]	0.5	0.03
Bones (mg/kg DM)				
Copper	0.40	0.41	0.01	0.40
Iron	123.3	114.5	7.2	0.41
Zinc	131.5	130.0	1.9	0.59

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[1]Standard error (SE) for n=32 birds.

Table III.17. Least-squares means for the main effect of minerals on tissue mineral concentration of broiler males raised to 56 d of age

	Dietary treatments[1]												
	Negative control	**Zn (mg/kg)**		**SE[2]**	**Cu (mg/kg)**		**SE[2]**	**As (mg/kg)**		**SE[2]**	**P-value**		
		120	**240**		**10**	**100**		**0**	**0.5**		**Zn**	**Cu**	**As**
Muscles (mg/kg DM)													
Copper	1.8	1.8	2.1	0.1	1.7[b]	2.2[a]	0.1	1.9	2.0	0.1	0.23	0.05	0.58
Iron	14.6	17.4	16.4	1.6	15.8	18.0	1.6	19.6[a]	14.2[b]	1.6	0.68	0.36	0.05
Zinc	27.3	25.8	24.4	0.8	25.2	25.0	0.8	24.6	25.6	0.8	0.25	0.83	0.38
Bones (mg/kg DM)													
Copper	0.4	0.4	0.4	0.01	0.4	0.4	0.01	0.4	0.4	0.01	0.94	0.21	0.18
Iron	92.8	120.5	126.0	10.4	129.5	114.0	10.4	136.2	110.3	10.4	0.72	0.42	0.12
Zinc	133.8	132.0	131.1	2.2	133.7	129.4	2.2	130.0	133.0	2.2	0.77	0.19	0.36

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[1]Negative control diets did not contain any added minerals. For the experimental diets, zinc (Zn) and copper (Cu) were included in the diets in the form of zinc sulfate and copper sulfate, respectively. Arsenic was added as Roxarsone.
[2]Standard error (SE) for n=18 birds.

Table III.18. Least-squares means for the main effect of minerals on tissue mineral concentration of broiler females raised to 56 d of age

	Dietary treatments[1]												
	Negative control	Zn (mg/kg)		SE[2]	Cu (mg/kg)		SE[2]	As (mg/kg)		SE[2]	P-value		
		120	240		10	100		0	0.5		Zn	Cu	As
Muscles (mg/kg DM)													
Copper	1.3	1.6	2.0	0.1	1.4[B]	2.2[A]	0.1	1.9	1.7	0.1	0.08	0.01	0.38
Iron	13.4	16.2	18.1	1.3	15.2	19.1	1.3	18.3	16.0	1.3	0.35	0.08	0.28
Zinc	24.6	22.9	24.3	0.5	22.9	24.3	0.5	23.9	23.2	0.5	0.09	0.10	0.38
Bones (mg/kg DM)													
Copper	0.4	0.4	0.4	0.01	0.4	0.4	0.01	0.4	0.4	0.01	0.23	0.23	0.59
Iron	79.5	105.6	12.9	7.1	111.0	118.5	7.1	126.3[a]	103.2[b]	7.1	0.11	0.47	0.05
Zinc	132.2	131.4	128.7	2.1	130.1	130.0	2.1	127.3	132.8	2.1	0.41	0.98	0.10

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Negative control diets did not contain any added minerals. For the experimental diets, zinc (Zn) and copper (Cu) were included in the diets in the form of zinc sulfate and copper sulfate, respectively. Arsenic was added as Roxarsone.
[2]Standard error (SE) for n=18 birds.

Table III.19. Least-squares means for the 2-way interaction effects of minerals on tissue mineral concentration of broiler males raised to 56 d of age

	Dietary treatments[1]																	
	Zn (mg/kg)					Zn (mg/kg)					Cu (mg/kg)					P-value		
	120		240		SE[2]	120		240		SE[2]	10		100		SE[2]	Zn*Cu	Zn*As	Cu*As
	Cu (mg/kg)					As (mg/kg)					As (mg/kg)							
	10	100	10	100		0	0.5	0	0.5		0	0.5	0	0.5				
Muscles (mg/kg DM)																		
Copper	1.8^{b}	1.8^{b}	1.5^{b}	2.6^{a}	0.2	2.0^{ab}	1.6^{b}	1.7^{ab}	2.4^{a}	0.2	1.7	1.7	2.0	2.3	0.2	0.03	0.03	0.48
Iron	16.2	18.6	15.3	17.5	2.3	22.3	12.5	16.8	16.0	2.3	19.7	11.9	19.5	16.6	2.3	0.96	0.09	0.32
Zinc	25.1	26.5	25.3	23.5	1.1	24.9	26.7	24.3	24.6	1.1	24.3	26.2	24.9	25.1	1.1	0.19	0.51	0.44
Bones (mg/kg DM)																		
Copper	0.4	0.4	0.4	0.4	0.01	0.4	0.4	0.4	0.4	0.01	0.4	0.4	0.4	0.4	0.01	0.21	0.18	0.86
Iron	138.1	103.0	120.9	131.1	14.7	134.5	106.6	138.0	114.1	14.7	148.9	110.2	123.6	110.5	14.7	0.16	0.89	0.41
Zinc	134.0	130.0	133.4	128.7	3.1	125.5^{b}	138.5^{a}	134.5^{ab}	127.6^{b}	3.1	131.7	135.7	128.3	130.4	3.1	0.91	0.01	0.75

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).

[1]For the dietary treatments, zinc (Zn) and copper (Cu) were included in the diets in the form of zinc sulfate and copper sulfate, respectively. Arsenic was added as Roxarsone.

[2]Standard error (SE) for n=9 birds.

Table III.20. Least-squares means for the 2-way interaction effects of minerals on tissue mineral concentration of broiler females raised to 56 d of age

	Dietary treatments[1]																	
	Zn (mg/kg)					Zn (mg/kg)					Cu (mg/kg)					P-value		
	120		240		SE[2]	120		240		SE[2]	10		100		SE[2]	Zn*Cu	Zn*As	Cu*As
	Cu (mg/kg)					As (mg/kg)					As (mg/kg)							
	10	100	10	100		0	0.5	0	0.5		0	0.5	0	0.5				
Muscles (mg/kg DM)																		
Copper	1.2	2.0	1.7	2.3	0.2	1.8	1.4	1.9	2.0	0.2	1.4	1.4	2.3	2.0	0.2	0.48	0.16	0.37
Iron	15.3	17.2	15.2	21.0	1.9	19.1	13.4	17.4	18.7	1.9	14.9	15.6	21.6	16.5	1.9	0.33	0.10	0.16
Zinc	22.3	23.4	23.4	25.2	0.8	23.3	22.4	24.5	24.1	0.8	22.8	23.0	25.1	23.5	0.8	0.63	0.76	0.24
Bones (mg/kg DM)																		
Copper	0.4	0.4	0.4	0.4	0.01	0.4	0.4	0.4	0.4	0.01	0.4	0.4	0.4	0.4	0.01	0.23	0.59	0.59
Iron	106.0	105.1	115.9	131.9	10.1	113.7	97.4	138.8	109.0	10.1	121.3	100.6	131.2	105.8	10.1	0.43	0.52	0.82
Zinc	131.9	130.8	128.3	129.2	3.0	126.3	136.4	128.2	129.3	3.0	130.4	129.8	124.2	135.8	3.0	0.75	0.18	0.08

[1]For the dietary treatments, zinc (Zn) and copper (Cu) were included in the diets in the form of zinc sulfate and copper sulfate, respectively. Arsenic was added as Roxarsone.
[2]Standard error (SE) for n=9 birds.

CHAPTER IV

Effect of dietary Zn and sex on performance of broilers

4.1. ABSTRACT

This study evaluated the effect of adding high levels of inorganic dietary zinc (Zn) on live performance of broilers raised to 42 d. A total of 288 d-old male and female Ross 344 × 708 broilers were raised sex-separate and randomly distributed among 3 dietary treatments with 12 replicate cages per treatment, and 8 birds per cage. Birds were fed diets containing no trace mineral premix and supplemented with either: 0, 120, or 240 mg Zn/kg diet. Feed intake and BW were measured and BW gain and adjusted feed conversion ratio (AdjFCR) were calculated. Males had greater ($P \leq 0.05$) BW at 28 and 42 d, greater ($P \leq 0.05$) feed intake and BW gain and improved ($P \leq 0.05$) AdjFCR from 1-42 d than females. Dietary Zn had no effect on feed intake of males, however, BW measured at 42 d was increased ($P \leq 0.05$) in males fed diets supplemented with 120 mg Zn/kg than females, also BW gain and AdjFCR measured from 1-42 d was improved ($P \leq 0.05$) in males fed diets supplemented with 120 mg Zn/kg. Dietary Zn had no effect on BW, feed intake, BW gain, or AdjFCR of females. Results indicated that supplementing high levels of dietary Zn did not enhance performance of broiler females while 120 mg Zn/kg improved performance in males.

Key words: Broiler, zinc, live performance

4.2. INTRODUCTION

Zinc (Zn) has been shown to be an essential trace element required for many metabolic and enzyme functions (Mills, 1983). Zinc finger proteins have played an integral role in regulating gene expression and consequently have impacted a wide variety of body functions including cell proliferation, hormone production, metabolism, appetite control, and immune development and response (Vallee and Falchuk, 1993; Shankar and Prasad, 1998; Underwood and Suttle, 1999; Fraker et al., 2000; Blanchard et al., 2001; Ibs and Rink, 2003; Predieri et al., 2003; Song et al., 2009). The significance of Zn as an essential trace mineral for agriculture developed slowly and was not definitely established until 1926 for green plants (Sommer and Lipman, 1926), and 1934 for mammals (Todd et al., 1934). Zinc was required to maintain many biological functions. A Zn deficiency in chickens caused leg abnormalities, poor feathering, and parakeratosis (O'Dell et al., 1958; Young et al., 1958). Supplementation of trace minerals such as Zn with large safety margins in broiler chicken diets has resulted in diets with several fold increases in some trace minerals as compared with NRC recommendations (NRC, 1994; Inal et al., 2001; Guclu et al., 2008). This supplementation has led to a high level of mineral excretion into the environment (Skrivan et al., 2006). Excreted minerals have been deposited in litter and accumulated for successive flocks to provide an additional source of trace minerals and other nutrients for each subsequent flock. The NRC (1994) estimated the Zn requirement for broiler chickens to be 40 mg/kg of diet. In addition, it was not surprising to find high concentrations of some trace minerals in the basal diets even without additional supplementation. Hence, it may have not been necessary to supplement these minerals at levels as high as those currently used by the industry (Pang et al., 2009). Several authors (Young et al., 2001; Bou et al., 2005; Brewer et al., 2012) have reported that live performance, carcass and parts yield, carcass composition and quality could be influenced by BW, strain, sex, nutrition, and environment. Therefore, this study aimed to investigate the influence of supplementing different levels of dietary Zn to broiler males and females raised to 42 d of age where litter consumption would not be a confounding factor.

