2004

Mineral utilization in poultry as affected by virginiamycin or mineral source

Tanika Ivel O'Connor-Dennie
Louisiana State University and Agricultural and Mechanical College, toconn2@lsu.edu

MINERAL UTILIZATION IN POULTRY AS AFFECTED BY VIRGINIAMYCIN OR MINERAL SOURCE

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Interdepartmental Program in
Animal and Dairy Sciences

by
Tanika O'Connor-Dennie
A.S., College of Agriculture, Science, and Education, Portland, Jamaica, 1997
B. S., Louisiana State University, 2002
August 2004

ACKNOWLEDGMENTS

This thesis could not have been written without the help of the whole Animal Science Department. Special thanks should be extended to Dr. L. Southern for putting up with one very stubborn Jamaican, because he could have given up a long time ago, but he saw the whole process through. Special thanks should also be extended to my committee members, Dr. T. Bidner and Dr. T. Lavergne, for giving me advice about the poultry and swine industries, life, and my options in the real world. I would like to thank Rob Payne, Brian Perkins, Melanie Roux, and the rest of the non-ruminant nutrition research group for helping me collect samples those early mornings and hot days and for providing guidance in both research and writing. Special thanks should be extended to Jason Shelton for being patient and answering all my questions however small they were and for helping me with lab work that appeared to have no end. He has shown me how a little patience can go a long way.

To my mother and sister, this is your degree and I would not have been here without you sending your support both emotionally and spiritually from Jamaica. You reminded me of my personal motto: *Age Quad Agis* which is Latin for what ever you do, do it to the best of your ability.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Four experiments were conducted to evaluate the effects of diets with reduced Ca or nonphytate P (nPP) levels with supplemental virginiamycin (Vm) on growth performance and bone response variables in chicks. All diets were corn-soybean meal based and all treatments were replicated six or eight times with five or six chicks each. Reducing dietary nPP from 0.45 to 0.15, 0.25, or 0.35% decreased most growth and bone response variables. The addition of Vm to diets containing 0.35% nPP and above increased both growth and bone response variables. The addition of Vm to diets containing 0.25% nPP and below had no affect or decreased growth and bone response variables. Reducing dietary Ca levels from 1.0 to 0.80 or 0.70% decreased bone response variables, and the addition of Vm increased these response variables at 0.70% Ca but not at 0.80% Ca.

Another experiment was conducted to determine the effect of supplemental Zn, Mn, and Cu in the inorganic or organic forms (metal-amino acid complexes) on egg production and egg quality during the post-molt production period. Nine hundred and sixty post-molt layers, were randomly assigned to five treatments with 12 replications of 16 layers each. The study lasted 32 wk and was divided into eight periods of 28 d each. The treatments consisted of: 1) Control (C) supplemented with inorganic trace minerals; 2) C + organic Zn (40 ppm), Mn (40 ppm), and Cu (7 ppm); 3) C + organic Zn (40 ppm); 4) C + inorganic Zn, Mn, and Cu to equal Diet 2; 5) C + inorganic Zn equal to Diet 3. The addition of trace minerals had variable effects on the egg production and quality during different periods. However, in the overall data, none of the forms of trace mineral supplementation affected any response criteria.

CHAPTER 1

INTRODUCTION

Antibiotics are compounds that can either be natural or synthetic in nature, and they are used to selectively target, kill, or inhibit the growth of microorganisms (Carlson and Fangman, 2004). Virginiamycin (Vm) is a cyclic polypeptide antibiotic complex from *Streptomyces virginiae* of the streptogramin class of antibiotics, and it is commonly used to treat infections caused by Gram-positive organisms and as a growth promoter in diets for cattle, swine, and poultry (Buresh et al., 1985a,b; Lee et al., 1996; Ives et al., 2002; Hansen et al., 2003).

Antibacterial agents may improve growth performance and nutrient utilization by thinning the small-intestinal epithelium or by decreasing the production of growth depressing toxins or metabolites by intestinal mircoflora (Feighner and Dashkevicz, 1987). Several researchers have reported the nutrient sparing effect of Vm on crude protein, Ca, and P in pigs and poultry (Buresh et al., 1985b; Singh et al., 2000; Agudelo et al., 2003).

The results reported in these studies prompted us to conduct a series of experiments that investigated the effects of Vm in diets with adequate or reduced Ca and nonphytate P on growth performance and bone response variables.

Another area of interest has been the effect of egg breakage and the incidence of dirty and ungradeable eggs on layer production. Klecker et al. (2002) estimated that 6 to 8% of all losses in egg production are a direct result of low eggshell quality. Eggshell breakage is directly related to the quality of the shell. The factors affecting eggshell quality include adequacy of nutrition, flock health problems, management practices, environmental conditions, and breeding (Emery et al., 1984; Lee, 1984; Hurtwitz, 1987; Solomon, 1991).

The eggshell is approximately 95% calcium carbonate, 0.3% P, 0.3% Mg, and traces of Na, K, Zn, Mn, Fe, and Cu (Butcher and Miles, 2002). The eggshell is made of a matrix consisting of interwoven protein fibers and spherical masses and interstitial calcite or calcium carbonate crystals (Tullet, 1987; Okubo et al., 1997). The organic membrane and its

relationship with the shell in addition to the mamillary and spongy matrix plays a critical role in eggshell strength and quality (Tullet, 1987; Nys et al., 1999; Lavelin et al., 2000). Therefore, shell thickness is the main factor, but not the only factor that determines strength (Butcher and Miles, 2002). Dietary manipulations through the inclusion of Ca, P, and vitamin D_3, have been the methods traditionally used to improve eggshell thickness. Recently however, focus has been directed to the inclusion of trace minerals that aid in the proper formation of the organic matrix (Frost et al., 1990; Frost et al., 1991; Rao et al., 1991; Butcher and Miles, 2002; Klecker et al., 2002; Mabe et al., 2003).

The results reported in these studies prompted us to conduct a study to compare the effectiveness of dietary supplementation of Zn, Mn, and Cu in the inorganic or organic forms (metal-amino acid complexes) on egg production and quality from post-molt hens.

CHAPTER 2

REVIEW OF LITERATURE

2.1 THE ROLE OF VIRGINIAMYCIN IN FOOD PRODUCING ANIMALS

Virginiamycin (Vm) is an antibiotic produced by a variant of the *Streptomyces virginiae* species. It was first isolated in 1955 by De Somer and Van Dijck (Miles et al., 1984). Virginiamycin marketed for use in domestic animals is actually a combination of two antibiotics, Vm m1 and Vm s1. Virginiamycin m1 binds to ribosomes and inhibits translation by itself, but it is more effective in combination with Vm s1 because cooperative binding of these two antibiotics acts synergistically to prevent protein synthesis within bacteria (Lee et al., 1996; Hansen et al., 2003). Virginiamycin is commonly used to treat Gram-positive organism infections. It also has been reported to have growth-promoting effects at subtherapeutic levels in diets for cattle, swine, and poultry (Buresh et al., 1985a,b; Proudfoot et al., 1990; Ives et al., 2002).

Parks et al. (2001) suggested that Vm controls microbial growth by acting on the mircoflora's biochemical processes in the cell, such as protein synthesis, by inhibiting the elongation of *Methonobacterium* and *Escherichia coli,* or by reducing lactic acid producing bacteria by 10 to 20 fold in the stomach. Also, antibacterial agents may improve growth performance and nutrient utilization by thinning the small-intestinal epithelium or by reducing the production of growth depressing toxins or metabolites by intestinal mircoflora (Feighner and Dashkevicz, 1987).

Virginiamycin inhibits bacterial protein synthesis by binding to the 50s ribosomal subunit that blocks normal peptide formation (Cocito et al., 1974; Parfait et al., 1978), which gives Vm a broad-spectrum action on bacteria such as *Lactobacillus*, *Bifidobacterium*, and *Streptococcus*. Cummings (2003) reported that antibiotics, such as Vm, reduce lactic-acid-producing bacteria, which predominate in the upper gastrointestinal tract of the broiler. While lactic acid producing bacteria (*Lactoballus*, *Stretocci,* and *Staphylococci*) help prevent Salmonella, they also are largely responsible for retarded growth seen in pigs and chickens (Cummings, 2003). The reduction in bacterial count may increase nutrient availability of the feed because there is less competition for the nutrient between the animal and the microflora.

2.2 VIRGINIAMYCIN AND NUTRIENT UTILIZATION

Several researchers have reported the nutrient sparing effects of Vm on crude protein, energy, Ca, Mn, and P in pigs and chickens. Pelura et al. (1980) reported that the addition of Vm to low protein diets for 42 d improved weight gain and feed efficiency in pigs. Agudelo et al. (2003) reported that the addition of Vm improved P, Ca, and Zn digestibilities by 28, 11, and 19%, respectively, and absolute retention by 33, 19, and 21%, respectively. Cervantes et al. (2002) reported that the addition of Vm to broiler diets marginally deficient in P increased body weight, improved feed conversion, and decreased mortality. These results agree with the findings of Buresh et al. (1985b) who reported that the addition of Vm to broiler diets with 0.47% total P or higher resulted in an increase in body weight and P utilization.

Ravindran et al. (1984) reported that the addition of Vm to high fiber diets improved retention of P, Mg, Cu, and Mn in pigs, but not in low fiber diets. They reported that the addition of Vm to high fiber diets slowed rate of passage from 20.6 h to 26.7 h. This slower rate of passage resulted in improved digestion coefficients for dry matter, crude protein, ash, and energy metabolization.

The nutrient sparing effects of Vm are not limited to high fiber, low crude protein diets, or diets with deficiencies in some essential minerals. Buresh et al. (1986) reported that the addition of Vm to diets increased methionine utilization as well as feed efficiency in turkey poults. This response agrees with the findings of Harms et al. (1986).

Henry et al. (1986a) reported that the addition of Vm to a diet low in Mn reduced intestinal weight in broilers, which was attributed to a thinning of the intestinal wall. They also reported that the addition of Vm increased Mn utilization regardless of the level of Mn supplementation. This increase in Mn utilization agrees with the hypothesis that Vm increases nutrient utilization by thinning the small-intestinal epithelium and by reducing intestinal mircoflora (Feighner and Dashkevicz, 1987). Henry et al. (1986a) indicated that a decrease in intestinal mass was important because the small-intestinal mucosa is the most rapidly regenerating tissue in the body, and maintenance of a greater intestinal mass would result in a greater utilization of nutrients by the intestinal mucosa.

They also suggested that decreased thickness of the mucosa may enhance nutrient absorption by the host.

Harms et al. (1986) reported that the addition of Vm increased body weight of birds in diets containing four different levels of energy, with the greatest increase occurring at the lowest energy level. The addition of Vm also improved feed efficiency, with a decrease in the kilocalories of ME required to produce one gram of broiler weight. This improvement in feed efficiency agrees with the findings of Buresh et al. (1985a) who reported that the addition of Vm to the diet improved body weight, feed efficiency, and dietary energy utilization in turkey poults with restricted access to feed. Belay and Teeter (1996) reported an increase in carcass weight, breast yield, fat, and protein gain as well as dry matter carcass energy content with the addition of Vm. They also reported that Vm increased dressing percentage, which was attributed to the decrease in intestinal weight. Virginiamycin also improved caloric efficiency of birds in a thermal neutral environment and supplemented with a high energy diet. Woodward et al. (1988) also reported an increase in both shell yield and ready-to-cook yield with the addition of Vm to broiler diets.

2.3 BIOAVAILABILITY OF TRACE MINERALS

Zinc, Mn, and Cu are important trace minerals in poultry diets; they are required for growth, bone development, feathering, enzyme structure and function, and appetite (Leach and Gross, 1983; Liu et al., 1994; Mohanna and Nys, 1999; Underwood and Suttle, 1999). Commercial corn-soybean diets may not provide enough trace minerals to meet the requirement for chicks because of the "chelating" effects of phytate found in natural plant protein sources such as corn and soybean meal (Yan et al., 2001). Therefore, supplemental trace minerals such as Zn, Mn, and Cu are often added to broiler diets in the form of sulfates, oxides, chelates, proteinates, and polysaccharides (Wedekind et al., 1992). Batal et al. (2001) reported that the two feed grade sources of Zn widely used in industry are ZnO (72% Zn) and Zn sulfate (36% Zn). Sulfates are highly water soluble, allowing reactive metal ions to promote free radical formation. This free radical formation results in a decreased nutritional value of the diet due to breakdown of vitamins, fats, and oils (Batal et al., 2001). The oxide form of Zn has been reported to have a decreased bioavailability. It has been

reported that ZnO has a bioavailability ranging from 61 to 77% relative to Zn sulfate, with tibia Zn being more responsive to Zn source than weight gain (Wedekind and Baker, 1990; Wedekind et al., 1992; Sandoval, 1997). Cromwell et al. (1989) reported that the inclusion of Cu above 125 ppm as Cu sulfate in the diet of weanling pigs increased daily gain, feed efficiency, and increased liver Cu levels at 250 ppm, while the addition of CuO at the same levels had no effect on growth variables or liver Cu levels. Aoyagi and Baker (1993a) reported that analytical grade Cu sulfate and Cu_2O increased bile Cu while CuO had no effect; they further stated that CuO had a bioavailability of zero in broilers. Ledoux et al. (1991) also stated that the bioavailability of CuO was zero in broilers compared with Cu sulfate, which has a bioavailability of 88.5% and Cu carbonate, which has a bioavailability of 54.3%, relative to Cu from acetate, which was set at 100%. Manganese sulfate has been reported to have a greater bioavailability in broilers compared with MnO and Mn carbonate (Black et al., 1984; Wong-Valle et al., 1989). Henry et al. (1986b) reported no difference in daily gain, feed intake, or feed conversion between broilers fed MnO and Mn sulfate; they did however report that MnO had a relative bioavailability of 79, 58, and 64% for bone, kidney, and liver responses compared with Mn sulfate, which was considered to have a relative bioavailability of 100% for all response variables.

Because of the relatively high bioavailability of the sulfate forms of trace minerals, they are often used as the standards by which bioavailabilities of organic trace minerals are compared (Baker and Halpin, 1987). Organic sources of trace minerals have been reported to have greater bioavailability, due to the ability of organic compounds such as amino acids to bind strongly to Zn and other divalent minerals under physiological pH conditions (Kidd et al., 1996). This strong bond between the mineral and its organic carrier prevents phytate from binding to the metals in the gastrointestinal tract while still being water soluble, thus facilitating mineral uptake into the mucosa of the small intestine (Cao et al., 2000). In the poultry industry, a great deal of attention has focused on trace mineral-amino acid complexes. Kidd et al. (1996), reported that Zn-Methionine (Zn-Met) differs from inorganic Zn, Zn proteinates, and Zn polysaccarides complexes because it is a specific amino acid complex; Zn sulfate complexed to DL-methionine. Zinc is coordinated between the

amino and carboxyl groups of methionine and vacant bonds are occupied by sulfate and water. This compound has a chemical ratio of methionine to Zn to sulfate of 1:1:1. Other trace mineral amino acid complexes have been reported to have similar molecular structure; thus, they may behave in a similar manner in the small intestine of the chicken.