4.3. MATERIALS AND METHODS

Birds and diets

This study was conducted from September 19 to October 31, 2012 at North Carolina State University, Prestage Department of Poultry Science, NC, USA. A total of 288 day-old male and female broiler chicks of Ross 344×708 strain were hatched from eggs collected from breeders at the site at 44 wk of age. Birds were feather-sexed and randomly distributed among 2 battery cages with a total of 36 cages. Birds were raised sex-separate with 8 birds per cage (56×68×36 cm for length×width×height, respectively). Birds were exposed to continuous light for the first 2 d, then to 23 h light and 1 h dark until 42 d of age. The initial room temperature was set at 32°C and gradually reduced to 25°C. Feed and water were provided for *ad libitum* consumption. Feed was covered with plastic screen wires to reduce feed spill and feed was stirred 2 times daily to stimulate uniform feed consumption. Diets were formulated to either meet or exceed the NRC (1994) requirements for broilers except for Zn. Table 1 shows the basal diet formulation and proximate composition for the starter and grower. Feed ingredients were selected to contain very low amounts of Zn to reduce the influence of any organically available Zn in the feed ingredients. Phytase was used to liberate Zn and phosphorus from phytate. Birds were fed crumbled starter from 1-18 d and pelleted grower from 19-42 d. One basal diet was formulated without including mineral premix. The three experimental diets were formulated from the same basal diet to ensure that birds had access to the same nutrients. Inorganic zinc sulfate ($ZnSO_4$) was added at the same level for both starter and grower. Zinc sulfate (Zinc Nacional, S.A., Mexico) was added to achieve a Zn concentration in the experimental diets of either 0, 120, or 240 mg Zn/kg diet. Diets were analyzed for their mineral contents using inductively coupled plasma optical emission spectrometer (Perkin Elmer ICP-OES 2000 DV). Analyzed Zn in the starter diets were 111, 156, and 233 mg/kg, and in the grower diets were 67, 160, and 249 for diets supplemented with 0, 120, and 240 mg Zn/kg, respectively. Analyzed iron (Fe) in the starter diets was 271, 240, and 218 mg/kg, and in the grower diets was 224, 226, and 177 for diets supplemented with 0, 120, and 240 mg Zn/kg, respectively. All methods regarding bird care in this experiment were approved by the NCSU Institutional Animal Care and Use Committee.

Live performance

Birds and feed were weighed by replicate cage at 1, 14, 28, and 42 d to determine BW gain, feed consumption, and adjusted feed conversion ratio (AdjFCR) which was calculated based upon BW of mortality. Mortality was monitored and recorded daily.

Statistical analysis

Data were analyzed as a randomized complete block design using the GLM procedure of SAS (SAS version 9.4, SAS Institute, Cary, NC, USA, 2014). Data was analyzed to assess the effect of sex, and then males and females data were analyzed separately to assess the effect of dietary treatments within each sex. Differences between means were separated using the LS means method and significance was reported at a probability level of $P \leq 0.05$. The experimental unit for statistical analysis of measured parameters was the cage.

4.4. RESULTS AND DISCUSSION

During this study, birds were raised in cages, so they did not have access to any wood shavings, or nutritional source by coprophagy, other than the feed and water. The BW was measured at 1, 14, 28, and 42 d of age. Sex did not affect (P = 0.58) BW measured at 1 and 14 d but increased BW of males at 28 and 42 d (P = 0.0001). Male and female BW at 42 d was 2969 and 2667 g, respectively (Table 2). Feed intake, BW gain and AdjFCR were measured from 1 – 14, 15 – 28, 29 – 42, 1 – 28, and 1 – 42 d. Feed intake from 1 – 42 d was 4346 and 3981 g for males and females, respectively. The BW gain measured for the period from 1 – 14 d was not affected by sex but was higher in males for all other periods. Adjusted feed conversion ratio (AdjFCR) was only affected by sex in the periods from 29 – 42 d (P = 0.001) and 1 – 42 d (P = 0.001) where males exhibited improved AdjFCR as compared to females. Values from 29 – 42 d were 1.69 and 1.82 g:g, and from 1 – 42 d values were 1.46 and 1.50 g:g for males and females, respectively.

Tables 3 and 4 show results for the influence of dietary Zn treatment on BW, feed intake, BW gain, and AdjFCR for males and females, respectively. Dietary Zn had no effect on BW of males measured at 1, 14, and 28 d but BW at 42 d was increased (P = 0.01) in males fed diets supplemented with 120 mg Zn/kg. The 42 d BW was 2902, 3057, and 2902 g for males supplemented with 0, 120, and 240 mg Zn/kg, respectively. Feed intake of males was not affected by dietary Zn treatment. On the other hand, BW gain was affected by dietary Zn treatment from 29 – 42 d (P = 0.03) and 1 – 42 d (P = 0.01). For both periods, males fed diets supplemented with 120 mg Zn/kg exhibited greater BW gain than females. For the 1 – 42 d period, AdjFCR was also better (P = 0.02) for males supplemented with 120 mg Zn/kg. Dietary Zn had no effect on BW, feed intake, BW gain, and AdjFCR of females. Results of the current study indicated that up to 42 d, Zn supplementation had no effect on feed intake. However, at 42 d and at similar feed intake birds supplemented with 120 mg Zn/kg gained 155 g more than non-supplemented and high supplemented birds (240 mg Zn/kg). Mehring et al. (1956) reported that growth and feed utilization of broilers fed diets containing Zn ranging from 36 – 814 mg/kg up to 9 wk was not affected by Zn level. Previous research showed that removal of trace minerals from broiler diets after 28 d had no effect on growth performance (Skinner et al., 1992; Deyhim and Teeter, 1993; Christmas et al., 1995). According to Bao et al. (2009), the optimal Zn requirements for broilers from 1—14 and 14—35 d of age were 58 and 68 mg/kg diet, respectively. On the other hand, Kim and Patterson (2004) reported reduced feed intake and BW gain of broilers supplemented with 1500 mg Zn/kg, which could be an indicator of increased Zn bioavailability and toxicity (Sandoval et al., 1998). Several studies reported improved live performance of broilers when fed supplemental Zn. Mohanna and Nys (1999) reported that dietary Zn concentration of 45 mg/kg was adequate to obtain optimum growth of broiler raised to 21 d regardless of Zn source. Huang et al. (2007) reported that BW gain and feed intake of broilers raised to 21 d were increased with dietary zinc level, and the maximum BW gain and feed intake were observed when dietary Zn concentration was 48.3 mg/kg. Burrell et al. (2004) reported improved BW gain when broilers were raised to 45 d and consumed diets formulated to contain 110 mg Zn/kg without affecting FCR. Liu et al. (2011) examined the effect of different sources and levels of Zn on performance of broilers raised to 42 d. Their results indicated that supplementation of 60, 120, or 180 mg Zn/kg increased feed intake and BW gain during the starter and grower periods as compared to non-supplemented birds regardless of the Zn source. Broilers raised to 29 d and fed either organic or inorganic trace minerals had greater feed intake and BW gain compared to non-supplemented birds (Bao et al., 2007). In their study, birds fed the non-supplemented basal that contained 20 mg Zn/kg exhibited Zn deficiency symptoms after 7 d of age (Bao et al., 2007). In the current study, dietary Zn concentration in the basal was 111 mg/kg in the starter and 67 mg/kg in the grower. Further, phytase was included in the basal diet, which might have increased the bioavailability of Zn (Revy et al., 2004; Shelton and Southern, 2006), and improved Zn utilization (Yi et al., 1996). Increased bioavailability have delayed the symptoms of Zn deficiency until 42 d in the birds fed the basal diet or the current might have a different requirement than previously utilized strains. Sunder et al. (2008) reported that a basal diet containing 111 mg Zn/kg was adequate to support broiler growth up to 28 d. In the current study, supplementing 120 mg Zn/kg to the basal diet containing 29 mg Zn/kg resulted in maximum BW and best FCR, which suggested that the basal diet may have not provided the minimum amount required for maximum growth. On the other hand, the high supplemented diets with 240 mg Zn/kg could have suffered from an antagonism with other minerals, thus affecting growth negatively.

4.5. CONCLUSION

Results indicated that supplementing high levels of dietary Zn did not enhance live performance of broiler females while 120 mg Zn/kg improved AdjFCR, BW and BW gain of males.

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Table IV.1. Composition and nutrient content of the basal diets

Item	Starter (1-18 d)	Grower (19-42 d)
Ingredients	(%)	
Corn	42.46	43.93
Wheat	27.00	28.00
Soybean meal, 48%	18.70	16.11
Acid casein	5.00	4.78
Spray-dried animal blood plasma	1.86	1.50
Poultry fat	1.50	2.22
Limestone	1.38	1.40
Monocalcium phosphate	0.93	0.94
Salt (NaCl)	0.50	0.50
DL-Methionine	0.23	0.19
L-Threonine	0.03	0.02
Choline chloride	0.20	0.20
Vitamin premix[1]	0.05	0.05
Coban (coccidiostat)[2]	0.05	0.05
Selenium premix[3]	0.03	0.03
Phytase[4]	0.03	0.03
Vermiculite (inert filler)[5]	0.05	0.05
Calculated nutrient content		
Metabolizable energy (kcal/g)	2.90	2.95
Crude protein (%)	21.6	20.0
Calcium (%)	0.75	0.75
Available Phosphorus (%)	0.30	0.30
Lysine (%)	1.25	1.14
Methionine (%)	0.59	0.53
Analyzed nutrient content, as fed		
Iron (mg/kg)	271	224
Zinc (mg/kg)	111	67

[1]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 1,984 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.
[2]Coccidiostat supplied monensin sodium at 90 mg/kg of feed.
[3]Selenium premix provided 0.2 mg Se (as Na_2SeO_3).
[4]Phytase enzyme added at 300 FTU/kg.
[5]Vermiculite was used as an inert filler to be replaced by inorganic zinc sulfate ($ZnSO_4$) in the experimental diets.