Wedekind et al. (1992) reported that the bioavailability of Zn-Met relative to Zn sulfate was decreased from 206 to 117% in a conventional corn-soybean diet versus a purified amino acid diet in broilers. They attributed this to the chelating effects of phytic acid in the more complex corn-soybean diet and the ability of organic complexes to compete more effectively against phytic acid in the small intestine of the animal, while Zn present in Zn sulfate forms such a strong bond with phytic acid in the small intestine that it is unavailable to the animal for absorption. Cao et al. (2000) reported that feed intake, daily gain, and bone Zn concentration were greatest in birds supplemented with organic Zn compared with those supplemented with inorganic Zn, which does not agree with the findings of Mohanna and Nys (1999) who reported that weight gain, feed intake, and feed conversion in broilers were not influenced by Zn sulfate or Zn-Met. Pimentel et al. (1991) reported that Zn source (ZnO or Zn-Met) had no affect on growth or tibia and liver Zn levels, but that broilers fed Zn-Met had higher levels of pancreatic Zn.

Guo et al. (2001) investigated the bioavailability of five organic Cu sources relative to Cu sulfate. They reported that the bioavailability of Cu-Lys, Cu amino acid, and Cu proteinate were all higher than Cu sulfate. Paik (2001) reported that pigs and broilers fed Cu-Met had increased daily gain and feed intake, while laying hen performance and eggshell quality were improved in layers fed Cu-Met compared with those fed Cu sulfate. Baker et al. (1991) reported that Cu from Cu_2O and Cu-Lys had the same bioavailability as Cu sulfate, but that CuO had a bioavailability of zero. Aoyagi and Baker (1993b) investigated the relative bioavailability of Cu in Cu-Met, Cu-Lys, and CuCl relative to Cu sulfate; they reported that weight gain and feed intake were not affected by Cu source, and there was no difference between the relative bioavailability of Cu-Met and Cu sulfate.

Smith et al. (1995) reported that the relative bioavailability of Mn proteinate and MnO were 120 and 91%, respectively, relative to Mn sulfate based on bone Mn. Baker and Halpin (1987)

reported that there was no difference between the bioavailability of Mn sulfate and Mn proteinate based on bone Mn levels, weight gain, or feed efficiency. Henry et al. (1989) reported that the relative bioavailability of MnO ranged from 86 to 96%, while Mn-Met had a relative bioavailability of 108 to 132% compared with Mn sulfate based on liver and kidney Mn concentrations.

2.4 TRACE MINERALS AND EGGSHELL QUALITY

Klecker et al. (2002) estimated that 6 to 8% of all losses in egg production are a direct result of low eggshell quality. Eggshell breakage is directly related to the quality of the shell, and shell quality has been shown to decrease with age of the flock (Hurwitz et al., 1998). As the hen ages, she begins laying larger eggs that require more shell. However, it is physiologically impossible to increase the amount of shell that is produced, resulting in thinner shells (Ensminger et al., 1990). Secondly, as the hen ages, the mobilization of calcium from bone is decreased, resulting in a decrease in the production of calcium carbonate. For these reasons, the expected eggshell quality declines with age (Butcher and Miles, 2002).

The dry eggshell is approximately 95% calcium carbonate, 0.3% P, 0.3% Mg, with traces of Na, K, Zn, Mn, Fe, and Cu (Butcher and Miles, 2002). The rest of the eggshell is made up of an organic matrix consisting of interwoven protein fibers and spherical masses and interstitial calcite or calcium carbonate crystals, which influence shell strength (Butcher and Miles, 2002). The organic matrix consists of two regions, the mamillary matrix, and the spongy matrix (Solomon, 1991). The mamillary matrix region is interconnected to protein fibers of the outer shell. Calcite crystals are oriented randomly within each mamillary matrix to form a cohesive mass. Matrix fibers pass through calcite crystals making the matrix an integral part of shell strength.

Trace minerals such as Zn, Mn, and Cu may affect eggshell quality by their catalytic properties for enzymes involved in the process of membrane and eggshell formation and their interaction with calcite crystals in the forming eggshell (Mabe et al., 2003). It also has been suggested that there may be an interaction between Ca and microminerals such as Zn and Mn (Klecker et al., 2002). The addition of Zn and Mn has been reported to increase the utilization of Ca in hens and to improve the qualitative parameters of the eggshell (Klecker et al., 2002). This

increase in Ca utilization is especially important considering that Ca utilization decreases from 80 to 50% as the hen enters the last stages of the laying cycle (Ensminger et al., 1990).

Manganese has been reported to activate the glycosyl transferases involved in the formation of mucopolysaccharides (components of proteoglycans), which contribute to the ability to resist compressive charges in the epiphyseal zone of the bone (Leach, 1976; Liu et al., 1994). Proteoglycans are present in the organic matrix that controls eggshell structure and texture; thus influencing its mechanical properties (Nys et al., 1999, Gautron et al., 2001). Hens fed Mn-deficient diets produce eggs with thinner shells, translucent areas, and abnormalities in eggshell ultrastructure, particularly in the mammillary layer (Leach and Gross, 1983). Hossain and Bertechini (1998) reported an increase in egg production and egg weight with the supplementation of Mn at levels between 50 and 70 mg/kg. However, de Faria et al. (1999) reported that the supplementation of Mn above the requirement had no effect on eggshell quality during the final phase of production.

Copper has an integral role in eggshell structural soundness; lysyl oxidase is a cuproenzyme used in the conversion of lysine to cross-linked desmosine and isodesmosine, which have crucial roles in eggshell formation (Mabe et al., 2003). Copper deficiency in hens results in abnormal eggshell formation (Baumgartner et al., 1978; Chowdhury, 1990). Baumgartner et al. (1978) reported that eggshells from Cu-deficient hens were characterized by an abnormal distribution of the shell membrane fibers due to alterations in lysine-derived cross-links, which results in egg shape deformation and abnormal mechanical properties.

Zinc also has been reported to improve eggshell quality. Zinc is a component of the carbonic anhydrase enzyme, which supplies the carbonate ions during eggshell formation (Innocenti et al., 2004). Inhibition of this enzyme results in lowered bicarbonate ion secretion and an increase in shell-less egg production (Nys et al., 1999). Balnave and Zhang (1993) reported that the addition of Zn to saline drinking water reduced the incidence of eggshell defects and improved shell breaking strength. These results agree with the findings of Moreng et al. (1992) who reported that the addition of organic Zn improved shell breaking strength compared with unsupplemented diets.

Balnave and Zhang (1993) reported that hens receiving supplemental Zn had decreased eggshell defects and an increased concentration of calcium-binding protein and carbonic anhydrase activity, compounds that aid in eggshell mineralization. This decrease in eggshell defects is in agreement with the findings of Moreng et al. (1992) who reported an increase in eggshell breaking strength, shell weight, and shell weight per unit of surface area. Sahin et al. (2002) reported that supplementation of CrPic and Zn sulfate increased egg weight, eggshell thickness, egg specific gravity, and Haugh units when layers were subjected to low ambient temperatures. Kita et al. (1997) reported that the addition of dietary Zn-Met did not improve egg quality in layers subjected to high ambient temperatures. Mabe et al. (2003) reported that dietary Zn, Mn, and Cu supplementation did not affect percentage eggshell, eggshell index, or eggshell stiffness. These results disagree with the findings of Klecker et al. (2002), who reported that the substitution of 20% to 40% supplemental inorganic Zn and Mn with their chelates resulted in an increase in laying hen performance, eggshell quality, eggshell strength, eggshell weight, and eggshell thickness. Lim and Paik (2003) reported variable effects with the addition of organic trace minerals on egg production. They reported that Cu-Met increased egg production, specific gravity, and eggshell strength and decreased the production of shell-less eggs, while the addition of Zn-Met decreased egg production. Fakler et al. (2002) reported that the production of undergrade eggs and egg efficiency were improved with the addition of Availa Zn ® (an organic Zn source) in the diets compared with the basal diet. Khajaren et al. (2002) also reported an improvement in egg production, egg quality, and shell quality in the second phase of egg production (38 to 65 wk of age) in layers fed Zn and Mn organic complexes.

2.5 SUMMARY

The review of the literature suggests that it may be possible to improve mineral utilization through unconventional methods of diet manipulation. Virginiamycin has been shown to improve the utilization of P, Ca, fiber, and methionine in cattle, swine, and poultry, as well as increase growth rate. The addition of trace minerals has been shown to improve egg production and egg quality due to the effects of trace minerals on the organic membrane of the eggshell. The source of the trace

mineral may determine how effectively additional trace mineral supplementation improves egg production and egg quality.

CHAPTER 3

THE EFFECT OF VIRGINIAMYCIN IN DIETS WITH ADEQUATE OR REDUCED DIETARY CALCIUM OR NONPHYTATE PHOSPHORUS FOR BROILERS

3.1 INTRODUCTION

Virginiamycin (Vm) is a cyclic polypeptide antibiotic complex from *Streptomyces virginiae* of the streptogramin class of antibiotics. It is commonly used to treat infections from Gram-positive organisms or as a growth promotant in cattle, swine, and poultry diets. The Vm complex consists of two major components, Vm factor m1 and s1, which act synergistically to prevent protein synthesis within bacteria (Lee et al., 1996). Parfait et al. (1978) suggested that Vm inhibits the elongation of *Methonobacterium* and *Escherichia coli* and other Gram-positive bacteria in the intestine, thus disrupting bacterial protein synthesis and growth. It also has been suggested that antibacterial agents improve growth performance and nutrient utilization by either thinning the epithelium of the small intestine or by reducing the production of growth depressing toxins or metabolites by intestinal mircoflora (Feighner and Dashkevicz, 1987).

Several researchers have reported the nutrient sparing effects of Vm on crude protein, energy, Ca, Mn, and P in pigs and chickens. Pelura et al. (1980) reported that the addition of Vm to low crude protein diets improved weight gain and feed efficiency in pigs. Agudelo et al. (2003) reported that Vm improved P, Ca, and Zn digestibility and retention in pigs when compared with those fed the control diet. Cervantes et al. (2002) reported that the addition of Vm to marginally deficient P diets for broilers increased body weight, improved feed conversion, and decreased mortality. These recent results agree with the findings of Buresh et al. (1985b), who reported that the addition of Vm to diets with 0.47 % total P or greater increased body weight and P utilization.

A significant proportion of the endogenous Ca and P in corn and soybean meal are bound to phytate (Harland and Oberleas, 1999; Jang et al., 2003). The phytate-bound minerals have a reduced bioavailability to non-ruminant animals, resulting in an increased need for inorganic P supplementation (Broz et al., 1994; Johnston and Southern, 2000). Moore et al. (1999) indicated that the biggest problem facing the poultry industry is P runoff into surrounding watersheds. If Vm

can increase P utilization as indicated by Buresh et al. (1985b) and Agudelo et al. (2003), then Vm could possibly reduce P runoff by increasing P bioavailability to the animal. Therefore, the objectives of these experiments (EXP) were to investigate the effects of Vm in diets with adequate or reduced levels of Ca and nonphytate P (nPP) on growth performance and bone response variables in broilers.

3.2 MATERIALS AND METHODS

3.2.1 General

The methods used in these EXP were approved by the Louisiana State University Agricultural Center Animal Care and Use Committee. Each EXP used female Ross x Ross broilers obtained from a commercial hatchery on d 0 posthatching. The broilers were allotted, wingbanded, and placed in environmentally controlled brooder batteries[1] with raised wire floors for the duration of the 0- to 18-d EXP.

The diets used for these EXP were corn-soybean meal (C-SBM) based and were formulated to provide 1.26% total Lys and 3,200 kcal of ME per kilogram of diet (Table 3.1). The diets met or exceeded the nutrient requirements suggested by the NRC (1994) except for Ca and nPP when appropriate. The reduced Ca and nPP levels were achieved by replacing limestone and monocalcium phosphate with sand.

Throughout all EXP, the chicks, feed, and water were checked twice daily, and the feed in mash form and water were provided *ad libitum*. At the termination of each EXP, all broilers and feed were weighed for calculation of daily gain (ADG), daily feed intake (ADFI), gain:feed (G:F), Ca intake, and nPP intake. Calcium intake was calculated by multiplying the Ca content of the diet by ADFI. Nonphytate phosphorus intake was calculated in the same manner. After weighing, three (EXP 1 and 3) or six (EXP 2 and 4) broilers per replicate were randomly selected for bone analyses. The left tibia was collected, pooled by replicate, and then frozen until further analyses.

[1] Petersime Incubator Company, Gettysburg, OH

Table 3.1 Percentage composition of the control diets, as-fed basis[a]

Ingredient	Experiment 1, 2, and 3	Experiment 4
Corn	52.46	52.46
Soybean meal (47.5% CP)	37.96	38.03
Vegetable oil	5.33	5.31
Monocalcium phosphate	1.65	1.65
Limestone	1.51	1.51
Salt	0.50	0.50
Trace mineral premix[b]	0.25	0.25
DL-Methonine	0.24	0.19
Choline chloride	0.05	0.05
Vitamin premix[c]	0.05	0.05
Calculated composition:		
ME, kcal/kg	3,200	3,200
Calcium, %	1.00	1.00
Phosphorous, %	0.73	0.73
Nonphytate phosphorous, %	0.45	0.45
Lysine, %	1.26	1.26
Tryptophan, %	0.28	0.28
Threonine, %	0.86	0.86
TSAA, %	0.91	0.91

[a] Virginiamycin was added to the basal diet at 9, 11, or 22 ppm to make the respective diets for each experiment. Monocalcium phosphate and limestone were replaced by sand to create low Ca or nonphytate P diets for each experiment.

[b] Provided per kilogram of diet: copper (copper sulfate•5 H_2O), 7.0 mg; iodine (potassium iodate), 1.0 mg; iron (ferrous sulfate•7 H_2O), 50 mg; manganese (manganese sulfate•H_2O), 100 mg; selenium (as sodium selenite), 0.15 mg; and zinc (zinc sulfate•7H_2O), 75 mg.

[c] Provided per kilogram of diet: vitamin A (retinyl acetate), 8,000 IU; vitamin D_3 (cholecalciferol), 3,000 IU; vitamin E (DL-alpha-tocopheryl acetate), 25 IU; menadione, 1.5 mg; vitamin B_{12} (cyanocobalamin), 0.02 mg; biotin, 0.1 mg; folacin (folic acid), 1 mg; niacin (nicotinic acid), 50 mg; pantothenic acid, 15 mg; pyridoxine (pyridoxine•HCl), 4 mg; riboflavin, 10 mg; and thiamin (thiamin mononitrate), 3 mg.

3.2.2 Experiments 1 and 2

In EXP 1 and 2, 216 broilers per EXP were allotted to six treatments with six replications of six chicks each. The average initial and final body weights for EXP 1 and 2 were 47 and 488, and 40 and 523 g, respectively. The treatments were: Diet 1) C-SBM with 1.00% Ca and 0.45% nPP (PC); Diet 2) C-SBM with 0.80% Ca and 0.45% nPP (0.80Ca); Diet 3) C-SBM with 1.00% Ca and 0.35% nPP (0.35nPP); and Diets 4 to 6) as Diets 1 to 3 supplemented with 11 (EXP 1) or 22 (EXP 2) ppm of Vm [2].

3.2.3 Experiment 3

In EXP 3, 240 broilers were allotted to six treatments with eight replications of five chicks each. The average initial and final weights were 45 and 462 g, respectively. The treatments were: Diet 1) C-SBM with 1.0% Ca and 0.45% nPP (PC); Diet 2) C-SBM with 0.70% Ca and 0.45% nPP (0.70Ca); Diet 3) C-SBM with 1.00% Ca and 0.25% nPP (0.25nPP); and Diets 4 to 6) as Diets 1 to 3 supplemented with 9 ppm of Vm.

3.2.4 Experiment 4

In EXP 4, 432 broilers were allotted to 12 treatments with six replications of six chicks each. The average initial and final weights were 37 and 432 g, respectively. Four levels of nPP (0.15, 0.25, 0.35, or 0.45%) and three levels of Vm (0, 11, or 22) were used in a 4 x 3 factorial arrangement.

3.2.5 Bone Analysis

After thawing the bones, bone breaking strength (BBS) was determined using a HD 250 Texture Machine[3] fitted with a three-point bend rig with a load cell capacity of 25 kg and cross-head speed of 100 mm/min. After determining BBS, fat was extracted from the tibias by a 36 h Soxhlet extraction in ethyl alcohol followed by a 36 h extraction with diethyl ether. The bones were dried at 110° C for 24 h and then weighed. The dry, defatted tibias were dry ashed in a muffle furnace at 560° C for 24 h. After cooling, tibia ash was weighed for determination of ash weight, bone ash

[2] Stafac 20, Phibro, Fairfield, NJ.
[3] Texture Technologies Corporation, Scarsdale, NY.

percentage (BAP), BBS per Ca intake (BBS/Ca), BBS per nPP intake (BBS/nPP), milligrams of tibia ash per chick (ASH), tibia ash per Ca intake (ASH/Ca), and tibia ash per nPP intake (ASH/nPP) for EXP 1, 2, and 4. Bone breaking strength per Ca or nPP intake were calculated by dividing the kilograms of BBS by total Ca or nPP intake in grams. Tibia ash was calculated for each treatment by dividing the ash weight of the tibias by the number of tibias. Tibia ash was then divided by total Ca or nPP intake to calculate ASH/Ca and ASH/nPP, respectively.

3.2.6 Statistical Analysis

Data were analyzed by analysis of variance procedures (Steel and Torrie, 1980) appropriate for completely randomized designs by the GLM procedure of SAS[4]. In all EXP, contrast statements were used to test the main treatment effects and any interactions of Ca, nPP, and Vm. The pen of chicks served as the experimental unit for all response variables.

3.3 RESULTS

3.3.1 Experiment 1

Reducing dietary Ca decreased ($P < 0.01$ to 0.07) G:F, Ca intake, BBS, BBS/nPP, ASH, and ASH/nPP (Table 3.2). Reducing dietary nPP decreased ($P < 0.01$) nPP intake, BBS, ASH, BAP, BBS/Ca, and ASH/Ca, but it increased ($P < 0.01$) ASH/nPP. The addition of Vm increased ($P < 0.04$ to 0.09) ADG, ADFI, Ca intake, nPP intake, BBS, BBS/Ca, BBS/nPP, and ASH/Ca (Figures 3.1 and 3.2). The addition of Vm to the 0.80Ca diet increased (Ca x Vm interaction, $P < 0.01$ to 0.09) ASH, ASH/nPP, and ASH/Ca. The addition of Vm to the 0.35nPP diets increased (nPP x Vm interaction, $P < 0.03$ to 0.10) ADG, ADFI, Ca intake, nPP intake, BBS, BBS/Ca, BBS/nPP, and ASH. These results suggest that Vm increases the availability or utilization of dietary Ca and nPP.

3.3.2 Experiment 2

Reducing dietary Ca decreased ($P < 0.01$ to 0.04) Ca intake and ASH/nPP, but it increased ($P < 0.02$) BBS/Ca and ASH/Ca (Table 3.3). Reducing dietary nPP decreased ($P < 0.01$ to 0.05) ADG, ADFI, G:F, Ca intake, nPP intake, BBS, BBS/Ca, BAP, ASH, and ASH/Ca, but it increased

[4] SAS Inst. Inc., Cary, NC.

Table 3.2 Growth performance and bone response variables of broilers fed varying levels of virginiamycin, calcium, and nonphytate phosphorus, Experiment 1[1]

Virginiamycin, ppm	0			11				*P* = F[2]				
										Ca x		nPP x
Diet	PC	0.80Ca	0.35nPP	PC	0.80Ca	0.35nPP	PSEM	Vm	Ca	Vm	nPP	Vm
Growth performance[3]												
ADG, g	24.71	23.64	24.09	24.68	24.58	26.19	0.59	0.05	NS	NS	NS	0.07
ADFI, g	33.98	32.16	32.10	33.39	34.26	34.94	0.86	0.06	NS	NS	NS	0.05
G:F, g/g	0.727	0.701	0.751	0.739	0.724	0.750	0.012	NS	0.07	NS	NS	NS
Ca intake, mg/d	340	257	321	334	274	349	8	0.06	0.01	NS	NS	0.03
nPP intake, mg/d	153	145	112	150	154	122	4	0.09	NS	NS	0.01	0.09
Bone response variables[4]												
BBS, kg	14.1	11.0	10.2	13.9	11.8	13.3	0.7	0.04	0.01	NS	0.01	0.03
BBS/Ca, kg/g	2.30	2.23	1.76	2.31	2.37	2.12	0.11	0.07	NS	NS	0.01	0.10
BBS/nPP, kg/g	5.11	3.96	5.04	5.13	4.22	6.05	0.27	0.07	0.01	NS	NS	0.06
Ash, %	57.81	57.18	56.39	58.21	57.47	56.96	0.45	NS	NS	NS	0.01	NS
ASH, mg	546	446	427	513	503	497	26	NS	0.04	0.09	0.01	0.05
ASH/Ca, mg/g	90	84	74	85	102	79	4	0.05	NS	0.01	0.01	NS
ASH/nPP, mg/g	199	149	211	189	181	226	9	NS	0.01	0.03	0.01	NS

[1] PC = corn-soybean meal diet (C-SBM) with 1.0% Ca and 0.45% nPP; 0.80Ca = C-SBM with 0.80% Ca and 0.45% nPP; 0.35nPP = C-SBM with 1.0% Ca and 0.35% nPP; ADG = average daily gain; ADFI = average daily feed intake; BBS = bone breaking strength; BBS/Ca = kg of BBS per g of total Ca intake; BBS/nPP = kg of BBS per g of total nPP intake; ASH, mg = mg of left tibia ash per bird; ASH/Ca = mg of ash per tibia per g of Ca intake; ASH/nPP = mg of ash per tibia per g of nPP intake.

[2] NS = not significant, $P > 0.10$.

[3] Data are means of six replicates of six broilers per replicate. Average initial and final body weight were 40 and 494 g and the experiment lasted from 0 to 18 d posthatching.

[4] Data are means of three tibias per replicate.

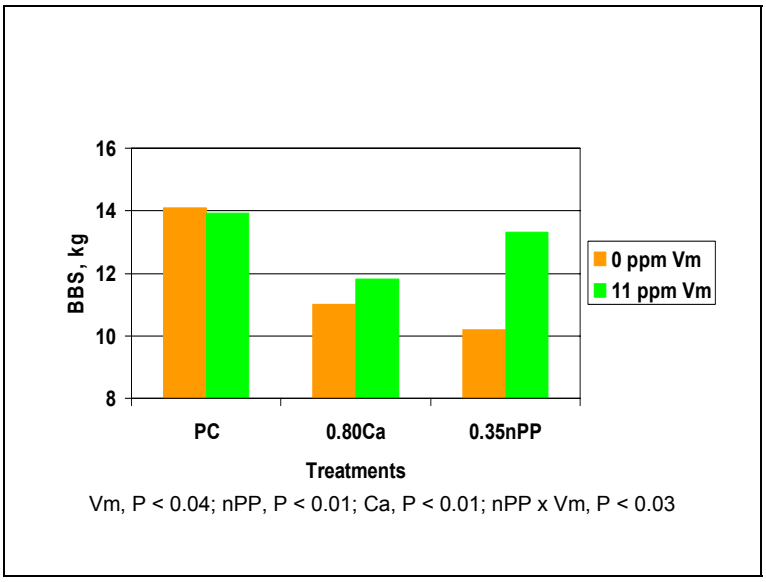

Figure 3.1. Bone breaking strength of broilers fed varying levels of virginiamycin, calcium, and nonphytate phosphorus, Experiment 1.

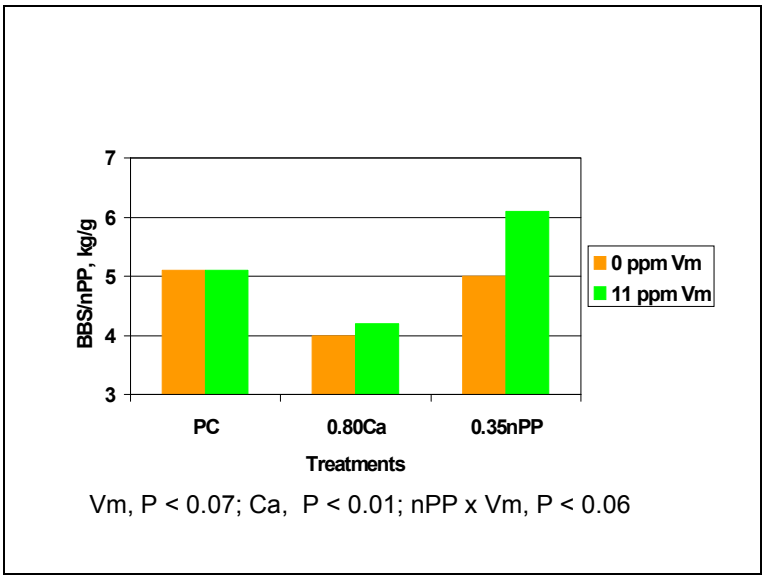

Figure 3.2. Bone breaking strength per nonphytate intake of broilers fed varying levels of virginiamycin, calcium, and nonphytate phosphorus, Experiment 1.

(*P* < 0.01 to 0.04) BBS/nPP and ASH/nPP. The addition of Vm increased (Vm, *P* < 0.02 to 0.07) BAP, ASH, ASH/Ca, and ASH/nPP, but there were no interactive effects of Vm and Ca or nPP. The lack of an interaction was because Vm had positive effects in the PC diet and in the reduced Ca and nPP diets.

3.3.3 Experiment 3

Reducing dietary Ca decreased ($P < 0.03$ to 0.06) G:F and BBS, while it increased ($P < 0.10$) ADFI (Table 3.4). Reducing dietary nPP decreased ($P < 0.01$) ADG, ADFI, and BBS. The addition of Vm increased ($P < 0.02$) BBS in birds fed the PC diet but decreased (nPP x Vm, $P < 0.06$) BBS in birds fed the 0.25nPP diet.

3.3.4 Experiment 4

Increasing dietary nPP increased (nPP linear, $P < 0.01$) ADG, ADFI, G:F, Ca and nPP intake, BBS, BBS/Ca, BAP, ASH, and ASH/Ca (Table 3.5). Increasing dietary nPP decreased ($P < 0.01$) BBS/nPP and ASH/nPP. The addition of Vm increased (Vm linear, $P < 0.03$ to 0.09) BBS, ASH, ASH/Ca, and ASH/nPP. The addition of 11 ppm Vm decreased (Vm quadratic, $P < 0.07$) BBS/Ca and BBS/nPP, but the 22 ppm addition increased these response variables (Vm quadratic, $P < 0.07$). The addition of Vm increased (nPP x Vm, $P < 0.05$) ADG and BBS in broilers fed diets containing 0.35% and 0.45% nPP but not in diets containing 0.15 or 0.25% nPP (Figure 3.3).

3.4 DISCUSSION

Increasing dietary Ca from 0.7% or 0.8% to 1.0% increased G:F and many bone response variables, but it did not affect ADG or ADFI. The lack of increase in ADG and ADFI as Ca levels were increased agrees with the findings of Applegate et al. (2003) who reported that increasing the level of Ca supplementation from 0.4 to 0.9 % had no effect or decreased ADG from 7 to 21 d or 14 to 24 d of age, respectively, but these data disagree with findings of other researchers who reported that ADG, ADFI, and G:F were increased as dietary Ca levels were increased (Onyango et al., 2003; Boling-Frankenbach et al., 2001).

Increasing dietary nPP increased all growth variables and many bone response variables. These results agree with the findings of other researchers. Onyango et al. (2003) reported that growth performance variables and tibia ash were increased linearly, and tibia bone mineral content and density were increased linearly and quadratically as dietary concentrations of P were increased.