Table IV.2. Least-squares means for the main effect of sex on live performance of broilers raised to 42 d of age

Age	Sex		SE[1]	P-value
	Male	Female		
(d)	**Body weight (g)**			
1	41	40	0.3	0.56
14	488	475	4.7	0.08
28	1594^{A}	1536^{B}	8.6	0.0001
42	2969^{A}	2667^{B}	17.8	0.0001
	Feed intake (g)			
1-14	528	514	5.3	0.07
1-28	2027^{A}	1956^{B}	12.4	0.01
1-42	4346^{A}	3981^{B}	20.3	0.0001
15-28	1499^{A}	1442^{B}	12.1	0.001
29-42	2319^{A}	2025^{B}	12.0	0.0001
	Body weight gain (g)			
1-14	446	435	4.6	0.08
1-28	1554^{A}	1496^{B}	8.6	0.0001
1-42	2929^{A}	2627^{B}	17.8	0.0001
15-28	1107^{A}	1061^{B}	11.5	0.01
29-42	1375^{A}	1131^{B}	18.9	0.0001
	Adjusted feed conversion ratio (g: g)			
1-14	1.22	1.21	0.01	0.86
1-28	1.34	1.34	0.01	0.79
1-42	1.46^{B}	1.50^{A}	0.01	0.001
15-28	1.42	1.41	0.03	0.77
29-42	1.69^{B}	1.82^{A}	0.02	0.001

^{A,B}Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Standard error (SE) for n=36 pens.

Table IV.3. Least-squares means for the main effect of zinc on live performance of broiler males raised to 42 d of age

Age	Dietary Zn[1] (mg/kg)			SE[2]	P-value
	0	120	240		Zn
(d)	**Body weight (g)**				
1	41	41	40	0.5	0.97
14	495	484	482	11.0	0.67
28	1584	1606	1593	15.0	0.62
42	2902^{B}	3057^{A}	2902^{B}	28.0	0.01
	Feed intake (g)				
1-14	535	527	522	12.0	0.72
1-28	2061	2012	2008	30.0	0.39
1-42	4372	4370	4297	46.0	0.45
15-28	1526	1485	1486	29.0	0.52
29-42	2311	2358	2289	22.0	0.14
	Body weight gain (g)				
1-14	455	443	442	11.0	0.65
1-28	1543	1565	1552	15.0	0.63
1-42	2908^{B}	3017^{A}	2861^{B}	28.0	0.01
15-28	1089	1123	1111	24.0	0.64
29-42	1365^{ab}	1451^{a}	1309^{b}	31.0	0.03
	Adjusted feed conversion ratio (g:g)				
1-14	1.22	1.21	1.21	0.01	0.91
1-28	1.38	1.32	1.33	0.03	0.37
1-42	1.47^{ab}	1.43^{b}	1.49^{a}	0.01	0.02
15-28	1.49	1.38	1.39	0.1	0.46
29-42	1.66	1.62	1.78	0.06	0.16

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Dietary Zn was included in the diets in the form of zinc sulfate.
[2]Standard error (SE) for n=18 pens.

Table IV.4. Least-squares means for the main effect of zinc on live performance of broiler females raised to 42 d of age

Age	Dietary Zn[1] (mg/kg)			SE[2]	P-value
	0	120	240		Zn
(d)	Body weight (g)				
1	40	41	40	0.5	0.67
14	470	480	475	6.0	0.49
28	1533	1548	1527	16.0	0.66
42	2627	2696	2679	26.0	0.17
	Feed intake (g)				
1-14	502	517	524	7.0	0.16
1-28	1958	1964	1946	10.0	0.50
1-42	3969	3989	3985	26.0	0.83
15-28	1455	1447	1423	11.0	0.17
29-42	2011	2026	2039	21.0	0.67
	Body weight gain (g)				
1-14	430	439	4325	6.0	0.50
1-28	1492	1507	1487	16.0	0.67
1-42	2587	2655	2639	26.0	0.17
15-28	1063	1068	1052	18.0	0.86
29-42	1095	1148	1152	31.0	0.38
	Adjusted feed conversion ratio (g:g)				
1-14	1.20	1.21	1.23	0.01	0.24
1-28	1.34	1.33	1.34	0.01	0.93
1-42	1.50	1.49	1.50	0.01	0.48
15-28	1.42	1.41	1.40	0.02	0.78
29-42	1.84	1.81	1.82	0.02	0.58

[1]Dietary Zn was included in the diets in the form of zinc sulfate.
[2]Standard error (SE) for n=18 pens.

CHAPTER V

Effect of dietary Zn and sex on carcass, meat quality, and ZPP/H ratio of broilers

5.1. ABSTRACT

The effect of adding high levels of inorganic dietary zinc (Zn) on carcass yield, breast meat quality, bone marrow color, and blood zinc protoporphyrin to heme ratio (ZPP/H) of broilers raised to 42 d was evaluated. A total of 288 day-old male and female Ross 344 × 708 broilers were raised sex-separate and randomly distributed among 3 dietary treatments, with 12 replicate cages per treatment and 8 birds per cage. Birds were fed diets containing no mineral premix, and supplemented with 0, 120, or 240 mg Zn/kg diet. Blood ZPP/H was measured weekly. At 42 d, 3 birds per cage were euthanized; carcasses were processed, and breast fillets, thighs, and femurs were collected for meat quality assessment. Deboned breast fillets were weighed, cooked to an internal temperature of 75ºC, and reweighed to determine cook yield. Tenderness was estimated on samples from the cooked breast fillets using a Warner-Bratzler shear device. Bone marrow was collected from the interior epiphyseal end caps of femurs. Marrow was minced and color was measured before and after cooking. Data was analyzed using GLM procedures of SAS to evaluate the effects of Zn and sex. Males had higher carcass weight but percentage dressing yield was comparable among males and females. Parts weights were heavier in males, while percentage yield was comparable except for legs yield that was greater in males while wings yield was greater in females. Dietary Zn had no effect on carcass weight and parts yield but absolute breast fillets weights was improved in males when Zn was added to the diets. Breast fillets cook yield and tenderness were not influenced by either sex or Zn. Raw breast fillets color was influenced by sex where males breasts were darker, more red, and less yellow in color. Raw thigh color of males was also lighter and less yellow compared to females. Raw marrow color measured immediately after harvest was more red (P = 0.03) and yellow (P = 0.01) in females. Cooked marrow with 1% Zn was more red (P = 0.01) in females. Lightness (L^*) and redness (a^*) of raw and cooked bone marrow collected from males was influenced by Zn where L^* was increased at 120 mg Zn/kg for both raw and cooked marrow, while a^* of cooked marrow was greater at 240 mg Zn/kg. Dietary Zn had no effect on the ZPP/H ratio. Females exhibited a higher ratio at 1, 7, and 42 d. The Zn and Fe concentration in breast muscle and femur were influenced by sex as males had more Fe in muscles and bones. Supplementing 120 mg Zn/kg resulted in increased Zn in muscles (P = 0.001) and reduced Zn in bones (P = 0.004) of males. For females, Zn concentration in bones was increased (P = 0.02) at 240 mg Zn/kg. Results indicated that supplementing 120 and 240 mg Zn/kg diet improved breast tender size, marrow a^*, and bone Zn concentration.

Key words: Broiler, zinc, carcass, meat color, ZPP/H

5.2. INTRODUCTION

The relative convenience of cooking chicken compared to red meat has made chicken more popular in recent years among US consumers, especially women (Haley, 2001). For poultry meat, appearance and texture were the two important attributes responsible for initial consumer meat evaluation as well as final product acceptance (Fletcher, 2002). Red and/or bloody discoloration of poultry meat, raw or cooked, has been a chronic yet sporadic problem for the poultry industry. Raw breast meat with red discoloration was objectionable to many customers, and cooked white or dark meat with a red defect was unacceptable to consumers due to the perception that it was undercooked.

Pigment deposition in birds has been reported to depend upon genetics, diet, health, and handling at processing (Fletcher, 2002). Therefore, the variation in the raw meat color affected the final color of a cooked product (Fletcher et al., 2000). Also, the commercial fast-growing broiler lines that have been specially bred for higher amounts of breast and thigh meat compared with layer or traditional chickens (Sandercock et al., 2009) could have a predisposition towards increased discoloration problem that contribute to consumer complaints.

Red marrow of the bones was reported to be a hemopoietic (blood forming) tissue (Koonz and Ramsbottom, 1947). It contained hemoglobin, which was likely the cause of the red color of the bone marrow (Brant and Stewart, 1950). Hemoglobin contributed to the color of meat since about 20-30% of hemoglobin remained in the carcass of well-bled birds (Froning, 1995). Also, myoglobin was the main pigment determining meat color (Koonz and Ramsbottom, 1947).

Zinc protoporphyrin (ZPP) was a normal metabolite that accumulated at trace amounts in erythrocytes during hemoglobin synthesis. In states of iron (Fe) deficiency, excess amounts of ZPP were formed as a byproduct of the heme biosynthetic pathway. This

ZPP response occurred due to the interaction between Fe and zinc (Zn) as ferrochelatase substrates, where Zn utilization increased when Fe supply has diminished (Labbe and Finch, 1981). The resulting high ZPP/H ratio in circulating erythrocytes reflected a state of relative iron-deficient erythropoiesis (Labbe and Rettmer, 1989). Formation of the ZPP molecule has been demonstrated to cause the irreversible bright red color of Italian Parma ham (Wakamatsu et al., 2004).

No research has been conducted to determine the effects of a diet that was high in Zn and low or normal in Fe on the subsequent meat quality of broilers, especially the prevalence of red discoloration in broiler breast meat. High Zn levels in the animal due to dietary supplements could produce elevated ZPP levels in the blood (hemoglobin) and meat (myoglobin), resulting in a red pigment resistant to cooking denaturation even at elevated endpoint temperatures above 85°C. Therefore, the aim of this research was to study the effect of different levels of dietary Zn at a fixed Fe level and sex on carcass yield, breast meat quality, meat and bone marrow color, and ZPP/H ratio.

5.3. MATERIALS AND METHODS

Birds and diets

A total of 288 day-old male and female broiler chicks of Ross 344×708 strain were feather-sexed and randomly distributed among 2 battery cages with a total of 36 cages. Birds were raised sex-separate with 8 birds per cage. Diets were formulated to either meet or exceed the NRC (1994) requirements for broilers except for Zn. Three experimental diets were formulated from the same basal diet to ensure that birds had access to similar nutrients.

Inorganic zinc sulfate ($ZnSO_4$) was added at the same level for both starter and grower. Experimental diets contained 0, 120, or 240 mg Zn/kg diet. The Fe concentration was fixed with values being 271, 240, and 218 mg/kg, and 224, 226, and 177 in the grower diets supplemented with 0, 120, and 240 mg Zn/kg, respectively. All methods regarding bird care in this experiment were approved by the NCSU Institutional Animal Care and Use Committee.