The purpose of this research was to determine whether Vm increased the availability of Ca and nPP beyond its effect as a growth promotant. In EXP 1, reducing nPP to 0.35% decreased BBS

Table 3.3 Growth performance and bone response variables of broilers fed varying levels of virginiamycin, calcium, and nonphytate phosphorus, Experiment 2[1]

Virginiamycin, ppm	0			22				*P* = F[2]				
										Ca x		nPP x
Diet	PC	0.80Ca	0.35nPP	PC	0.80Ca	0.35nPP	PSEM	Vm	Ca	Vm	nPP	Vm
Growth performance[3]												
ADG, g	27.81	28.04	25.66	28.68	28.29	26.21	0.79	NS	NS	NS	0.01	NS
ADFI, g	35.20	35.88	32.92	36.23	35.83	33.78	1.09	NS	NS	NS	0.04	NS
G:F, g/g	0.791	0.782	0.78	0.792	0.789	0.782	0.007	NS	NS	NS	0.01	NS
Ca intake, mg/d	349	287	329	362	287	338	11	NS	0.01	NS	0.05	NS
nPP intake, mg/d	157	161	115	163	161	118	5	NS	NS	NS	0.01	NS
Bone response variables[4]												
BBS, kg	18.2	18.5	14.6	19.8	18.8	16.5	1.0	NS	NS	NS	0.01	NS
BBS/Ca, kg/g	2.91	3.59	2.47	3.02	3.62	2.75	0.14	NS	0.01	NS	0.01	NS
BBS/nPP, kg/g	6.46	6.38	7.05	6.72	6.43	7.86	0.30	NS	NS	NS	0.01	NS
Ash, %	55.49	55.3	53.9	55.71	55.2	54.97	0.26	0.07	NS	NS	0.01	NS
ASH, mg	539	515	426	605	546	492	27	0.02	NS	NS	0.01	NS
ASH/Ca, mg/g	86	100	72	93	106	80	4	0.04	0.02	NS	0.01	NS
ASH/nPP, mg/g	192	177	205	206	188	227	8	0.02	0.04	NS	0.04	NS

[1] PC = corn-soybean meal (C-SBM) diet with 1.0% Ca and 0.45% nPP; 0.80Ca = C-SBM with 0.80% Ca and 0.45% nPP; 0.35nPP = C-SBM with 1.0% Ca and 0.35% nPP; ADG = average daily gain; ADFI = average daily feed intake; BBS = bone breaking strength; BBS/Ca = kg of BBS per g of total Ca intake; BBS/nPP = kg of BBS per g of total nPP intake; ASH, mg = mg of left tibia ash per bird; ASH/Ca = mg of ash per tibia per g of Ca intake; ASH/nPP = mg of ash per tibia per g of nPP intake.

[2] NS = not significant, $P > 0.10$.

[3] Data are means of six replicates of six broilers per replicate. Average initial and final body weight were 47 and 524 g and the experiment lasted from 0 to 18 d posthatching.

[4] Data are means of six tibias per replicate.

Table 3.4 Growth performance and bone response variables of broilers fed varying levels of virginiamycin, calcium, and nonphytate phosphorus, Experiment 3[1]

Virginiamycin, ppm	0			9				*P* = F[2]				
										Ca x		nPP x
Diet	PC	0.70Ca	0.25nPP	PC	0.70Ca	0.25nPP	PSEM	Vm	Ca	Vm	nPP	Vm
Growth performance[3]												
ADG, g	24.16	24.67	21.02	24.34	24.96	20.41	0.62	NS	NS	NS	0.01	NS
ADFI, g	34.30	35.37	29.83	33.49	35.50	28.93	0.93	NS	0.10	NS	0.01	NS
G:F, g/g	0.704	0.698	0.706	0.727	0.703	0.708	0.008	NS	0.06	NS	NS	NS
Bone response variables[4]												
BBS, kg	12.7	10.4	9.0	14.3	13.8	8.1	0.7	0.02	0.03	NS	0.01	0.06

[1] PC = corn-soybean meal diet (C-SBM) with 1.0% Ca and 0.45% nPP; 0.70Ca = C-SBM with 0.70% Ca and 0.45% nPP; 0.25nPP = C-SBM with 1.0% Ca and 0.25% nPP; ADG = average daily gain; ADFI = average daily feed intake; BBS = bone breaking strength.

[2] NS = not significant, $P > 0.10$.

[3] Data are means of six replicates of six broilers per replicate. Average initial and final body weight were 45 and 462 g and the experiment lasted from 0 to 18 d posthatching.

[4] Data are means of three tibias per replicate.

Table 3.5 Growth performance and bone response variables of broilers fed varying levels of virginiamycin and nonphytate phosphorus, Experiment 4[1]

Virginiamycin, ppm	0				11				22				
nPP level	0.15	0.25	0.35	0.45	0.15	0.25	0.35	0.45	0.15	0.25	0.35	0.45	PSEM
Growth performance[2]													
ADG, g[7,8,9]	14.55	22.95	25.92	28.16	13.49	21.79	26.87	28.81	10.75	23.09	27.49	30.11	0.76
ADFI, g[7,8]	21.45	29.9	33.48	34.75	18.4	28.11	35.95	35.6	19.29	29.64	36.51	36.32	1.61
G:F, g/g[7]	0.678	0.768	0.775	0.813	0.729	0.775	0.755	0.81	0.615	0.779	0.762	0.831	0.025
Ca intake, mg/d[7,8]	215	299	335	347	184	281	359	356	193	296	365	363	16
nPP intake, mg/d[7]	32	75	117	156	28	70	126	160	29	74	128	163	5
Bone response variables[3]													
BBS, kg[5,7,9]	5.6	8.9	14.4	18.7	3.9	8.0	14.8	19.9	5.2	8.9	16.7	21.2	0.8
BBS/Ca, kg/g[6,7,8]	1.45	1.66	2.39	2.99	1.16	1.58	2.31	3.11	1.61	1.67	2.47	3.25	0.13
BBS/nPP, kg/g[6,7,8]	9.66	6.64	6.82	6.64	7.76	6.31	6.59	6.91	10.73	6.67	7.09	7.23	0.61
Ash, %[7,8]	42.76	49.70	53.81	55.38	41.70	48.37	53.15	57.86	43.26	48.46	53.67	56.54	0.91
ASH, mg[4,7,8]	241	311	401	549	224	284	431	665	259	323	463	620	25
ASH/Ca, mg/g[5,7,8]	64	58	66	88	69	56	67	105	80	61	71	95	6
ASH/nPP, mg/g[5,7,8]	425	231	190	195	457	224	192	233	536	242	203	211	32

[1] ADG = average daily gain; ADFI = average daily feed intake; BBS = bone breaking strength; BBS/Ca = kg of BBS per g of total Ca intake; BBS/nPP = kg of BBS per g of total nPP intake; ASH, mg = mg of left tibia ash per bird; ASH/Ca = mg of ash per tibia per g of Ca intake; ASH/nPP = mg of ash per tibia per g of nPP intake.

[2] Data are means of six replicates of six broilers per replicate. Average initial and final body weight were 36 and 432 g and the experiment lasted for 18 d.

[3] Data are means of six tibias per replicate.

[4] Vm linear ($P < 0.03$).

[5] Vm linear ($P < 0.09$).

[6] Vm quadratic ($P < 0.07$).

[7] nPP linear ($P < 0.01$).

[8] nPP quadratic ($P < 0.01$).

[9] nPP x Vm linear ($P < 0.05$).

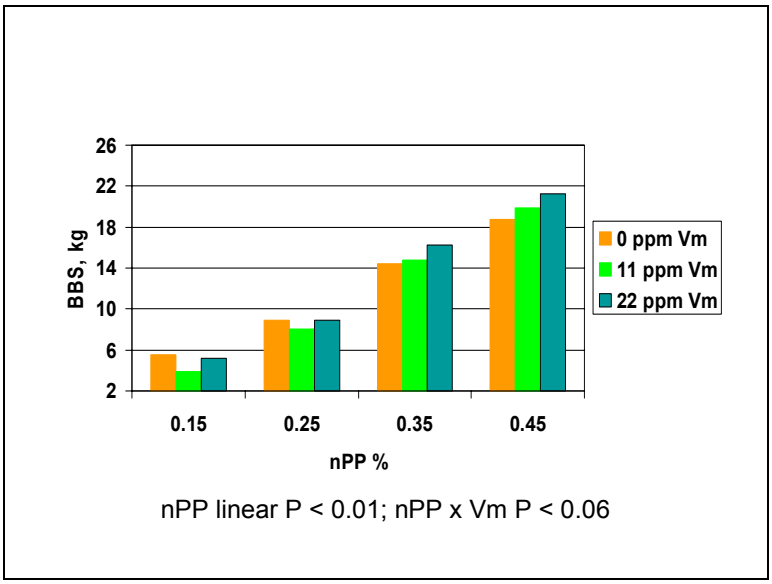

Figure 3.3. Bone breaking strength of broilers fed varying levels of virginiamycin and nonphytate phosphorus, Experiment 4

and ASH and the addition of Vm increased these response variables. However, Vm also increased feed intake and thus the increase in BBS and ASH could be due to an increase nPP intake and not an increased nPP utilization. However, BBS/nPP was also increased suggesting that Vm increases nPP utilization. The addition of Vm also increased ASH/Ca, but the effect was not significant. In EXP 2, reducing nPP to 0.35% decreased growth and all bone response variables, and the addition of Vm improved or tended to improve many of these response variables. However, Vm was just as effective in the PC diet as the diet deficient in nPP. In EXP 3, reducing nPP levels to 0.25% decreased ADG, ADFI, and BBS. The addition of Vm did not improve any of these response variables, and in fact, Vm decreased BBS in birds fed 0.25% nPP. In EXP 4, decreasing dietary nPP levels decreased ADG and BBS and there were significant Vm by nPP interactions. Virginiamycin decreased or did not affect ADG or BBS in birds fed 0.15 or 0.25% nPP, respectively, but Vm increased ADG and BBS in birds fed 0.35 or 0.45% nPP.

The results of this research suggest that Vm may not affect Ca utilization beyond its effects as a growth promotant. However, these results are inconclusive because we never had clear evidence of a Ca deficiency. In EXP 1 (0.80% Ca) and EXP 3 (0.70% Ca), BBS was decreased in

the low Ca diets. In EXP 1, the addition of Vm did not affect BBS in chicks fed the low Ca diet, and in EXP 3, BBS was increased by the addition of Vm in birds fed the low Ca diet and the PC diet.

Buresh et al. (1985b) reported that Vm decreased the amount of P needed to increase body weight. Agudelo et al. (2003) reported a 28% increase in P digestibility, an 11% increase in Ca digestibility, and an increase in absolute retention of P, Ca, Zn, and Mg in limit-fed pigs given Vm. Henry et al. (1986a) reported that the addition of Vm increased Mn utilization regardless of the level of Mn supplementation and reduced intestinal weight in broilers. The reduction of intestinal weight was attributed to a thinning of the intestinal wall.

There are several theories behind the nutrient sparing effects of Vm. One hypothesis for the nutrient sparing effects of Vm is that it acts on the mircoflora's biochemical processes in the cell (Parks et al., 2001). Virginiamycin inhibits bacterial protein synthesis by binding to the 50s ribosomal subunit and blocks normal peptide formation (Cocito et al., 1974; Parfait et al., 1978). This inhibition of protein synthesis gives Vm a broad-spectrum action on bacteria such as *Lactobacillus*, *Bifidobacterium*, and *Streptococcus*, which may be responsible for retarded growth in broilers (Cummings, 2003). Thus, by eliminating these harmful small intestine microflora, Vm may cause more nutrients to be available for the animal and not diverted to microflora.

The failure of Vm to increase the growth and bone response variables at dietary nPP levels below 0.35% was not an anticipated response. One possible explanation for the lack of response to Vm at nPP levels of 0.25% and below could be an imbalance of the Ca:nPP ratio. Hulan et al. (1985) reported that as the Ca:nPP ratio was increased from 2.53:1 to 3.38:1 in broiler diets, body weight was decreased. Larger Ca:tP ratios have been reported to decrease the effectiveness of phytase in broilers at nPP levels of 0.27% (Qian et al., 1997). The increase in the Ca:nPP ratio in EXP 3 and 4 from 2.22:1 in the PC to 6.67:1 in the 0.15nPP could have similar effects on the effectiveness of Vm. Buresh et al. (1985b) reported that Vm did not affect body weight below 0.47% P, while the addition of Vm to diets above 0.47% P resulted in an increase in body weight, feed intake, and P utilization, which is consistent with our findings.

The results of this research are inconclusive, especially with regard to Ca utilization, in that no clear deficiency of Ca was demonstrated. In one EXP but not another, Vm seemed to improve nPP utilization when dietary nPP level was 0.35%. Virginiamycin had no or negative effects when the level of nPP was at or below 0.25%. In another EXP, Vm improved many of the response variables in birds fed the 0.35% nPP diet, but it was equally effective at 0.45% nPP, which is at or above the requirement. The implications of this increased utilization are numerous, such as a potential reduction in the amount of Ca and nPP that needs to be supplemented to C-SBM diets and a reduced loss of these nutrients to the environment. Further research is needed to ascertain the mode of action of Vm and its potential to increase the utilization of Ca and nPP in C-SBM diets and to investigate the relationship between Ca:nPP ratio and Vm.

CHAPTER 4

THE EFFECTS OF ORGANIC SOURCES OF ZINC, MANGANESE, AND COPPER ON EGG PRODUCTION AND QUALITY IN LAYING HENS

4.1 INTRODUCTION

Approximately 8% of all losses in egg production are a direct result of low eggshell quality (Klecker et al., 2002). These losses have a huge impact on the poultry industry (Bell and Weaver, 2002). Eggshell breakage is directly related to the quality of the shell, which is affected by adequacy of nutrition, management practices, environmental conditions, and breeding (Emery et al., 1984; Solomon, 1991).

Additional trace mineral supplementation may improve eggshell quality and egg production. Fakler et al. (2002) reported that the production of marketable eggs and egg efficiency were improved in hens supplemented with organic Zn, and Khajaren et al. (2002) reported that organic Zn and Mn improved egg production, egg quality, and shell quality in hens 38 to 65 wk of age.

Leach and Gross (1983) reported that hens fed Mn-deficient diets produced eggs with thinner shells, translucent areas, and abnormalities in eggshell ultrastructure, particularly in the mammillary layer. Baumgartner et al. (1978) reported that hens fed a Cu-deficient diet produced eggs that had abnormal distribution of shell membrane fibers, egg shape deformation, and abnormal mechanical properties due to alterations in lysine-derived cross-links. The addition of Zn to saline drinking water has been reported to reduce the incidence of eggshell defects and improve shell breaking strength (Moreng et al., 1992; Balnave and Zhang, 1993).

Therefore, an experiment (EXP) was conducted to compare the effect of dietary supplementation of Zn, Mn, and Cu in the inorganic or organic forms (metal-amino acid complexes) on egg production and egg quality during the post-molt period in commercial layers.

4.2 MATERIALS AND METHODS

4.2.1 Experimental Hens and Diets

The Louisiana State University Agricultural Center Animal Care and Use Committee approved all methods pertaining to animal care. Nine hundred and sixty Hy-Line W-36 layers[5] at 63 wk of age were allotted to five treatments with 12 replications. Each replication consisted of four adjoining cages housing four birds per cage resulting in 16 layers per replication. The treatments consisted of: 1) Control (C) diet supplemented with trace minerals; 2) C + organic Zn, Mn, and Cu; 3) C + organic Zn; 4) C + inorganic Zn, Mn, and Cu; 5) C + inorganic Zn. Zinc, Mn, and Cu were added to provide 40, 40, or 7 ppm, respectively, in the organic (Availa Zn, Mn, or Cu)[6] or inorganic (sulfate) form. All diets were corn-soybean based and formulated to provide 2.85, 3.85, or 4.10% Ca; 0.79, 0.88, and 0.82% total lysine, and 3,101, 3,188, and 3,195 kcal/kg of ME for Molt 1, Molt 2 and peaking diet, respectively (Table 4.1). All diets were formulated to meet or exceed the nutrient requirements for laying hens (NRC, 1994).

The layers were placed in an environmentally controlled tunnel ventilated house with galvanized wire metal pens (52.1 cm x 34.3 cm x 30.5 cm) at the Louisiana State University Agricultural Center Poultry Farm. The house contained 640 cages with an under-floor flush system and was maintained at approximately 30^{o} C during the day and 18^{o} C during the night throughout the EXP. Each 24-h period consisted of 16 h of incandescent light and 8 h of darkness. The EXP lasted 32 wk and started after the birds had been molted once. The hens were fed phase 1 post-molt diet for 7 d, phase 2 post-molt diet for 18 d, and switched to peaking-formula diet for the remainder of the project.

4.2.2 Response Variables

Hen day production (HDP) was assessed daily and then divided into eight time periods of 28 d each. Hen day production is the total egg production for a period divided by the total hen days for that period multiplied by 100. The same method was used to calculate the percentage of dirty eggs

[5] Hy-Line International, West DesMoines, IA
[6] Zinpro Corp., Eden Prairie, Mn 55344

Table 4.1 Percentage composition of layer diets, as-fed basis

Ingredient	Molt 1[a]	Molt 2 [b]	Peaking [c]
Corn	70.74	60.74	62.40
Soybean meal (47.5% CP)	20.20	23.99	21.78
Limestone [d]	6.48	9.09	9.94
Monocalcium phosphate	1.88	1.88	1.42
Tallow	0.00	3.54	3.82
Salt	0.35	0.37	0.35
Mineral premix [e]	0.10	0.10	0.10
DL-methonine	0.06	0.11	0.08
Choline chloride	0.05	0.05	0.05
Vitamin premix [f]	0.05	0.05	0.05
Additive [g]	0.10	0.10	0.10
Calculated composition			
ME, kcal/kg	3,101	3,188	3,195
Calcium, %	2.85	3.85	4.10
Phosphorous, %	0.73	0.73	0.62
Nonphytate phosphorous, %	0.50	0.50	0.40
Chlorine, %	0.26	0.27	0.26
Potassium, %	0.68	0.73	0.69
Lysine, %	0.79	0.88	0.82
Tryptophan, %	0.17	0.19	0.18
Threonine, %	0.58	0.62	0.58
TSAA, %	0.60	0.66	0.61

[a] Molt 1 layer diet was fed for 1 wk.

[b] Molt 2 layer diet was fed for 18 d.

[c] Peaking layer diet was fed for the remainder of the project

[d] Limestone was provided as one half fine granula (< 0.32 cm) and one half course (0.32 cm to 0.64 cm).

[e] Provided per kilogram of diet: copper (copper sulfate•5 H_2O), 10 mg; iodine (potassium iodate), 1.0 mg; iron (ferrous sulfate•7 H_2O), 50 mg; manganese (manganese sulfate•H_2O), 60 mg; selenium (as sodium selenite), 0.15 mg; and zinc (zinc sulfate•7H_2O), 60 mg.

[f] Provided per kilogram of diet: vitamin A (retinyl acetate), 8,000 IU; vitamin D_3 (cholecalciferol), 3,000 IU; vitamin E (DL-alpha-tocopheryl acetate), 25 IU; menadione, 1.5 mg; vitamin B_{12} (cyanocobalamin), 0.02 mg; biotin, 0.1 mg; folacin (folic acid), 1 mg; niacin (nicotinic acid), 50 mg; pantothenic acid, 15 mg; pyridoxine (pyridoxine•HCl), 4 mg; riboflavin, 10 mg; and thiamin (thiamin mononitrate), 3 mg.

[g] Supplemental trace minerals in the from of organic or inorganic Zn, Mn, and Cu to provide 40 ppm Zn and Mn, and 7 ppm Cu for Diets 2 and 4, organic or inorganic Zn to provide 40 ppm Zn for Diets 3 and 5, respectively.

(eggs with any adhering feces or blood), cracked eggs (eggs that were cracked or checked with or without intact membranes), and shell-less eggs (eggs with soft outer shell). Daily feed intake (ADFI) was calculated every 28 d and fresh diets were provided every 14 d. Specific gravity (SG) was determined every 28 d on eight randomly selected eggs per replicate by the method of Holder and Bradford (1979). The eggs were submerged in graded levels of salt concentrations ranging from 1.060 to 1.085 using increments of 0.005, eggs that did not float at 1.085 where assumed to have a SG of 1.090. Egg weight (EW) was obtained every 28 d on the eggs used for SG. Egg mass (EM) was calculated by dividing HDP by 100 then multiplying by EW. Feed conversion was calculated by dividing EM by ADFI.

4.2.3 Statistical Analysis

All the data for each period were analyzed with the GLM procedure of SAS[7] appropriate for a completely randomized design. The overall data were analyzed using the MIXED procedure of SAS appropriate for repeated measures. Treatment differences were considered significant at $P < 0.10$.

4.3 RESULTS

The addition of trace minerals, regardless of source, had variable effects on egg production and quality measures when individual periods were analyzed (Appendices A and B). However, there was no effect of dietary treatment on any response variable when the overall data were analyzed (Table 4.2).

4.4 DISCUSSION

The trace minerals Zn, Mn, and Cu all have an integral role in eggshell formation (Mabe et al., 2003). Thus, we conducted this EXP to determine if additional Zn, Mn, and Cu supplementation in the inorganic and organic form would affect hen production responses.

Organic sources of these trace minerals have been reported to have greater bioavailability than inorganic trace minerals because they prevent phytate from binding to the metals in the gastrointestinal tract while still being water soluble; thus, facilitating mineral uptake in the small intestine (Kidd et al., 1996; Cao et al., 2000).

[7]SAS Inst. Inc., Cary, NC.

Table 4.2 Overall egg production responses [1]

Treatment	HDP [2,a]	Dirty [3,a]	Cracked [3,a]	Shell-less[3,a]	SG [4,a]	EW [4,a]	ADFI[a]	EM [5,a]	Feed conversion [6,a]
	%	%	%	%		g	g	g	g/g
Control (C)	76.70	4.61	1.01	0.25	1.080	64.64	98.83	49.71	0.51
C + Org Zn, Mn, Cu	76.97	3.77	1.03	0.18	1.079	64.61	98.17	49.80	0.51
C + Org Zn	76.93	3.91	0.88	0.16	1.080	64.18	97.21	49.48	0.52
C + Inorg Zn, Mn, Cu	77.44	3.79	1.11	0.18	1.081	65.15	99.21	50.54	0.51
C + Inorg Zn	77.30	3.99	0.92	0.18	1.080	64.77	97.99	50.15	0.52
PSEM	0.88	0.41	0.16	0.06	0.001	0.40	1.13	0.58	0.01

[1]Data are means of 12 replications of 16 hens (four cages of four hens each) per replication for the entire 224 d period. HDP = hen day production, SG = specific gravity, EW = egg weight, ADFI = average daily feed intake, EM = egg mass.

[2] Total eggs produced divided by total hen days multiplied by 100.

[3] Data calculated by dividing total eggs collected intact, dirty, cracked, or shell-less by total hen days.

[4] Means of eight eggs per replicate.

[5] EM = Hen day production divided by 100 multiplied by egg weight.

[6] Feed conversion = egg mass divided by feed intake.

[a] Significant period effect, $P < 0.01$.

The addition of trace minerals, regardless of source, had no effect on HDP during any period. These findings do not agree with those of Klecker et al. (2002) who reported an increase in egg production for layers fed diets supplemented with Mn and Zn chelates. Sahin et al. (2002) also reported that layers fed supplemental Cr and Zn, either individually or together, had an increased egg production or improved feed efficiency. Lim and Paik (2003) reported that the addition of organic Cu, Zn, and Mn had variable effects on egg production and egg quality. The addition of organic Cu increased egg production, while the addition of organic Zn decreased egg production. They further reported that the combination of organic Zn and Mn tended to decrease egg production, while the combination of organic Zn, Mn, and Cu did not affect egg production. Lim and Paik (2003) also reported that shell-less and broken egg production were highest in the organic Zn, Mn, and Cu combination, but lowest in hens fed the diet with organic Cu. They suggested that the antagonistic relationship between Cu and Zn could be the reason for the decrease in eggshell quality seen in hens fed the diet with organic Zn, Mn, and Cu. These findings are similar to those of Lima et al. (2000) who reported that the addition of Zn and Mn in the organic or inorganic form did not improve egg production. Kita et al. (1997) also reported that the addition of organic Zn to diets of layers subjected to high ambient temperature did not affect egg production, eggshell quality, or egg weight. Mabe et al. (2003) reported that the addition on Zn, Mn, and Cu did not affect eggshell percentage in older hens (60 to 73 wk) regardless of source, while inorganic sources of Zn, Mn, and Cu slightly decreased eggshell percentage in a younger group of hens (32 to 45 wk). Klecker et al. (2002) reported an increase in laying hen performance, eggshell quality, eggshell strength, and eggshell weight when 20% and 40% of supplemental inorganic Mn and Zn were replaced with the organic forms.

Balnave and Zhang (1993) reported that the addition of Zn to saline drinking water reduced the incidence of eggshell deformities. This response is consistent with the role Zn plays in eggshell formation. Zinc is a component of the carbonic anhydrase enzyme, which supplies carbonate ions during eggshell formation; inhibition of this enzyme results in an increase in the production of shell-less eggs (Nys et al., 1999; Innocenti et al., 2004).

The addition of trace minerals in the organic and inorganic forms had variable effects on egg production and egg quality measures. Other than the decrease in the percentage dirty and shell-less eggs early in the EXP, there were no consistent effects of mineral or source on any other response criteria. This lack of response is evident in the overall data, where no significant effects were observed. This lack of results may be due to the fact that our trace mineral mix provided adequate trace minerals, and that supplementation with additional trace minerals is not warranted. This theory is consistent with the findings of Dale and Strong (1998) who reported that additional Mn and Zn supplementation above the NRC requirement for post-molt hens had no effect on eggshell quality. Another possible reason why the results of this EXP differ from the results of other researchers is the length of this study which, lasted 32 wk, compared with the 13 wk for Mabe et al. (2003) and 8 wk for Lim and Paik (2003). The beneficial effects of additional trace mineral supplementation were not evident after the early stages of the EXP. The age of the hens may also have contributed to the results obtained in this study. Shell quality has been shown to decrease with age of the flock. According to Ensminger et al. (1990), egg size increases as the hen ages, but it is impossible to increase shell production and deposition, which results in thinner shells. The efficiency of Ca mobilization from the bone also decreases with age and this leads to a decrease in the production of calcium carbonate for shell production resulting in a decrease in SG and egg quality as the hen ages (Butcher and Miles, 2002). More long-term studies need to be conducted on the effects of trace mineral supplementation on egg production and eggshell quality of older hens that have been previously molted before any recommendations can be made.

CHAPTER 5

SUMMARY AND CONCLUSIONS

The purpose of the first research project was to determine whether Vm increased the availability of Ca and nPP beyond its effect as a growth promotant in diets formulated to be deficient in Ca and nPP. Decreasing dietary Ca to 0.70 or 0.80% from 1.0% failed to decrease growth in the first three EXP, it did however decrease BBS in EXP 3. These results agree with the role Ca plays in bone formation. Decreasing nPP decreased most growth and bone responses in all four EXP.

The addition of Vm to the marginally deficient Ca diets overcame some of the negative responses associated with feeding these diets. The addition of Vm to diets containing 0.35% nPP and above increased growth and bone response variables. The addition of Vm to diets with less than 0.35% nPP decreased some growth and bone response variables, which was not an anticipated response.

The results, although inclusive, seem to point to an improved utilization of nPP and, to a lesser extent, Ca in broilers fed diets supplemented with Vm. The implications of this increased utilization are numerous, such as a potential reduction in the amount of Ca and nPP that needs to be supplemented to corn-soybean meal (C-SBM) diets and a reduced loss of these nutrients to the environment. Further research is needed to ascertain the mode of action of Vm and its potential to increase the utilization of Ca and nPP in C-SBM diets and to investigate the relationship between Ca:nPP ratio and Vm.

The purpose of the second research project was to compare the effect of dietary supplementation of Zn, Mn, and Cu in the inorganic or organic forms on egg production and egg quality during the post-molt period in commercial layers.

The addition of trace minerals in the organic and inorganic forms had variable effects on egg production and egg quality measures with few consistent responses. There were no consistent effects of mineral or source on any other response criteria other than the decrease in the percentage dirty and shell-less eggs early in the EXP. This lack of response is evident in the overall data, where no significant effects were observed. This lack of results may be due to the fact that our trace

mineral mix provided adequate trace minerals, and that supplementation with additional trace minerals is not warranted. Additional research is needed to investigate the effects of trace mineral supplementation in the organic and inorganic forms on egg production and egg quality in post-molt hens with diets not adequate in the trace minerals being investigated.

REFERENCES

Agudelo, J. H., M. D. Lindemann, G. L. Cromwell, and R. D. Nimmo. 2003. Virginiamycin influences mineral digestibility in pigs. J. Anim. Sci. 81(Suppl. 2):82. (Abstr.)

Aoyagi, S., and D. H. Baker. 1993a. Bioavailability of copper in analytical-grade and feed-grade inorganic copper sources when fed to provide copper at levels below the chick's requirement. Poult. Sci. 72:1075-1083.

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

Applegate, T. J., R. Angel, and H. L. Classen. 2003. Effect of dietary calcium, 25-hydroxycholecalciferol, or bird strain on small intestinal phytase activity in broiler chickens. Poult. Sci. 82:1140-1148.

Baker, D. H., and K. M. Halpin. 1987. Research note: Efficacy of a manganese-protein chelate compared with that of manganese sulfate for chicks. Poult. Sci. 66:1561-1563.

Baker, D. H., J. Odle, M. A. Funk, and T. M. Wieland. 1991. Research note: Bioavailability of copper in cupric oxide, cuprous oxide, and in a copper-lysine complex. Poult. Sci. 70:177-179.