Carcass and parts yield

At 42 d of age, 3 birds per cage were fasted for 12 h, and transported from Prestage Department of Poultry Science to NCSU pilot processing plant (10 km) in plastic cages each containing 8 birds. Birds were weighed (fasted live weight) electrically stunned using 25 milliamps for 11 s, killed by exsanguination, and allowed to bleed for 90 s. Birds were then scalded at 55°C for 90 s in a rotary scalder, picked for 30 s in a drum picker, and manually eviscerated. Carcasses were dressed by removing giblets (liver, gizzard, and heart), oil gland, crop, proventriculus, neck, head, feet, lungs, and viscera, to determine the hot WOG weight (without giblets). Carcasses were then chilled overnight by immersion in ice chilled water at 0 to 4°C, and manually deboned on stationary cones the following day. The legs, thighs, breast fillets (*Pectoralis major*), breast tenders (*Pectoralis minor*), wings, fat pad, and ribs, back, and skin were weighed. The carcass yield was calculated for both the hot and chilled WOG as a percentage of the fasted live weight. Parts yield was calculated as a percentage of the chilled WOG weight. Femur bones were collected from the thighs for bone marrow harvest and bone minerals concentration assessment.

Cook yield

Breast fillets (*Pectoralis major*) were weighed, placed on aluminum pans, and cooked in a forced air oven (SilverStar Southbend, Model SLES/10sc, gas type, NC, USA). Fillets were cooked to an internal temperature of 75°C (approximately 25 min) as measured by a Therma Plus thermocouple with a 10-cm needle temperature probe (ThermoWorks Model 221-071, UT, USA). The cooked fillets were cooled to room temperature and re-weighed to determine cook yield as a percentage of the cooked weight relative to the raw weight.

Shear force

Cooked breast fillets samples were evaluated for texture using a Warner-Bratzler shear device (Warner-Bratzler meat shear, Bodine Electric Company, Chicago, USA). Two samples per breast fillets (2×2×2 cm) were sheared in a direction perpendicular to the muscle fiber. Crosshead speed was 4 mm/sec. The maximum force measured when cutting the samples was expressed in kg.

Color

Skin was removed from thighs and breast fillets. Color was measured on the surface of raw thigh muscle and on the medial side of both raw and cooked breast fillets. Color was measured by the CIE L*a*b* system using a Minolta Chroma Meter CR-400 (Konica Minolta Sensing, Inc., Japan). A measuring area of 10 mm and illuminant D65 and 2° standard observer were used. Colorimeter was calibrated using a white tile (reference number 13033071; Y = 93.9, x = 0.3156, y = 0.3318). Triplicate measurements were taken for each sample. CIE lightness (L^*), redness (a^*), and yellowness (b^*) were measured.

Red discoloration induction

This test was conducted according to the procedure described by Smith and Northcutt (2004a) which aimed to resemble the effect of bone marrow leakage to adjacent meat. Collected femurs were pooled by treatment and sex. Bone marrow was collected by removing the exterior bone from the epiphyseal end caps, and the porous hard bone marrow was collected and manually minced using mortar and pestle until it turned into a thick granular paste. Boneless, skinless broiler breast fillets were obtained fresh (refrigerated) from a local retail store. The breast meat contained up to 5% retained water (Nature's Place breast meat, USA). External fat was trimmed and connective tissue removed. Cleaned meat was chopped manually and blended for 30 s in a food processor (KitchenAid Model KFP600WH, MI, USA). Glass tubes open at both ends were used (OD = 20 mm, ID = 17 mm × 200 mm length). For each tube, 10 g of minced meat was divided into 2 equal parts. One part meat was put into the tube at one end, followed by 1 g of minced bone marrow, then the second part of the meat was added and each tube was sealed with a #2 rubber stopper. Three replicate tubes were prepared for each treatment per sex. Tubes were placed in a circulating water bath (Model MW-1120A-1, Blue M Electric Company, IL, USA) set at 95°C. Tubes were removed immediately when temperature reached 75°C (approximately 2 min) as measured by a Therma Plus thermocouple with a 125-cm hard-wired temperature probe (Probe Type K, ThermoWorks Model 221-071, UT, USA). Tubes were placed in an ice water bath until the temperature reached 4°C (approximately 45 min). Stoppers were removed from the glass tubes and the cooked plugs were pushed out of the tubes (plug diameter = 16 mm).

Plugs were separated manually into 2 parts at the contact surface with bone marrow and the excess marrow was cleaned off the meat surface. The CIE lightness (L*), redness (a*), and yellowness (b*) were measured at the contact surface of the meat with bone marrow in replicate.

Bone marrow color

The CIE color for lightness (L*), redness (a*), and yellowness (b*) of raw bone marrow paste was measured immediately and 1 h after harvest. Half of each sample was then cooked following the procedure described above. Cooked bone marrow color was measured in replicate. The other half of raw bone marrow paste was weighed and mixed with 1% $ZnSO_4$ by weight and then stored at 4°C overnight. Color of raw bone marrow mixed with $ZnSO_4$ was measured the following day. Bone marrow paste was cooked following the same procedure and color of the cooked bone marrow mixed with $ZnSO_4$ was measured in replicate.

Blood ZPP/H ratio

To determine the ZPP/H ratio baseline, 10 males and 10 females were killed after hatching and blood samples were collected from the heart using non-heparinized syringes. The ZPP/H concentration in whole blood was measured immediately using a hematofluorometer (Aviv hematofluorometer Model 206D, Aviv Biomedical, NJ, USA). This instrument measured the ratio of ZPP fluorescence to heme absorption. A drop of whole blood was placed on a microscope cover glass (Aviv Biomedical, NJ, USA) for insertion into the ZPP hematofluorometer and fluorescence was measured in µmol/mol heme with an assumed hematocrit of 42%. Blood samples were collected from one bird per cage weekly. Blood samples were collected from the heart up to 21 d, after that, blood samples were collected from the wing via the brachial vein.

Tissue minerals concentration

Concentrations of Zn and Fe were determined in breast muscle and bones using inductively coupled plasma optical emission spectrometer (Perkin Elmer ICP-OES 2000 DV). Breast meat samples were prepared by mincing the meat and drying at 70°C for 72 h before being finely ground. Femurs were cleaned of any exterior adhering tissues, dried at 105°C, then soaked in ethyl ether (Ethyl Oxide, Diethyl Ether $C_4H_{10}O$) for 48 h to extract the fat, and then analyzed for mineral concentration.

Statistical analysis

Data were analyzed as a randomized complete block design using the GLM procedure of SAS (SAS version 9.4, SAS Institute, Cary, NC, USA, 2014). The model tested the main effects of sex and dietary Zn effect within each sex by analyzing data for males and females separately. Differences between means were separated using the LS means method and significance was reported at a probability level of $P \leq 0.05$. The experimental unit for statistical analysis of measured parameters was the cage.

5.4. RESULTS AND DISCUSSION

Carcass and parts yield

Results for fasted live weight, carcass and parts weight and yield are presented in Tables 1, 2, and 3. Fasted live BW was greater in males than females ($P = 0.0001$). Values were 2905 and 2566 g for males and females, respectively. Hot WOG weight was measured immediately after evisceration and chilled WOG weight was measured after carcasses were chilled by immersion in an ice water tank overnight. Both weights were increased in males ($P = 0.0001$). However, dressing yield calculated for both hot and chilled WOG weights were comparable among males and females (Table 1). Dietary Zn had no effect on carcass weights and dressing yield of males and females (Table 3). Parts of legs, thighs, breast fillets (*Pectoralis major*), breast tenders (*Pectoralis minor*), wings, fat pad, and ribs, back, and skin were weighed. All parts weights were heavier in males except for the fat pad which was comparable while wings yield was increased in females ($P = 0.01$) and legs yield was greater ($P = 0.001$) in males (Table 1). Breast tenders weights increased ($P = 0.02$) as dietary Zn increased (Table 2). Values were 105, 113, and 119 g for dietary Zn of 0, 120, and 240 mg Zn/kg, respectively. The breast tenders yield, on the other hand was not affected by dietary Zn in males. Dietary Zn supplementation had no effect on parts weights and yield in females (Table 3). Several investigators reported results that were in agreement with the present results. Rossi et al. (2007) reported that supplementing up to 60 mg organic Zn/kg to a basal diet containing 60 mg inorganic Zn/kg did not affect carcass yield of broilers raised to 42 d. Also, thigh, drumstick, and breast yields were comparable among different dietary Zn levels. However, our results showed that breast tenders yield increased as dietary Zn level increased in males. Hess et al. (2001) fed different dietary sources of Zn to broilers raised to 42 d in the presence of 40 mg supplemental Zn/kg from either Zn-methionine, Zn-lysine, or a mixture of both; their results did not show any difference in carcass and parts yield. In another study, Skinner et al. (1992) reported that carcass yield was not affected by either mineral or vitamin premix removal from broiler diets fed from 42-49 d. Collins and Moran (1999) examined the effect of supplementing manganese at either 0 or 180 mg/kg and zinc at either 0 or 150 mg/kg on live performance and carcass quality of two diverse broiler strains raised to 49 d. Their results showed that carcass and parts weight and yield were not affected by either manganese or Zn. Sunder at al. (2008) reported that parts yield of broilers slaughtered at 28 d were not affected by increasing dietary Zn supplementation from 10 to 320 mg/kg. Bou et al. (2005) fed broilers 0, 300, or 600 mg Zn/kg by supplementing zinc sulfate to a basal diet containing 37 mg Zn/kg. Their results showed that carcass weight was not affected by Zn level even though live BW of birds supplemented with 600 mg Zn/kg was decreased. They suggested that for broiler production purposes, the upper tolerable Zn supplementation should be set between 600 and 1,000 mg/kg. Liu et al. (2011) fed different levels and sources of Zn to broilers and reported that Zn supplementation had no effect on carcass, breast, or thigh yields. Zhao et al. (2010) investigated the effect of chelated trace minerals supplementation on breast meat yield of male and female broilers slaughtered at 52 d and found that mineral supplementation (40 mg/kg Zn) improved breast tender yield in both males and females. Lopez et al. (2011) studied the effects of strain and sex on meat characteristics of broilers slaughtered at 42 d and reported that males had greater carcass and breast weights and lower dressing percentage and breast meat yield when compared to females. Musa et al. (2006) investigated the effect of sex and breed on broiler carcass characteristics and showed that males had higher dressing percentage, carcass, and breast muscle weights but leg muscle weight was not affected by sex. Salakova et al. (2009) reported that males had increased carcass and breast weights, while Kidd et al. (2005) reported that male broilers had a lower dressing percentage when compared to females. Young et al. (2001) reported that female carcasses produced higher percentage yields of forequarters, breasts, and filets but lower yields of drumsticks, which were similar to the findings of Abdullah et al. (2010). However, these findings were not consistent with the current findings, where females produced an increased percentage yield of wings and decreased percentage yield of legs as compared to males while sex had no effect on carcass dressing percentage.