Balnave, D., and D. Zhang. 1993. Research note: Responses of laying hens on saline drinking water to dietary supplementation with various zinc compounds. Poult. Sci. 72:603-606.

Batal, A. B., T. M. Parr, and D. H. Baker. 2001. Zinc bioavailability in tetrabasic zinc chloride and the dietary zinc requirement of young chicks fed a soy concentrate diet. Poult. Sci. 80:87-90.

Baumgartner, S., D. J. Brown, E. Salevsky, Jr, and R. M. Leach, Jr. 1978. Copper deficiency in the laying hen. J. Nutr. 108:804-811.

Belay, T., and R. G. Teeter. 1996. Virginiamycin and caloric density effects on live performance, blood serum metabolite concentration, and carcass composition of broilers reared in thermoneutral and cycling ambient temperatures. Poult. Sci. 75:1383-1392.

Bell, D. D., and W. D. Weaver. 2002. Commercial Chicken Meat and Egg Production. 5th ed. Kluwer Academic Publishers. NY.

Black, J. R., C. B. Ammerman, P. R. Henry, and R. D. Miles. 1984. Biological availability of manganese sources and effects of high dietary manganese on tissue mineral composition of broiler-type chicks. Poult. Sci. 63:1999-2006.

Boling-Frankenbach, S. D., J. L. Snow, C. M. Parsons, and D. H. Baker. 2001. The effect of citric acid on the calcium and phosphorus requirements of chicks fed corn-soybean meal diets. Poult. Sci. 80:783–788.

Broz, J., P. Oldale, A. - H. Perrin-Voltz, G. Rychen, J. Schulze, and C. S. Nunes. 1994. Effects of supplemental phytase on performance and phosphorus utilisation in broiler chickens fed a low phosphorus diet without addition of inorganic phosphates. Br. Poult. Sci. 35:273-280.

Buresh, R. E., R. D. Miles, and R. H. Harms. 1985a. Influence of virginiamycin on energy utilization when turkey poults were fed ad libitum or restricted. Poult. Sci. 64:1041-1042.

Buresh, R. E, R. D. Miles, and R. H. Harms. 1985b. Influence of virginiamycin on phosphorus utilization by broiler chicks. Poult. Sci. 64:757-758.

Buresh, R. E., R. H. Harms, and R. D. Miles. 1986. A differential response in turkey poults to various antibiotics in diets designed to be deficient or adequate in certain essential nutrients. Poult. Sci. 65:2314–2317.

Butcher, G. D., and R. Miles. 2002. Subject: Concepts of eggshell quality. http://edis.ifas.ufl.edu/VM013. Accessed Sept. 2002.

Carlson, M., and T. J. Fangman. 2004. Subject: Swine antibiotics and feed additives: food safety considerations. http://muextension.missouri.edu/ xplor/agguides/ansci/g02353.htm. Accessed Apr. 2004.

Cao, J., P. R. Henry, R. Guo, R. A. Holwerda, J. P. Toth, R. C. Littell, R. D. Miles, and C. B. Ammerman. 2000. Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. J. Anim. Sci. 78:2039-2054.

Cervantes, H., K. Bafundo, P. Ewing, G. Pesti, and R. Bakalli. 2002. Dietary supplementation with virginiamycin or phytase improves phosphorus utilization in broiler chickens. Poult. Sci. 81 (Suppl. 1):150. (Abstr.)

Chowdhury, S. D. 1990. Shell membrane system in relation to lathyrogen toxicity and copper deficiency. World's Poult. Sci. J. 46:153–169.

Cocito, C., H. O. Voorma, and L. Bosch. 1974. Interference of virginiamycin M with the initiation and the elongation of peptide chains in cell-free systems. Biochem. Biophys. Acta. 340:285-298.

Cromwell, G. L., T. S. Stahly, and H. J. Monegue. 1989. Effects of source and level of copper on performance and liver copper stores in weanling pigs. J. Anim. Sci. 67: 2996-3002.

Cummings, T. S. 2003. Subject: Understanding competitive exclusion and the rationale for using growth promotant antibiotics in microflora management. http://www.cvm.msstate.edu/Poultry/Documents/Microflora%20Management.pdf. Accessed Jan. 2003.

Dale, N., and C. F. Strong, Jr. 1998. Inability to demonstrate an effect of eggshell # 49 on shell quality in older laying hens. J. Appl. Poult. Res. 7:219-224.

de Faria, D. E., O. M. Junqueira, N. K. Sakomura, and A. E. Santana. 1999. Effect of different levels of manganese and phosphorus on the performance and eggshell quality of laying hens. Rev. Bras. Zoot. 28:105-112.

Emery, D. A., P. Vohra, R. A. Ernst, and S. R. Morrison. 1984. The effect of cyclic and constant ambient temperatures on feed consumption, egg production, egg weight, and shell thickness of hens. Poult. Sci. 63:2027-2035.

Ensminger, M. E., J. M. Oldfield, and W. W. Heinemann. 1990. Feed and Nutrition. 2nd ed. Ensminger Pub. Co., Clovis, CA.

Fakler, T., T. L. Ward, and H. J. Kuhl. 2002. Zinc amino acid complexes (Availa Zn®) improve layer production and egg quality. Poult. Sci. 81(Suppl. 1):120. (Abstr.)

Feighner, S. D., and M. P. Dashkevicz. 1987. Subtherapeutic levels of antibiotics in poultry feeds and their effects on weight gain, feed efficiency, and bacterial cholyltaurine hydrolase activity. Appl. Environ. Microbiol. 53:331-336.

Frost, T. J., D. A. Roland, Sr, and G. G. Untawale. 1990. Influence of vitamin D3, 1 alpha-hydroxyvitamin D3, and 1,25-dihydroxyvitamin D3 on eggshell quality, tibia strength, and various production parameters in commercial laying hens. Poult. Sci. 69:2008-2016.

Frost, T. J., D. A. Roland, Sr, and D. N. Marple. 1991. The effects of various dietary phosphorus levels on the circadian patterns of plasma 1,25-dihydroxycholecalciferol, total calcium, ionized calcium, and phosphorus in laying hens. Poult. Sci. 70:1564-1570.

Gautron J., M. T. Hincke, M. Panheleux, J. M. Garcia-Ruiz, T. Boldicke, and Y. Nys. 2001. Ovotransferrin is a matrix protein of the hen eggshell membranes and basal calcified layer. Connect. Tissue Res. 42:255-267.

Guo, R., P. R. Henry, R. A. Holwerda, J. Cao, R. C. Littell, R. D. Miles, and C. B. Ammerman. 2001. Chemical characteristics and relative bioavailability of supplemental organic copper sources for poultry. J. Anim. Sci. 79:1132-1141.

Hansen, J.L., P. B. Moore, and T. A. Steitz. 2003. Structures of five antibiotics bound at the peptidyl transferase center of the large ribosomal subunit. J. Mol. Biol. 330:1061-1075.

Harland, B. F., and D. Oberleas. 1999. Phytic acid complex in feed ingredients. Pages 69-75 in Phytase in Animal Nutrition and Waste Management: A BASF Reference. BASF Corp., Mount Olive, NJ.

Harms, R. H., N. Ruiz, and R. D. Miles. 1986. Influence of virginiamycin on broilers fed four levels of energy. Poult. Sci. 65:1984-1986.

Henry, P. R., C. B. Ammerman, and R. D. Miles. 1986a. Influence of virginiamycin and dietary manganese on performance, manganese utilization, and intestinal tract weight of broilers. Poult. Sci. 65:321-324.

Henry, P. R., C. B. Ammerman, and R. D. Miles. 1986b. Bioavailability of manganese sulfate and manganese monoxide in chicks as measured by tissue uptake of manganese from conventional dietary levels. Poult. Sci. 65:983-986.

Henry, P. R., C. B. Ammerman, and R. D. Miles. 1989. Relative bioavailability in a manganese-methionine complex for broiler chicks. Poult. Sci. 68:107-112.

Holder, D. P., and M. V. Bradford. 1979. Relationship of specific gravity of chicken eggs to number of cracked eggs observed and percent shell. Poult. Sci. 58:250–251.

Hossain, S.M., and A. G. Bertechini. 1998. Effects of varying levels of magnesium and available phosphorus on performance of layers. Anim. Feed Sci. Technol. 71:363-368.

Hulan, H. W., G. De Groote, G. Fontaine, G. Demunter, K. B. McRae, and F. G. Proudfoot. 1985. The effect of different totals and ratios of dietary calcium and phosphorus on the performance and incidence of leg abnormalities of male and female broiler chickens. Poult. Sci. 64:1157-1169.

Hurwitz, S. 1987. Effect of nutrition on egg quality. Pages 235-254 in: Egg Quality-Current Problems and Recent Advances. R. G. Wells and C. G. Belyavin, Butterworths, London, UK.

Hurwitz, S., E. Wax, Y. Nisenbaum, M. Ben-Moshe, and I. Plavnik. 1998. The response of laying hens to induced molt as affected by strain and age. Poult. Sci. 77:22-31.

Innocenti, A., S. Zimmerman, J. G. Ferry, A. Scozzafava, and C. T. Supuran. 2004. Carbonic anhydrase inhibitors. Inhibition of the zinc and cobalt gamma-class enzyme from the archaeon Methanosarcina thermophila with anions. Bioorg. Med. Chem. Lett. 14:3327-3331.

Ives, S. E., E. C. Titgemeyer, T. G. Nagaraja, A. del Barrio, D. J. Bindel, and L. C. Hollis. 2002. Effects of virginiamycin and monensin plus tylosin on ruminal protein metabolism in steers fed corn-based finishing diets with or without wet corn gluten feed. J. Anim. Sci. 80:3005-3015.

Jang, D. A., J. G. Fadel, K. C. Klasing, A. J. Mireles, Jr., R. A. Ernst, K. A. Young, A. Cook, and V. Raboy. 2003. Evaluation of low-phytate corn and barley on broiler chick performance. Poult. Sci. 82:1914-1924.

Johnston, S. L., and L. L. Southern. 2000. The effect of varying mix uniformity (simulated) of phytase on growth performance, mineral retention, and bone mineralization in chicks. Poult. Sci. 79:1485-1490.

Khajarern, S., C. J. Rapp, T. L. Ward, A. B. Johnson, and T. Fakler. 2002. Effect of zinc and manganese amino acid complexes (Availa® Z/M) on layer production and egg quality. Poult. Sci. 81(Suppl. 1):120. (Abstr.)

Kidd, M. T., P. R. Ferket, and M. A. Qureshi. 1996. Zinc metabolism with special reference to its role in immunity. World's Poult. Sci. J. 52:309-324.

Kita, K., I. Hohmura, and J. Okumura. 1997. Influence of dietary zinc methionine supplementation on eggshell quality in laying hens under hot climate environment. Jpn. Poult. Sci. 34:21-26.

Klecker, D., L. Zeman, P. Jelinek, and A. Bunesova. 2002. Effect of manganese and zinc chelates on the quality of eggs. Acta Univ. Agric. et Silvic. Mendel. Brun. 50:59-68.

Lavelin, I., N. Meiri, and M. Pines. 2000. New insight in eggshell formation. Poult. Sci. 79:1014-1017.

Leach, R. M. , Jr, and J. R. Gross. 1983. The effect of manganese deficiency upon the ultrastructure of the eggshell. Poult. Sci. 62:499-504.

Leach, R. M., Jr. 1976. Metabolism and function of manganese. Pages 235–247 in Trace Elements in Human Health and Disease. Vol. II, A. S. Prasad, ed., Academic Pr, New York.

Ledoux, D. R., P. R. Henry, C. B. Ammerman, P. V. Rao, and R. D. Miles. 1991. Estimation of the relative bioavailability of inorganic copper sources for chicks using tissue uptake of copper. J. Anim. Sci. 69:215-222.

Lee, K. 1984. Feed restriction during the growing period, forced molt, and egg production. Poult. Sci. 63:1895-1897.

Lee, C., M. Minami, S. Sakuda, T. Nihira, and Y. Yamada. 1996. Stereospecific reduction of virginiamycin m_1 as the virginiamycin resistance pathway in streptomyces virginiae. Antimicrob. Agents Chemother. 40:595-601.

Lim, H. S., and K. Paik. 2003. Effects of supplementary mineral methionine chelates (Zn, Cu, Mn) on the performance and eggshell quality of laying hens. Asian-Australas. J. Anim. Sci.12:1804-1808.

Lima, F. R., I. Mabe, and R. Albuquerque. 2000. Zinc and manganese additional supplementation may benefit egg production and quality. Poult. Sci. 79(Suppl.1):46. (Abstr.)

Liu, A. C., B. S. Heinrichs, and R. M. Leach, Jr. 1994. Influence of manganese deficiency on the characteristics of proteoglycans of avian epiphyseal growth plate cartilage. Poult. Sci. 73:663-669.

Mabe, I., C. Rapp, M. M. Bain, and Y. Nys. 2003. Supplementation of a corn-soybean meal diet with manganese, copper, and zinc from organic or inorganic sources improves eggshell quality in aged laying hens. Poult. Sci. 82:1903-1913.

Miles, R. D., D. M. Janky, and R. H. Harms.1984. Virginiamycin and broiler performance. Poult. Sci. 63:1218-1221.

Mohanna, C., and Y. Nys. 1999. Effect of dietary zinc content and sources on the growth, body zinc deposition and retention, zinc excretion and immune response in chickens. Br. Poult. Sci. 40:108-114.

Moore, P. A., Jr., T. C. Daniel, and D. R. Edwards. 1999. Reducing phosphorus runoff and improving poultry production with alum. Poult. Sci. 78:692-698.

Moreng, R. E., D. Balnave, and D. Zhang. 1992. Dietary zinc methionine effect on eggshell quality of hens drinking saline water. Poult. Sci. 71:1163-1167.

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

Nys, Y., M. T. Hincke, J. L. Arias, J. M. Garica-Ruiz, and S. E. Solomon. 1999. Avian eggshell mineralization. Poult. Avian Biol. Rev. 10:143-166.

Okubo, T., S. Akachi, and H. Hatta. 1997. Structure of hen eggs and physiology of egg laying. Pages 1-24 in: Hen Eggs-Their Basic and Applied Science. T. Yamamoto, L. R. Juneja, H. Hatta, and M. Kim, CRC press, Boca Raton, Florida, USA.

Onyango, E. M., P. Y. Hester, R. Stroshine, and O. Adeola. 2003. Bone densitometry as an indicator of percentage tibia ash in broiler chicks fed varying dietary calcium and phosphorus levels. Poult. Sci. 82:1787–1791.