Cook yield and shear force

Results of the breast fillets cook yield and tenderness are presented in Tables 4, 5, and 6. Raw and cooked breast fillets were heavier (P = 0.001) in males but the cook yield was comparable among males and females with cook yield values of 77.2 and 76.3 % for males and females, respectively (Table 4). Shear force values were also comparable among males and females. Dietary Zn had no effect on fillets weights, cook yield, or shear force in either males or females (Tables 5 and 6, respectively). The effect of dietary Zn supplementation on broiler meat quality parameters has not been adequately investigated, and it has remained unclear why Zn affected meat quality. In this research, cook yield was not affected by either dietary Zn or sex. Brewer et al. (2012) did not find any difference in cook loss of breast fillets due to sex in broilers. On the other hand, Northcutt et al. (2001) reported that sex affected cook yield and Poole et al. (1999) found that males had higher cook yield as compared to females, while Abdullah et al. (2010) reported that females had less cook loss that indicated higher cook yield as compared to males.

Salim et al. (2012) investigated the effect of adding 25 mg/kg organic Zn on meat texture of male and female broilers and reported that Zn had no effect on texture but male muscles required greater shear force. Liu et al. (2011) reported that dietary Zn supplementation decreased shear force in thigh muscle, and decreased drip loss in breast and thigh muscles. Salakova et al. (2009) reported that males had more tender meat as compared to females. Similarly, Abdullah and Matarneh (2010) reported reduced shear force values for breast fillet collected from males as compared to females. Other researchers reported that females possessed more tender breast meat as compared to males (Lyon et al., 1992; Musa et al., 2006). On the other hand, several investigations (Simpson and Goodwin, 1975; Ngoka et al., 1982; Lyon and Wilson, 1986; Poole et al., 1999; Northcutt et al., 2001; Abdullah et al., 2010; Lopez et al., 2011) reported that bird sex had no effect on breast fillet tenderness. In the current study, we were unable to detect any differences between males and females with respect to tenderness.

Color

The CIE lightness (L^*), redness (a^*), and yellowness (b^*) were measured for raw and cooked breast fillets, cooked breast meat, and raw thigh, results are presented in Tables 7, 8, and 9. Raw breast fillets harvested from females were lighter ($P = 0.001$), less red ($P = 0.04$), and more yellow ($P = 0.001$) in color as compared to males (Table 7). Values were for L^* 50.19 and 54.00, a^* 2.37 and 1.98, and b^* 5.78 and 6.83, for males and females, respectively.

However, when color was measured on the cooked breast fillets, the color coordinates were comparable among males and females. Raw thighs of males were lighter (P = 0.01) and less yellow (P = 0.0001) as compared to females but a* was comparable among males and females (Table 7). Lightness (L*) of cooked breast meat with bone marrow was increased (P = 0.05) in females, while b* was comparable among males and females. Redness (a*) of cooked breast meat with bone marrow was increased relative to a* of raw and cooked breast fillets and raw thighs with males having higher (P = 0.02) a* as compared to females (Table 7). Values were 19.42 and 15.71 for males and females, respectively. Feeding different levels of dietary Zn did not affect color coordinates of the breast fillets and thighs of both males and females (Tables 8 and 9, respectively). Raw breast fillets and thighs had similar L* and a* values, however, b* was higher for raw breast fillets, indicating more yellowness as compared to raw thighs.

Bone marrow color results are presented in Tables 10, 11, and 12. Females had more red (P = 0.03) and more yellow (P = 0.01) color compared to males when raw bone marrow color was measured immediately after harvest but only L* was higher (P = 0.01) in females when color was measured 1 h after harvest, and when bone marrow was cooked, L* (P = 0.001) and b* (P = 0.001) color coordinates remained higher in females (Table 10). Cooked bone marrow color values were for L* 38.58 and 40.35, a* 10.90 and 11.90, and b* 7.35 and 8.18, for males and females, respectively. Adding 1% of $ZnSO_4$ to the raw bone marrow decreased a* and b* for both males and females, with no differences observed between them but when bone marrow was cooked, the value of *a** was higher (*P* = 0.01) for females (Table 10).

Lightness (*L**) of raw bone marrow without added $ZnSO_4$ measured immediately after harvest increased (*P* = 0.04) by adding dietary Zn only in males, while *a** and *b** were not affected by dietary Zn. When color was measured 1 h after harvest, all color coordinates were decreased, and the decrease in *L** was larger when Zn was supplemented to the diets. Values for *L** measured immediately after harvest in males were 36.74, 41.00, and 38.58, and values measured 1 h after harvest were 36.01, 37.69, and 33.67for dietary Zn of 0, 120, and 240 mg Zn/kg, respectively (Table 11). The *a** and *b** of raw bone marrow without added Zn measured 1 h after harvest in males increased when dietary Zn was supplemented but cooking decreased (*P* = 0.001) *a** the most when dietary Zn was supplemented at 120 mg/kg, while *L** decreased (*P* = 0.03) as dietary Zn increased (Table 11). In females, raw bone marrow *L** measured 1 h after harvest decreased (*P* = 0.05) as dietary Zn increased (Table 12). Upon cooking, both *a** and *b** were reduced by increasing the dietary Zn in females. Adding 1% $ZnSO_4$ to the raw bone marrow increased *L** for all treatments as compared to the raw bone marrow without added $ZnSO_4$, while *a** and *b** were reduced. Cooking the raw bone marrow mixed with 1% $ZnSO_4$ only affected *L** (*P* = 0.05) in males and *a** (*P* = 0.01) in females where *a** values decreased as dietary Zn increased (Tables 11 and 12). Color has been considered to be the first criterion upon which consumers rely to make purchase decisions (Mancini and Hunt, 2005). The pigment responsible for color of meat was the heme protein in myoglobin in combination with residual quantities of hemoglobin that remained in muscles after slaughter. In the current research, we were unable to detect the effect of high dietary Zn on breast meat color but the effect was clear on bone marrow color, possibly due to higher content of heme in bone marrow as compared to muscles. Smith and Northcutt (2003) reported that approximately 11% of selected fully-cooked retail chicken products evaluated for bloody/red defect were affected. One cause for this discoloration was determined to be related to bone marrow (Smith and Northcutt, 2004b). The bones from younger animals, such as broilers, have not been completely calcified and the epiphyses of such bones characteristically have spongy walls that could be more susceptible to breakage. Thus, the marrow fluid containing hemoglobin could escape through the porous walls of the bones and migrate to adjacent muscle imparting a dark appearance to the meat when cooked. Liu et al. (2011) reported that dietary Zn supplementation decreased yellowness value in raw breast muscle of broilers slaughtered at 42 d. Salakova et al. (2009) reported that cooked breast meat was significantly lighter, more red, and more yellow than the raw meat which was consistent with our findings concerning breast meat color. They also reported that female raw breast meat was lighter and more yellow as compared to males, which was also similar to findings of the current research. On the other hand, Ngoka et al. (1982) reported no effect of sex on color $L*$, $a*$, and $b*$ attributes in turkeys. Similarly, Wheeler et al. (1999) did not observe any differences in breast muscle $L*$ values between sexes in turkeys. Lopez et al. (2011) did not find any difference in color of raw breast fillets when measured on the dorsal side. However, when color was measured on the ventral side of the breast fillet females exhibited increased $b*$ values which was consistent with the present findings.

Blood ZPP/H ratio

Blood ZPP/H ratio was measured weekly and is reported in Tables 13, 14, and 15. To establish a baseline for ZPP/H, a total of 10 males and 10 females were used. The average ZPP/H ratio in the males was 37.2 µmol/mol and 47.1 µmol/mol for females. Females exhibited an increased (P = 0.001) ZPP/H ratio at 7 d (Table 13). Dietary Zn had no effect on ZPP/H ratio in males (Table 14). However, in females ZPP/H ratio was numerically (P = 0.08) decreased as dietary Zn increased (Table 15). Biologically important interactions between and among chemically similar metal ions such as Zn and Fe were proposed by Hill and Matrone (1970). Iron (atomic number 26, atomic weight 55.85) and Zn (atomic number 30, atomic weight 65.37) were in the first transition series of the periodic table of the elements and had identical outer electron shell configurations. Thus, it was expected that Zn and Fe interacted and possibly that excess Zn affected Fe metabolism, possible changes included gut secretion of Fe, altered heme synthesis, or increased red blood cell turnover as suggested by Settlemire and Matrone (1967).

This was the first study of its kind that investigated the effect of dietary Zn on color of bone marrow and blood ZPP/H ratio. The lack of other reports impaired our ability to interpret our results. Measuring ZPP/H ratio has been generally practiced as a screening test to detect Fe deficiency and lead poisoning, mainly in humans, and it had been used only once with ducks to test for lead poisoning (Roscoe and Nielsen, 1979). A high ZPP/H ratio indicated that Zn had replaced Fe in the heme, thus changing the color of hemoglobin and myoglobin.

The stable bright red color of Italian Parma ham was believed to be a result of Zn-protoporphyrin complex that was produced during the maturation process. This complex has been found to be very stable and did not change with either heat or light exposure (Wakamatsu et al., 2004). It has been suggested that since Zn was the second most abundant cation in skeletal muscles (Kagawa, 2001) that it had replaced the Fe in the heme, which resulted in the stable bright red color. We were unable to detect this effect in broiler breast meat, possibly due to the low myoglobin content of chicken breast muscle as compared to red meat (Young and West, 2001). Additionally, the Zn level in feed may have not been sufficient to induce the red color in breast meat. Numata and Wakamatsu (2009) conducted a study to summarize the causes of natural red pigments in food and food products and found that during a long curing process Zn present in tissues replaced the Fe in myoglobin and any remaining hemoglobin. The resulting zinc protoporphyrin molecule (ZPP) was a very heat stable red pigment. This process has now been patented for producing red pigments for food use that were heat stable (Numata and Wakamatsu, 2009).

Tissue mineral concentration

The effect of sex and dietary Zn on Zn and Fe concentrations in breast fillets (*Pectoralis major*) and femurs are presented in Tables 16, 17, and 18. Generally, mineral concentrations were greater in bones than muscles. Mineral concentrations in meat and bones were affected by sex (Table 16). In breast meat, females had reduced (P = 0.03) Fe concentration but increased (P = 0.0001) Zn concentration (Table 16). Values of Fe concentration in breast meat were 12.9 and 12.3 mg/kg and Zn concentration 20.7 and 21.5

mg/kg for males and females, respectively. In femurs, females had lower Fe and Zn concentrations (P = 0.0001) compared to males. Values of Fe concentration in femurs were 123.9 and 97.2 mg/kg, and Zn concentration 180.0 and 134.6 mg/kg for males and females, respectively.

Dietary Zn did not affect Fe concentration in either breast meat or femurs of males (Table 17). However, in breast meat, Zn concentration was the greatest at the dietary Zn level of 120 mg/kg. In femurs, Zn concentration increased (P = 0.004) with increasing dietary Zn level. Values of Zn concentration in breast meat of males were 20.2, 21.2, and 20.7 mg/kg and in femurs 171.5, 172.3, and 196.4 mg/kg at dietary Zn levels of 0, 120, and 240 mg/kg, respectively. In females, both Fe and Zn in breast meat were influenced by dietary Zn where Fe was the lowest (P = 0.001) at dietary Zn of 120 mg/kg, while Zn in breast meat decreased (P = 0.001) as dietary Zn increased. On the other hand, Zn concentration in femurs of females followed the same trend as in males and increased (P = 0.02) as dietary Zn increased (Table 18). It has been reported that Zn from bone can be utilized when Zn deficiency occurred. Thus, bone appeared to be a functional reserve for Zn, which could be readily mobilized for any immediate need (Harland et al., 1975). Mavromichalis (2000) reported that 55% of total body Zn was found in skeletal muscles and bones. Bones had the highest concentration of Zn (144.0 µg Zn/g DM) with most of remaining tissues having a Zn concentration between 50 and 120 µg Zn/g DM in chickens. Similarly, Hambidge et al. (1986) reported a range of 100-250 µg Zn/g DM for bone and a range of 30-250 µg Zn/g DM for most other tissues in mammals. Sandoval et al. (1998) evaluated the effect of dietary Zn on tissue concentrations in chicks and reported that increasing dietary Zn supplementation resulted in an increased Zn concentration in bone, liver, kidney, and muscle. They reported that bone accumulated more Zn while muscle Zn concentration was less sensitive to dietary Zn changes than other tissues. Similarly, Stahl el al. (1989) reported that chicks fed 2000 mg Zn/kg accumulated more Zn in their tissues (liver, pancreas, tibia) but exhibited decreased tibia Fe, and liver and pancreas copper (Cu) concentrations. Shelton and Southern (2006) reported that there was a reduction in Zn, Manganese (Mn), and Cu levels in the tibia of broilers fed diets not containing a trace mineral premix.

There have been several reports where Zn retention in different chicken tissues increased when birds were fed high doses of dietary Zn from 4 d to 3 wk of age (Williams et al., 1989; Emmert and Baker, 1995). In addition, increased Zn content in plasma and tibia were found when the dietary Zn content was greater than the Zn requirements for growth of chickens (Mohanna and Nys, 1999). Sunder at al. (2008) reported a linear increase in Zn concentration in bone, liver, and kidney with increasing dietary Zn supplementation from 10 to 320 mg/kg in broilers slaughtered at 28 d. Gajula et al. (2011) reported similar results when dietary Zn was increased to 160 mg/kg. On the other hand, Zhao et al. (2010) did not find any difference in tibia Zn when broilers were fed different sources of trace minerals. In a study that compared Zn content in males and females, Salim et al. (2012) reported that supplementing organic Zn increased Zn content in thigh meat of both male and female broilers without producing any difference between males and females.

5.5. CONCLUSION

Results of this study indicated that supplementing 120 and 240 mg Zn/diet increased breast tender size, bone marrow *a**, and bone Zn concentration. Breast and thigh color were not affected by dietary Zn although bone marrow *a** was increased by dietary supplementation with Zn. It was possible that Fe levels in body and diet were sufficient to prevent replacement of Zn in the myoglobin and hemoglobin. It was also possible that levels of myoglobin and hemoglobin in chicken breast and thigh were not great enough to result in a clear effect. Since bone marrow *a** was influenced, it would be beneficial to test bone-in breast meat and measure the color at the connection between bone and meat since bone marrow could influence the color of those specific locations.

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Table V.1. Least-squares means for the main effect of sex on carcass and parts weights and yield of broilers raised to 42 d of age

	Sex		**SE**[1]	**P-value**
	Male	**Female**		
Carcass weight (g) and yield (%)				
Fasted live weight	2905[A]	2566[B]	19.8	0.0001
Hot WOG[2] weight	2197[A]	1947[B]	17.6	0.0001
Chilled WOG weight	2298[A]	2050[B]	18.4	0.0001
Hot carcass yield[3]	75.6	75.9	0.3	0.57
Chilled carcass yield	79.1	79.9	0.3	0.10
Parts weight (g)				
Breast fillets	558[A]	505[B]	8.8	0.0001
Breast tenders	112[A]	102[B]	2.1	0.001
Wings	224[A]	206[B]	2.1	0.0001
Legs	263[A]	222[B]	3.0	0.0001
Thighs	362[A]	319[B]	4.5	0.0001
Ribs, back, and skin	730[A]	641[B]	8.4	0.0001
Fat pad	31	31	1.8	0.75
Parts yield[4] (%)				
Breast fillets	24.2	24.6	0.3	0.36
Breast tenders	4.9	5.0	0.1	0.59
Wings	9.8[B]	10.1[A]	0.1	0.01
Legs	11.4[A]	10.8[B]	0.1	0.001
Thighs	15.7	15.6	0.2	0.54
Ribs, back, and skin	31.8	31.3	0.3	0.26
Fat pad	1.3	1.5	0.1	0.09

[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Standard error (SE) for n=106 birds.
[2]WOG weight is carcass weight without giblets (heart, liver, gizzard, and neck).
[3]Hot carcass yield calculated based on hot WOG weight as a percentage of fasted live weight, and chilled carcass yield calculated based on chilled WOG weight as a percentage of fasted live weight.
[4]Yield of parts calculated based on chilled WOG weight.

Table V.2. Least-squares means for the main effect of zinc on carcass and parts weights and yield of broiler males raised to 42 d of age

Variable	Dietary Zn[1] (mg/kg)			SE[2]	P-value
	0	120	240		Zn
Carcass weight (g) and yield (%)					
Fasted live weight	2861	2923	2933	34.2	0.29
Hot WOG[3] weight	2154	2212	2227	28.0	0.17
Chilled WOG weight	2252	2314	2331	29.0	0.14
Hot carcass yield[4]	75.3	75.7	75.9	0.3	0.30
Chilled carcass yield	78.7	79.2	79.5	0.3	0.25
Parts weight (g)					
Breast fillets	533	560	581	14.6	0.08
Breast tenders	105^{b}	113^{ab}	119^{a}	3.3	0.02
Wings	222	227	225	3.0	0.62
Legs	260	259	270	4.7	0.20
Thighs	354	365	366	7.7	0.46
Ribs, back, and skin	730	739	721	12.2	0.57
Fat pad	32	30	30	3.0	0.88
Parts yield[5] (%)					
Breast fillets	23.6	24.2	24.9	0.5	0.20
Breast tenders	4.7	4.9	5.1	0.1	0.11
Wings	9.9	9.8	9.6	0.1	0.37
Legs	11.5	11.2	11.6	0.2	0.30
Thighs	15.7	15.8	15.7	0.3	0.98
Ribs, back, and skin	32.4^{a}	32.0^{ab}	30.9^{b}	0.4	0.04
Fat pad	1.4	1.3	1.3	0.1	0.73

^{a,b}Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[1]Dietary Zn was included in the diets in the form of zinc sulfate.
[2]Standard error (SE) for n=53 birds.
[3]WOG weight is carcass weight without giblets (heart, liver, gizzard, and neck).
[4]Hot carcass yield calculated based on hot WOG weight as a percentage of fasted live weight, and chilled carcass yield calculated based on chilled WOG weight as a percentage of fasted live weight.
[5]Yield of parts calculated based on chilled WOG weight.

Table V.3. Least-squares means for the main effect of zinc on carcass and parts weights and yield of broiler females raised to 42 d of age

Variable	Dietary Zn[1] (mg/kg)			SE[2]	P-value
	0	120	240		Zn
Carcass weight (g) and yield (%)					
Fasted live weight	2547	2555	2595	34.0	0.57
Hot WOG[3] weight	1938	1951	1951	33.0	0.95
Chilled WOG weight	2044	2049	2057	35.0	0.97
Hot carcass yield[4]	76.1	76.3	75.2	0.7	0.47
Chilled carcass yield	80.2	80.2	79.3	0.8	0.60
Parts weight (g)					
Breast fillets	509	500	507	16.0	0.92
Breast tenders	98	106	101	3.6	0.25
Wings	201	208	210	4.1	0.26
Legs	219	221	225	5.6	0.78
Thighs	311	322	324	7.6	0.45
Ribs, back, and skin	649	638	637	16.8	0.86
Fat pad	31	34	29	3.1	0.57
Parts yield[5] (%)					
Breast fillets	24.8	24.4	24.6	0.6	0.86
Breast tenders	4.8	5.2	4.9	0.2	0.19
Wings	9.9	10.1	10.2	0.2	0.30
Legs	10.7	10.8	10.9	0.2	0.77
Thighs	15.3	15.7	15.8	0.3	0.52
Ribs, back, and skin	31.7	31.1	31.0	0.6	0.69
Fat pad	1.5	1.7	1.4	0.2	0.54

[1]Dietary Zn was included in the diets in the form of zinc sulfate.
[2]Standard error (SE) for n=53 birds.
[3]WOG weight is carcass weight without giblets (heart, liver, gizzard, and neck).
[4]Hot carcass yield calculated based on hot WOG weight as a percentage of fasted live weight, and chilled carcass yield calculated based on chilled WOG weight as a percentage of fasted live weight.
[5]Yield of parts calculated based on chilled WOG weight.

Table V.4. Least-squares means for the main effect of sex on cook yield and texture of breast fillets harvested from broilers raised to 42 d of age

	Sex		SE[1]	P-value
	Male	**Female**		
Raw breast weight (g)	279^{A}	256^{B}	4.3	0.001
Cooked breast weight (g)	215^{A}	196^{B}	3.5	0.001
Cook loss (%)	22.8	23.7	0.4	0.14
Cook yield (%)	77.2	76.3	0.4	0.14
Shear force (kg)	2.7	2.7	0.1	0.93

^{A,B}Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Standard error (SE) for n=53 birds.