Paik, I. 2001. Application of chelated minerals in animal production. Asian-Australas. J. Anim. Sci. 14:191-198.

Parfait, R., M. P. de Bethume, and C. Cocito. 1978. A spectrofluorimetric study of the interaction between virginiamycin s and bacterial ribosomes. Mol. Gen. Genet. 166:45–51.

Parks, C. W., J. L. Grimes, P. R. Ferket, and A. S. Fairchild. 2001. The effect of mannanoligosaccharides, bambermycins, and virginiamycin on performance of large white male market turkeys. Poult. Sci. 80:718–723.

Pelura, J., III, J. L. Krider, T. R. Cline, C. Reisert, and L. B. Underwood. 1980. Virginiamycin, protein and lysine responses of young swine. J. Anim. Sci. 50:767-772.

Pimentel, J. L., M. E. Cook, and J. L. Greger. 1991. Research note: Bioavailability of zinc-methionine for chicks. Poult. Sci. 70:1637-1639.

Proudfoot, F. G., E. D. Jackson, H. W. Hulan, and C.D.C. Salisbury. 1990. The response of male chicken broilers to the dietary addition of virginiamycin. Poult. Sci. 69:1713-1717.

Qian, H., E. T. Kornegay, and D. M. Denbrow. 1997. Utilization of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol, and the calcium:total phosphorus ratio in broiler diets. Poult. Sci. 76:37-46.

Ravindran, V., E. T. Kornegay, and K. E. Webb, Jr. 1984. Effects of fiber and virginiamycin on nutrient absorption, nutrient retention and rate of passage in growing swine. J. Anim. Sci. 59:400-408.

Rao S. K., D. A. Roland, Sr, and J. I. Orban. 1991. Influence of dietary cholecalciferol, calcium, and phosphorus on urinary calcium in commercial leghorn hens. Poult. Sci. 70:1921-1927.

Sahin, N., M. Onderci, and K. Sahin. 2002. Effects of dietary chromium and zinc on egg production, egg quality, and some blood metabolites of laying hens reared under low ambient temperature. Biol. Trace Elem. Res. 85:47-58.

Sandoval, M., P. R. Henry, C. B. Ammerman, R. D. Miles, and R. C. Littell. 1997. Relative bioavailability of supplemental inorganic zinc sources for chicks. J. Anim. Sci. 75:3195–3205.

Singh, M., R. K. Srivastava, S. S. Chauhan, and K. S. Singh. 2000. Responses of virginiamycin and bactracin methylene disalicylate on the weight gains and nutrient utilization of broiler chicken. Indian J. Poult. Sci. 35:272-275.

Smith, M. O., I. L. Sherman, L. C. Miller, and K. R. Robbins. 1995. Relative biological availability of manganese from manganese proteinate, manganese sulfate, and manganese monoxide in broilers reared at elevated temperatures. Poult. Sci. 74:702-707.

Solomon, S. E. 1991. Egg and Eggshell Quality. Wolfe Publishing LTD, London England.

Steel, R.G.D., and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd ed. McGraw-Hill Book Company, New York.

Tullett, S. G. 1987. Egg shell formation and quality. Pages 123-146 in: Egg Quality-Current Problems and Recent Advances. R. G. Wells and C. G. Belyavin, Butterworths, London, UK.

Underwood, E. J., and N. F. Suttle. 1999. The Mineral Nutrition of Livestock. 3rd ed. CAB publishing, New York, NY.

Wedekind, K. J., A. E. Hortin, and D. H. Baker. 1992. Methodology for assessing zinc bioavailability: efficacy estimates for zinc-methionine, zinc sulfate, and zinc oxide. J. Anim. Sci. 70:178-187.

Wedekind, K. J., and D. H. Baker. 1990. Zinc bioavailability in feed-grade sources of zinc. J. Anim. Sci. 68:684-689.

Wong-Valle, J., C. B. Ammerman, P. R. Henry, P. V. Rao, and R. D. Miles. 1989. Bioavailability of manganese from feed grade manganese oxides for broiler chicks. Poult. Sci. 68:1368-1373.

Woodward, S. A., R. H. Harms, R. D. Miles, D. M. Janky, and N. Ruiz. 1988. Research note: Influence of virginiamycin on yield of broilers fed four levels of energy. Poult. Sci. 67:1222-1224.

Yan, F., J. H. Kersey, and P. W. Waldroup. 2001. Phosphorus requirements of broiler chicks three to six weeks of age as influenced by phytase supplementation. Poult. Sci. 80:455-459.

APPENDIX A

EGG PRODUCTION RESPONSES FOR PERIODS 1 THROUGH 8

Table A.1 Egg production responses for Periods 1 and 2[1]

Treatment	HDP [2] %	Dirty [3] %	Cracked [3] %	Shell-less[3] %	SG [4]	EW [4] g	ADFI g	EM [5] g	Feed conversion [6] g/g
Period 1									
Control (C)	45.83	6.52[a]	0.56[a]	0.17	1.082	60.81	92.78[a]	27.83	0.30
C + Org Zn, Mn, Cu	46.98	4.19[b]	0.63[a]	0.14	1.082	62.27	93.74[a]	29.27	0.31
C + Org Zn	46.29	4.97[b,c]	0.26[b]	0.06	1.082	60.92	96.66[b]	28.21	0.29
C + Inorg Zn, Mn, Cu	46.60	4.57[b,c]	0.45[a,b]	0.06	1.081	62.41	94.64[a]	29.09	0.31
C + Inorg Zn	47.31	5.58[a,c]	0.42[a,b]	0.05	1.082	61.66	93.12[a]	29.12	0.31
PSEM	1.51	0.54	0.12	0.06	0.001	1.08	0.84	1.01	0.01
Period 2									
Control (C)	82.85	7.66[a]	0.59	0.12[a]	1.084	63.65	105.78	52.73	0.50
C + Org Zn, Mn, Cu	83.39	5.95[b]	0.50	0.07[a,b]	1.084	62.93	103.58	52.48	0.51
C + Org Zn	84.71	5.88[b]	0.57	0.07[a,b]	1.083	62.61	103.89	53.04	0.51
C + Inorg Zn, Mn, Cu	84.13	5.73[b]	0.62	0.02b[b]	1.083	63.05	104.20	53.04	0.51
C + Inorg Zn	83.89	6.19[a,b]	0.45	0.02[b]	1.084	62.71	103.88	52.61	0.51
PSEM	0.82	0.67	0.14	0.04	0.001	0.45	1.10	0.65	0.01

[1]Data are means of 12 replications of 16 hens (four cages of four hens each) per replication for a period of 28 d. HDP = hen day production, SG = specific gravity, EW = egg weight, ADFI = average daily feed intake, EM = egg mass.

[2] Total eggs produced divided by total hen days multiplied by 100.

[3] Data calculated by dividing total eggs collected intact, dirty, cracked, or shell-less by total hen days.

[4] Means of eight eggs per replicate.

[5] EM = Hen day production divided by 100 multiplied by egg weight.

[6] Feed conversion = egg mass divided by feed intake.

[a,b,c] Means with different superscripts differ, $P < 0.10$.

Table A.2 Egg production responses for Periods 3 and 4[1]

Treatment	HDP [2]	Dirty [3]	Cracked [3]	Shell-less[3]	SG [4]	EW [4]	ADFI	EM [5]	Feed conversion [6]
	%	%	%	%		g	g	g	g/g
Period 3									
Control (C)	84.94	5.81	0.88	0.12[a,c]	1.081	63.70	105.37	54.10	0.52
C + Org Zn, Mn, Cu	85.96	4.53	0.95	0.04[a,b]	1.081	63.88	105.36	54.91	0.52
C + Org Zn	86.10	4.75	0.83	0.04[a,b]	1.081	64.10	108.02	55.19	0.51
C + Inorg Zn, Mn, Cu	86.68	4.84	0.98	0.00[a]	1.081	64.49	108.13	55.90	0.52
C + Inorg Zn	86.39	4.90	0.82	0.17[c]	1.082	63.40	106.30	54.76	0.52
PSEM	0.95	0.59	0.21	0.04	0.001	0.51	1.29	0.71	0.01
Period 4									
Control (C)	83.92	4.57	0.70	0.10	1.079	65.66[a]	105.07	55.10	0.52
C + Org Zn, Mn, Cu	84.15	4.01	1.20	0.11	1.080	65.43[a,b]	103.75	55.06	0.53
C + Org Zn	83.73	3.88	1.10	0.13	1.080	64.23[b]	104.26	53.79	0.52
C + Inorg Zn, Mn, Cu	84.82	4.66	0.83	0.07	1.080	65.21[a,b]	104.17	55.30	0.53
C + Inorg Zn	84.36	4.54	0.96	0.27	1.080	64.55[a,b]	104.22	54.44	0.52
PSEM	0.95	0.57	0.22	0.08	0.001	0.53	1.24	0.76	0.01

[1]Data are means of 12 replications of 16 hens (four cages of four hens each) per replication for a period of 28 d. HDP = hen day production, SG = specific gravity, EW = egg weight, ADFI = average daily feed intake, EM = egg mass.

[2] Total eggs produced divided by total hen days multiplied by 100.

[3] Data calculated by dividing total eggs collected intact, dirty, cracked, or shell-less by total hen days.

[4] Means of eight eggs per replicate.

[5] EM = Hen day production divided by 100 multiplied by egg weight.

[6] Feed conversion = egg mass divided by feed intake.

[a,b,c] Means with different superscripts differ, $P < 0.10$.

Table A.3 Egg production responses for Periods 5 and 6[1]

Treatment	HDP [2]	Dirty [3]	Cracked [3]	Shell-less[3]	SG [4]	EW [4]	ADFI	EM [5]	Feed conversion [6]
	%	%	%	%		g	g	g	g/g
Period 5									
Control (C)	81.14	2.69	1.04	0.27	1.079	66.14[a,b]	97.14	53.62	0.55[a,b]
C + Org Zn, Mn, Cu	80.45	2.56	1.01	0.12	1.079	65.79[a]	96.03	52.93	0.55[a,b]
C + Org Zn	80.89	2.82	0.77	0.24	1.079	65.30[a]	97.40	52.81	0.54[b]
C + Inorg Zn, Mn, Cu	82.44	2.41	1.16	0.11	1.078	66.39[a,b]	96.71	54.72	0.57[b]
C + Inorg Zn	81.24	2.67	0.95	0.18	1.079	67.28[b]	97.52	54.65	0.56[a,b]
PSEM	1.09	0.37	0.26	0.07	0.001	0.51	1.33	0.74	0.01
Period 6									
Control (C)	80.17	3.04	1.04	0.31	1.077	65.65[a,b]	103.21	52.59	0.51
C + Org Zn, Mn, Cu	80.27	2.52	1.24	0.15	1.076	65.34[a]	102.19	52.44	0.51
C + Org Zn	79.56	2.50	1.01	0.18	1.077	64.81[a]	101.86	51.51	0.51
C + Inorg Zn, Mn, Cu	81.27	2.87	1.39	0.30	1.088	65.90[a,b]	102.97	53.54	0.52
C + Inorg Zn	80.69	2.46	1.30	0.19	1.077	66.79[b]	102.77	53.88	0.52
PSEM	1.35	0.42	0.29	0.10	0.005	0.51	1.13	0.83	0.01

[1]Data are means of 12 replications of 16 hens (four cages of four hens each) per replication for a period of 28 d. HDP = hen day production, SG = specific gravity, EW = egg weight, ADFI = average daily feed intake, EM = egg mass.

[2] Total eggs produced divided by total hen days multiplied by 100.

[3] Data calculated by dividing total eggs collected intact, dirty, cracked, or shell-less by total hen days.

[4] Means of eight eggs per replicate.

[5] EM = Hen day production divided by 100 multiplied by egg weight.

[6] Feed conversion = egg mass divided by feed intake.

[a,b,c] Means with different superscripts differ, $P < 0.10$.

Table A.4 Egg production responses for Period 7 and 8[1]

Treatment	HDP [2]	Dirty [3]	Cracked [3]	Shell-less[3]	SG [4]	EW [4]	ADFI	EM [5]	Feed conversion [6]
	%	%	%	%		g	g	g	g/g
Period 7									
Control (C)	77.65	2.90	1.35	0.35	1.077	65.69[a,c]	99.64[a]	51.00	0.51
C + Org Zn, Mn, Cu	78.73	3.30	1.19	0.32	1.078	65.27[a]	98.58[a,c]	51.35	0.52
C + Org Zn	77.80	3.34	1.46	0.18	1.078	65.96[a,c]	96.42[c]	51.33	0.53
C + Inorg Zn, Mn, Cu	78.56	2.69	1.88	0.37	1.078	66.96[c]	99.17[a,c]	52.63	0.53
C + Inorg Zn	78.10	2.69	1.34	0.22	1.077	66.12[a,c]	99.16[a,c]	51.65	0.52
PSEM	1.30	0.51	0.34	0.11	0.001	0.55	1.23	0.95	0.01
Period 8									
Control (C)	77.09	3.66	1.93	0.58	1.076	65.79	81.69[a]	50.71	0.64[a]
C + Org Zn, Mn, Cu	75.80	3.08	1.51	0.51	1.074	65.97	82.13[a]	49.98	0.62[a]
C + Org Zn	76.33	3.15	1.08	0.35	1.075	65.52	69.12[b]	49.93	0.75[b]
C + Inorg Zn, Mn, Cu	75.00	2.58	1.59	0.46	1.075	66.82	83.68[a]	50.12	0.62[a]
C + Inorg Zn	76.36	2.90	1.09	0.34	1.076	65.68	76.96[a,b]	50.13	0.68[a,b]
PSEM	1.58	0.50	0.28	0.22	0.001	0.57	4.12	1.02	0.04

[1]Data are means of 12 replications of 16 hens (four cages of four hens each) per replication for a period of 28 d. HDP = hen day production, SG = specific gravity, EW = egg weight, ADFI = average daily feed intake, EM = egg mass.

[2] Total eggs produced divided by total hen days multiplied by 100.

[3] Data calculated by dividing total eggs collected intact, dirty, cracked, or shell-less by total hen days.

[4] Means of eight eggs per replicate.

[5] EM = Hen day production divided by 100 multiplied by egg weight.

[6] Feed conversion = egg mass divided by feed intake.

[a,b,c] Means with different superscripts differ, $P < 0.10$.