Table V.5. Least-squares means for the main effect of zinc on cook yield and texture of breast fillets harvested from broiler males raised to 42 d of age

Variable	Dietary Zn[1] (mg/kg)			SE[2]	P-value
	0	120	240		Zn
Raw breast weight (g)	270	280	288	7.5	0.26
Cooked breast weight (g)	210	215	222	5.9	0.37
Cook loss (%)	22.3	23.3	22.9	0.6	0.57
Cook yield (%)	77.7	76.7	77.1	0.6	0.57
Shear force (kg)	2.7	3.0	2.5	0.2	0.10

[1]Dietary Zn was included in the diets in the form of zinc sulfate.
[2]Standard error (SE) for n=53 birds.

Table V.6. Least-squares means for the main effect of zinc on cook yield and texture of breast fillets harvested from broiler females raised to 42 d of age

Variable	Dietary Zn[1] (mg/kg)			SE[2]	P-value
	0	120	240		Zn
Raw breast weight (g)	259	251	259	7.5	0.66
Cooked breast weight (g)	200	188	199	6.4	0.35
Cook loss (%)	22.8	24.9	23.4	0.7	0.10
Cook yield (%)	77.2	75.1	76.6	0.7	0.10
Shear force (kg)	2.8	2.5	2.9	0.2	0.26

[1]Dietary Zn was included in the diets in the form of zinc sulfate.
[2]Standard error (SE) for n=53 birds.

Table V.7. Least-squares means for the main effect of sex on color of breast fillets and thighs harvested from broilers raised to 42 d of age

	Sex		SE[1]	P-value
	Male	**Female**		
Raw breast fillets color				
*L**	50.19^{B}	54.00^{A}	0.5	0.001
*a**	2.37^{a}	1.98^{b}	0.1	0.04
*b**	5.78^{B}	6.83^{A}	0.2	0.001
Cooked breast fillets color				
*L**	78.05	79.45	0.6	0.12
*a**	3.36	3.31	0.1	0.77
*b**	16.37	16.83	0.3	0.22
Cooked breast meat with marrow				
*L**	49.98^{b}	52.45^{a}	0.9	0.05
*a**	19.42^{a}	15.71^{b}	1.0	0.02
*b**	14.25	13.65	0.7	0.57
Raw thigh color				
*L**	54.62^{A}	53.30^{B}	0.4	0.01
*a**	2.73	2.81	0.1	0.66
*b**	1.00^{B}	1.72^{A}	0.1	0.0001

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Standard error (SE) for n=106 birds for all variables except *L**, *a**, and *b** for cooked breast meat with marrow SE for n=36 tubes.

Table V.8. Least-squares means for the main effect of zinc on color of breast fillets and thighs harvested from broiler males raised to 42 d of age

Variable	Dietary Zn[1] (mg/kg)			SE[2]	P-value
	0	120	240		Zn
Raw breast fillets color					
*L**	50.15	49.72	50.73	0.9	0.73
*a**	2.46	2.54	2.08	0.2	0.32
*b**	5.63	5.75	6.00	0.3	0.71
Cooked breast fillets color					
*L**	78.56	76.90	78.77	1.3	0.55
*a**	3.51	3.46	3.10	0.2	0.38
*b**	17.01	15.82	16.27	0.4	0.09
Cooked breast meat with marrow					
*L**	48.86	51.40	49.68	1.6	0.52
*a**	20.21	17.20	20.85	1.9	0.37
*b**	14.86	14.18	13.71	1.3	0.82
Raw thigh color					
*L**	53.92	55.37	54.57	0.6	0.18
*a**	2.61	2.60	3.01	0.2	0.24
*b**	1.00	0.86	0.94	0.1	0.92

[1]Dietary Zn was included in the diets in the form of zinc sulfate.
[2]Standard error (SE) for n=53 birds for all variables except *L**, *a**, and *b** for cooked breast meat with marrow SE for n=18 tubes.

Table V.9. Least-squares means for the main effect of zinc on color of breast fillets and thighs harvested from broiler females raised to 42 d of age

Variable	Dietary Zn[1] (mg/kg)			SE[2]	P-value
	0	120	240		Zn
Raw breast fillets color					
*L**	52.86	54.99	54.19	0.8	0.21
*a**	1.86	1.77	2.31	0.2	0.22
*b**	6.46	7.23	6.80	0.4	0.36
Cooked breast fillets color					
*L**	78.70	80.09	79.62	0.8	0.47
*a**	3.46	3.08	3.38	0.2	0.44
*b**	16.88	16.95	16.63	0.5	0.89
Cooked breast meat with marrow					
*L**	52.23	53.84	51.27	1.4	0.46
*a**	17.94	13.74	15.46	1.6	0.20
*b**	13.64	14.00	13.36	1.4	0.95
Raw thigh color					
*L**	53.60	53.17	53.13	0.7	0.86
*a**	2.73	2.57	3.02	0.2	0.52
*b**	1.68	1.71	1.76	0.2	0.98

[1]Dietary Zn was included in the diets in the form of zinc sulfate.

[2]Standard error (SE) for n=53 birds for all variables except *L**, *a**, and *b** for cooked breast meat with marrow SE for n=18 tubes.

Table V.10. Least-squares means for the main effect of sex on color of bone marrow harvested from broilers raised to 42 d of age

	Sex		SE[1]	P-value
	Male	Female		
Raw marrow color without added Zn immediately after harvest (0 h)				
*L**	38.76	40.39	0.6	0.09
*a**	32.51^{b}	35.72^{a}	0.9	0.03
*b**	14.10^{B}	17.03^{A}	0.6	0.01
Raw marrow color without added Zn 1 h after harvest				
*L**	35.79^{B}	39.30^{A}	0.7	0.01
*a**	30.10	29.62	0.8	0.67
*b**	12.28	11.89	0.4	0.49
Raw marrow color with 1% added Zn				
*L**	39.80	41.47	1.1	0.32
*a**	14.01	12.92	0.9	0.43
*b**	3.87	3.60	0.4	0.66
Cooked marrow color without added Zn				
*L**	38.58^{B}	40.35^{A}	0.4	0.001
*a**	10.90	11.90	0.5	0.13
*b**	7.35^{B}	8.18^{A}	0.2	0.001
Cooked marrow color with 1% added Zn				
*L**	42.28	42.23	1.2	0.98
*a**	6.03^{B}	9.12^{A}	0.6	0.01
*b**	5.41	5.37	0.2	0.82

^{a,b}Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
^{A,B}Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Standard error (SE) for n=12 tubes for all variables except *L**, *a**, and *b** for cooked marrow color without added Zn SE for n=36 tubes.

Table V.11. Least-squares means for the main effect of zinc on color of bone marrow harvested from broiler males raised to 42 d of age

Variable	Dietary Zn[1] (mg/kg)			SE[2]	P-value
	0	120	240		Zn
Raw marrow color without added Zn immediately after harvest (0 h)					
*L**	36.74[b]	41.00[a]	38.58[ab]	0.6	0.04
*a**	30.73	34.27	32.53	1.6	0.40
*b**	13.19	15.03	14.08	0.9	0.44
Raw marrow color without added Zn 1 h after harvest					
*L**	36.01	37.69	33.67	0.8	0.07
*a**	27.65[C]	32.58[A]	30.08[B]	0.3	0.01
*b**	10.92[b]	13.40[a]	12.52[a]	0.3	0.02
Raw marrow color with 1% added Zn					
*L**	41.02	10.90	37.47	2.5	0.59
*a**	14.21	13.92	13.89	2.2	0.99
*b**	3.83	3.81	3.97	1.1	0.99
Cooked marrow color without added Zn					
*L**	39.00[a]	39.54[a]	37.19[b]	0.6	0.03
*a**	10.29[B]	9.47[B]	12.92[A]	0.5	0.001
*b**	7.07	7.70	7.28	0.2	0.17
Cooked marrow color with 1% added Zn					
*L**	38.93[b]	45.89[a]	41.02[ab]	1.1	0.05
*a**	6.52	5.71	5.87	0.3	0.31
*b**	5.44	5.21	5.58	0.2	0.48

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A-C]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Dietary Zn was included in the diets in the form of zinc sulfate.
[2]Standard error (SE) for n=6 tubes for all variables except *L**, *a**, and *b** for cooked marrow color without added Zn SE for n=18 tubes.

Table V.12. Least-squares means for the main effect of zinc on color of bone marrow harvested from broiler females raised to 42 d of age

Variable	Dietary Zn[1] (mg/kg)			SE[2]	P-value
	0	120	240		Zn
Raw marrow color without added Zn immediately after harvest (0 h)					
*L**	40.52	40.54	40.11	0.7	0.89
*a**	33.89	36.79	36.48	1.3	0.34
*b**	15.94	18.36	16.79	1.0	0.33
Raw marrow color without added Zn 1 h after harvest					
*L**	41.00^{a}	39.00^{ab}	38.00^{b}	0.5	0.05
*a**	28.18	31.00	29.68	0.6	0.09
*b**	11.35	12.47	11.84	0.4	0.29
Raw marrow color with 1% added Zn					
*L**	40.75	43.93	39.74	0.8	0.07
*a**	12.24	11.17	15.37	0.8	0.06
*b**	3.54	2.78	4.47	0.4	0.13
Cooked marrow color without added Zn					
*L**	40.98	40.66	39.40	0.5	0.07
*a**	14.89^{A}	10.45^{B}	10.64^{B}	0.5	0.0001
*b**	8.90^{A}	8.10^{B}	7.55^{C}	0.2	0.001
Cooked marrow color with 1% added Zn					
*L**	41.62	41.29	43.80	1.8	0.61
*a**	11.44^{A}	8.96^{B}	6.98^{C}	0.4	0.01
*b**	5.37	5.69	5.05	0.2	0.21

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A-C]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Dietary Zn was included in the diets in the form of zinc sulfate.
[2]Standard error (SE) for n=6 tubes for all variables except *L**, *a**, and *b** for cooked marrow color without added Zn SE for n=18 tubes.

Table V.13. Least-squares means for the main effect of sex on whole blood ZPP/H ratio harvested from broilers raised to 42 d of age

	Sex		SE[1]	P-value
	Male	**Female**		
ZPP/H (µmol/mol) at 42% Hematocrit				
1 d	37.2	47.1	-	-
7 d	43.6^{B}	55.2^{A}	2.3	0.001
14 d	47.4	49.2	3.2	0.71
21 d	33.6	38.9	2.0	0.09
28 d	31.8	36.3	1.8	0.09
35 d	29.8	34.2	1.9	0.11
42 d	30.8^{b}	37.1^{a}	2.0	0.03
ZPP/H (µmol/mol) at 35% Hematocrit				
14 d	29.2	31.1	2.2	0.09
21 d	20.9	24.5	1.3	0.772
28 d	19.3	21.9	1.3	0.644
35 d	23.7	26.6	1.3	0.720
42 d	24.8^{b}	30.3^{a}	1.8	0.627

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Standard error (SE) for n=36 birds.

Table V.14. Least-squares means for the main effect of zinc on whole blood ZPP/H ratio harvested from broiler males raised to 42 d of age

Variable	Dietary Zn[1] (mg/kg)			SE[2]	P-value
	0	120	240		Zn
ZPP/H (µmol/mol) at 42% Hematocrit					
1 d	37.2	37.2	37.2	-	-
7 d	47.5	43.7	39.0	4.0	0.36
14 d	50.6	41.8	51.0	6.2	0.53
21 d	29.7	38.8	32.2	3.7	0.23
28 d	34.2	34.0	27.7	3.7	0.41
35 d	32.7	28.0	28.8	2.6	0.41
42 d	34.2	26.7	31.7	3.0	0.22
ZPP/H (µmol/mol) at 35% Hematocrit					
14 d	32.4	25.2	30.8	4.1	0.46
21 d	18.8	23.2	20.8	2.6	0.51
28 d	19.8	20.3	17.8	2.8	0.80
35 d	26.3	21.8	22.8	1.8	0.22
42 d	27.2	22.2	25.0	2.1	0.43

[1]Dietary Zn was included in the diets in the form of zinc sulfate.
[2]Standard error (SE) for n=18 birds.

Table V.15. Least-squares means for the main effect of zinc on whole blood ZPP/H ratio harvested from broiler females raised to 42 d of age

Variable	Dietary Zn[1] (mg/kg)			SE[2]	P-value
	0	120	240		Zn
ZPP/H (µmol/mol) at 42% Hematocrit					
1 d	47.1	47.1	47.1	-	-
7 d	51.8	60.3	53.0	3.9	0.29
14 d	53.7	48.0	46.0	5.3	0.58
21 d	41.4	39.0	35.8	2.9	0.45
28 d	39.8	32.7	36.3	2.0	0.08
35 d	30.0	39.2	33.5	3.8	0.26
42 d	38.2	37.6	35.5	4.0	0.88
ZPP/H (µmol/mol) at 35% Hematocrit					
14 d	35.2	29.7	28.5	3.8	0.43
21 d	26.6	24.0	23.3	1.7	0.44
28 d	24.0[a]	19.2[b]	22.7[a]	1.3	0.05
35 d	23.7	30.2	25.8	2.4	0.19
42 d	32.5	30.0	28.3	7.0	0.77

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[1]Dietary Zn was included in the diets in the form of zinc sulfate.
[2]Standard error (SE) for n=18 birds.

Table V.16. Least-squares means for the main effect of sex on tissue mineral concentration of broilers raised to 42 d of age

	Sex		SE[1]	P-value
	Male	**Female**		
Muscles (mg/kg DM)				
Iron	12.9[a]	12.3[b]	0.2	0.03
Zinc	20.7[B]	21.5[A]	0.1	0.0001
Bones (mg/kg DM)				
Iron	123.9[A]	97.2[B]	3.4	0.0001
Zinc	180.0[A]	134.6[B]	4.3	0.0001

[a,b]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B]Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Standard error (SE) for n=54 birds.

Table V.17. Least-squares means for the main effect of zinc on tissue mineral concentration of broiler males raised to 42 d of age

Variable	Dietary Zn[1] (mg/kg)			SE^2	P-value
	0	120	240		Zn
Muscles (mg/kg DM)					
Iron	12.9	13.2	12.6	0.3	0.35
Zinc	20.2^B	21.2^A	20.7^B	0.2	0.001
Bones (mg/kg DM)					
Iron	124.3	127.7	119.6	4.8	0.51
Zinc	171.5^B	172.3^B	196.4^A	4.2	0.004

^{A,B}Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).

[1]Dietary Zn was included in the diets in the form of zinc sulfate.

[2]Standard error (SE) for n=27 birds.

Table V.18. Least-squares means for the main effect of zinc on tissue mineral concentration of broiler females raised to 42 d of age

Variable	Dietary Zn[1] (mg/kg)			SE[2]	P-value
	0	120	240		Zn
Muscles (mg/kg DM)					
Iron	13.0^{A}	11.5^{B}	12.5^{A}	0.2	0.001
Zinc	21.9^{A}	21.8^{A}	20.9^{B}	0.2	0.001
Bones (mg/kg DM)					
Iron	91.3	91.2	109.0	6.7	0.15
Zinc	130.8^{ab}	112.8^{b}	160.1^{a}	9.7	0.02

^{a,b}Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
^{A,B}Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1]Dietary Zn was included in the diets in the form of zinc sulfate.
[2]Standard error (SE) for n=27 birds.

SUMMARY AND CONCLUSIONS

The effects of dietary trace minerals on live performance, carcass yield, and meat quality of broilers were investigated. Results presented in Chapters 2 and 3 showed that males, in comparison to females, exhibited increased BW, BW gain, feed intake, and mortality-adjusted feed conversion ratio (AdjFCR) but females exhibited lower mortality, as expected. Supplementation of feed with 240 mg/kg Zn improved AdjFCR in the starter period of males and grower period of females but at no other times. Supplementation of feed with 100 mg/kg Cu was beneficial for females only during the finisher period. Furthermore, the combined supplementation of 240 mg/kg Zn and 10 mg/kg Cu improved AdjFCR in the starter period for both males and females. Roxarsone supplementation as an arsenic (As) source improved AdjFCR in the finisher period only when supplemented in combination with 100 mg/kg Cu in males. Roxarsone supplementation also improved fasted live BW and hot carcass weight in females but there was no effect in males. Mineral supplementation (Zn, Cu, and As) did not affect absolute and relative weight of parts or muscle redness. Raw bone marrow redness was decreased when Zn was supplemented at 240 mg/kg for both males and females. However, the supplementation of 120 mg/kg Zn in combination with 100 mg/kg Cu increased raw bone marrow redness in both males and females. Copper supplementation increased Cu content in muscle tissue of males and females while Fe was reduced in muscles of males and bones of females when Roxarsone (As) was supplemented. Results presented in Chapters 4 and 5 showed that dietary Zn had no effect on feed intake of males but BW gain and AdjFCR measured from 1-42 d was improved in males fed diets supplemented with 120 mg Zn/kg. Dietary Zn had no effect on BW, feed intake, BW gain, or AdjFCR of females. Dietary Zn had no effect on carcass weight and parts yield other than breast fillet weights that were increased in males when Zn was added to the diets. Raw breast fillets color was darker, more red, and less yellow in males as compared to females. Dietary Zn had no effect on raw breast fillet color harvested from males and females. Raw marrow color measured immediately after harvest was more red and more yellow in females. Dietary Zn supplementation at 120 mg/kg produced more red color of raw bone marrow collected from males when measured 1 h after harvest. Dietary Zn had no effect on the measured ZPP/H ratio except at 28 d where it was numerically reduced by 120 mg Zn/kg feed. Dietary Zn supplementation at 120 mg/kg feed increased Zn content in muscle tissue while 240 mg/kg increased Zn content in bones of both males and females.

Results indicated that supplementing high levels of dietary Zn did not affect breast meat redness but increased bone marrow redness. The Fe levels in the body, diet, and the previously used litter of this experiment could have been sufficient to prevent replacement of Zn in myoglobin and hemoglobin. It was also possible that levels of myoglobin and hemoglobin in chicken breast and thigh were not great enough to produce a clear effect.

In this study, we were unable to reproduce the problem of red/bloody discoloration of breast meat in broilers using trace minerals in feed but the increased marrow redness due to Zn demonstrated the possibility that muscle in contact with bones could be affected by higher redness values.

This investigation into plausible reasons for the problem of red discoloration of white meat revealed several potential causative factors. The sporadic nature of red discoloration problem in the broiler industry could be related to the practice of not changing the house litter between flocks. This common practice could have allowed minerals in house litter to accumulate and create a higher concentration of As from Roxarsone and lower concentration of Fe since monocalcium phosphate has been used in combination with phytase and DDGS (P source) as a means of reducing feed cost, which indirectly reduced the amount of Fe in feed and consequently in litter. Furthermore, the co-precipitation of As and Fe would have rendered Fe less abundant as well as less available and allowed Zn to more easily exert its effect on meat color. Therefore, sporadic clusters of the problem would disappear when the litter was changed and then reappear as litter became older. The vast broiler production volume where possibly 400 houses in a single complex were used and litter was not changed simultaneously aid to the nature of this being a sporadic problem. Based on industry complaints, the problem has been frequently observed in birds slaughtered at lighter BW (2 kg) compared to heavier BW (5 kg). This observation also suggested a relationship between minerals in litter and red meat discoloration. The industry has reared broilers at a high stocking density to decrease costs, which would eventually result in caking of the litter towards the end of each broiler production cycle, thus limiting coprophagy by birds. However, at a smaller slaughter BW, coprophagy and consumption of minerals would still be evident as caking of litter may not have yet started.

Therefore, differences in management practices related to litter, broiler stocking density, and feed formulation between the industry and educational research farm probably resulted in lower Fe in the industry setting. Thus, high Zn could have manifested its effect on meat color under commercial conditions, especially if As were present. The ratio of Zn:Fe should be considered when formulating feed as a ratio higher than 3:1 would result in Fe deficiency and Zn could cause red discoloration in meat. It was concluded that additional Fe may have to be added to broiler diets to help avoid this problem.

In the chicken broiler industry, males and females have often been raised together. Therefore, it would not be possible to determine whether the problem was sex-related under commercial conditions. However, based on findings of the current research where broilers were grown sex-separate, it was evident that sex played a major role in the red discoloration problem probably because males approach sexual maturity before females. This would have caused the hematocrit to increase coincident with more hemoglobin being moved from bone marrow to blood in circulation in males. Sexual maturity would be advanced to an even earlier age due to the relatively high protein concentration in some commercial diets. This was evident as meat from roosters used in canning has also often exhibited the red discoloration problem.

Several white muscle defects and myopathy have been reported. According to the literature, these problems spiked in the 1970s and 2000s concurrent with increased feed prices. It was suggested that producers were driven to use less expensive feed and use alternative feed ingredients (e.g. DDGS) to control costs. One major consequence of feeding less expensive feed was that the essential amino acids (e.g. lysine, methionine) became a primary concern when formulating these diets while the non-essential amino acids (e.g. arginine, glycine, proline) were neglected despite their essential role in connective tissue formation, which may have contributed to the emerging muscle defects.

The spectacular advancements in genetics witnessed by the broiler industry has resulted in broilers with higher growth rate while the role of nutrition has become even more critical in supporting the increased growth demands of what may have become a relatively fragile animal. Profit-driven decisions related to formulating feed in a least-cost manner while neglecting the essentiality of non-essential amino acids in nutrition would eventually be evidenced by increased condemnation at the processing plant and increased consumer complaints.