APPENDIX B

EFFECT OF TRACE MINERAL SUPPLEMENTATION ON RESPONSE VARIABLES

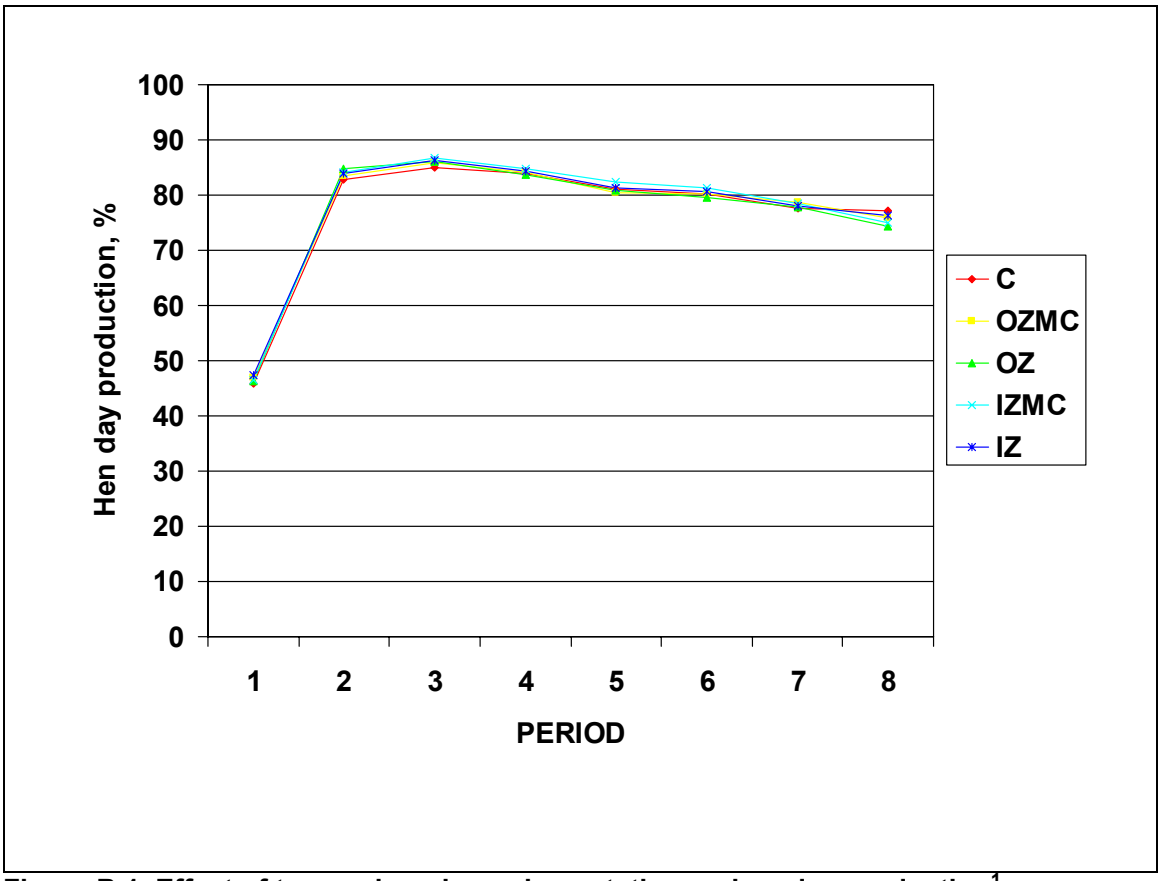

Figure B.1. Effect of trace mineral supplementation on hen day production[1]

[1]Data are means of 12 replications of 16 hens (four cages of four hens each) per replication. C = corn soybean meal (C-SBM) control; OZMC = C-SBM with organic Zn, Mn, and Cu; OZ = C-SBM with organic Zn; IZMC = C-SBM with inorganic Zn, Mn, and Cu; OZ = C-SBM with inorganic Zn.

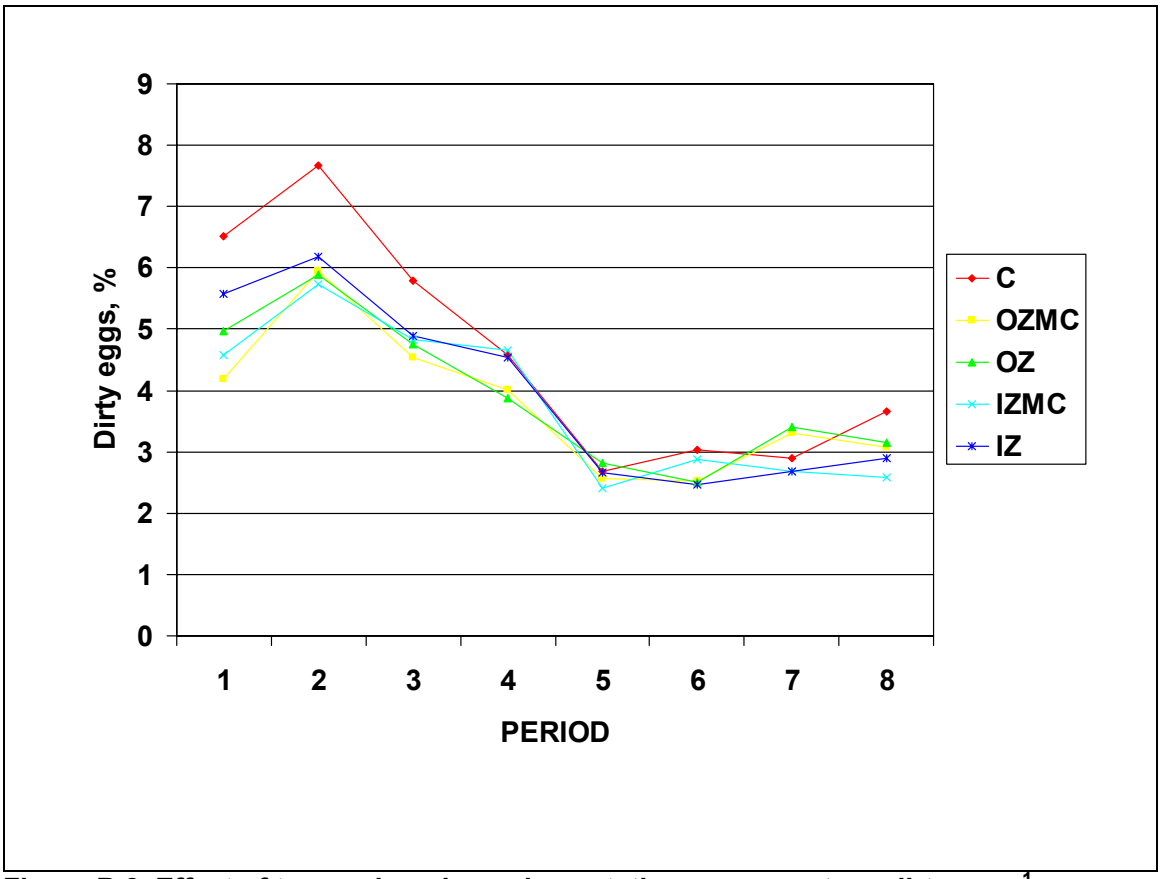

Figure B.2. Effect of trace mineral supplementation on percentage dirty eggs[1]

[1]Data are means of 12 replications of 16 hens (four cages of four hens each) per replication. C = corn soybean meal (C-SBM) control; OZMC = C-SBM with organic Zn, Mn, and Cu; OZ = C-SBM with organic Zn; IZMC = C-SBM with inorganic Zn, Mn, and Cu; OZ = C-SBM with inorganic Zn.

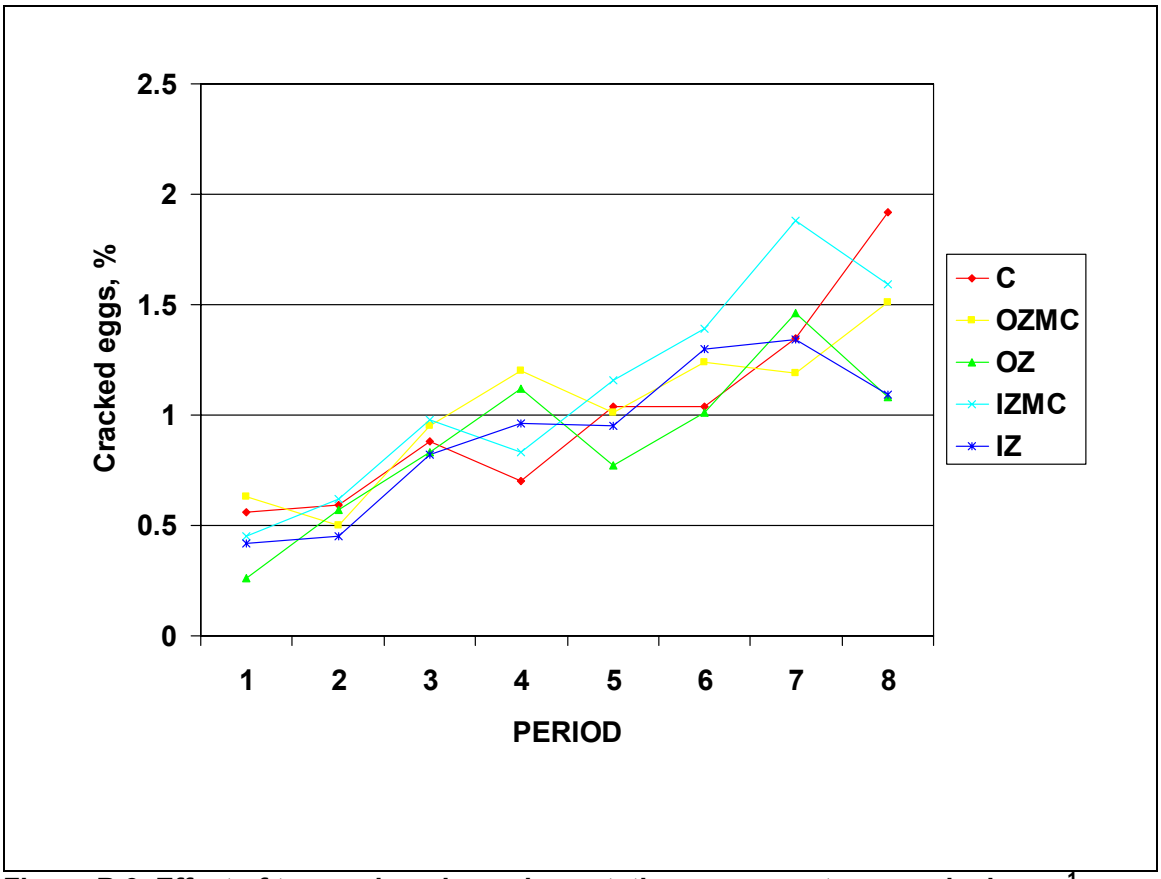

Figure B.3. Effect of trace mineral supplementation on percentage cracked eggs[1]

[1]Data are means of 12 replications of 16 hens (four cages of four hens each) per replication. C = corn soybean meal (C-SBM) control; OZMC = C-SBM with organic Zn, Mn, and Cu; OZ = C-SBM with organic Zn; IZMC = C-SBM with inorganic Zn, Mn, and Cu; OZ = C-SBM with inorganic Zn.

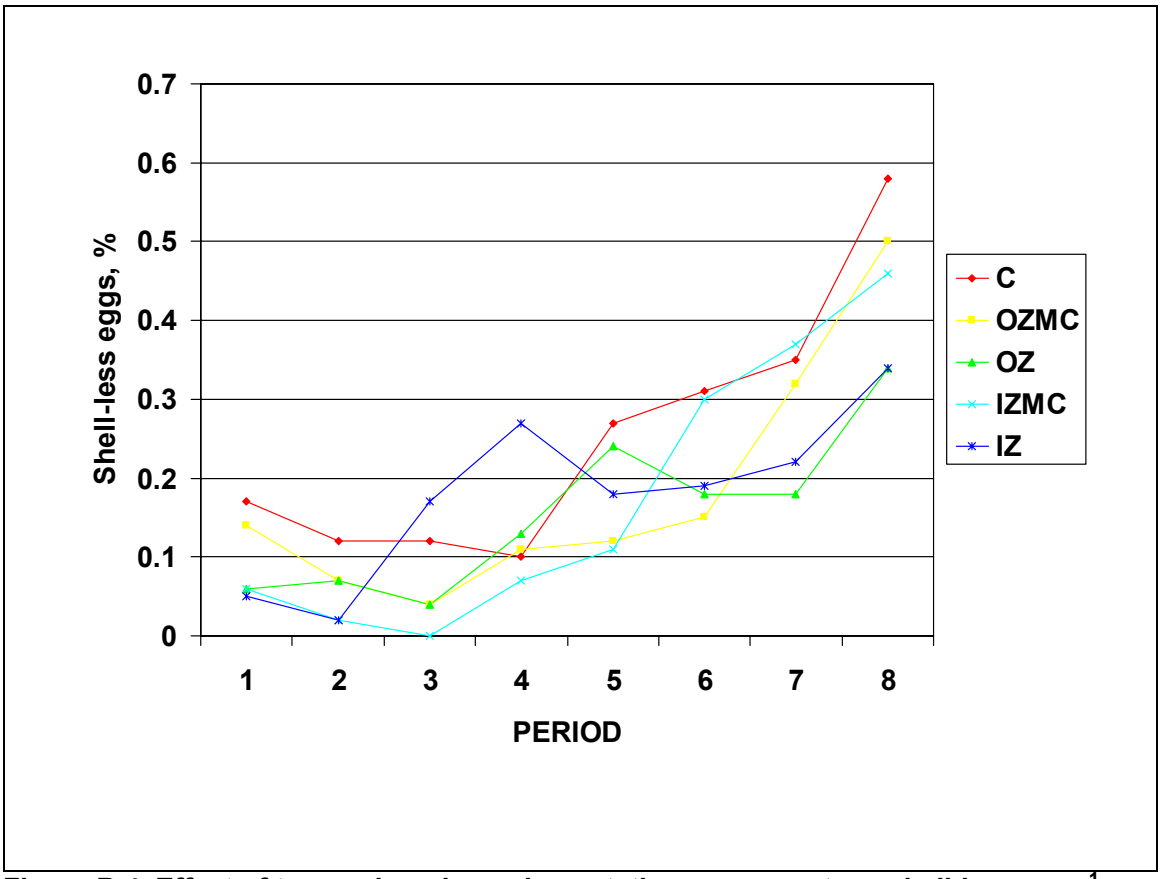

Figure B.4. Effect of trace mineral supplementation on percentage shell-less eggs[1]

[1]Data are means of 12 replications of 16 hens (four cages of four hens each) per replication. C = corn soybean meal (C-SBM) control; OZMC = C-SBM with organic Zn, Mn, and Cu; OZ = C-SBM with organic Zn; IZMC = C-SBM with inorganic Zn, Mn, and Cu; OZ = C-SBM with inorganic Zn.

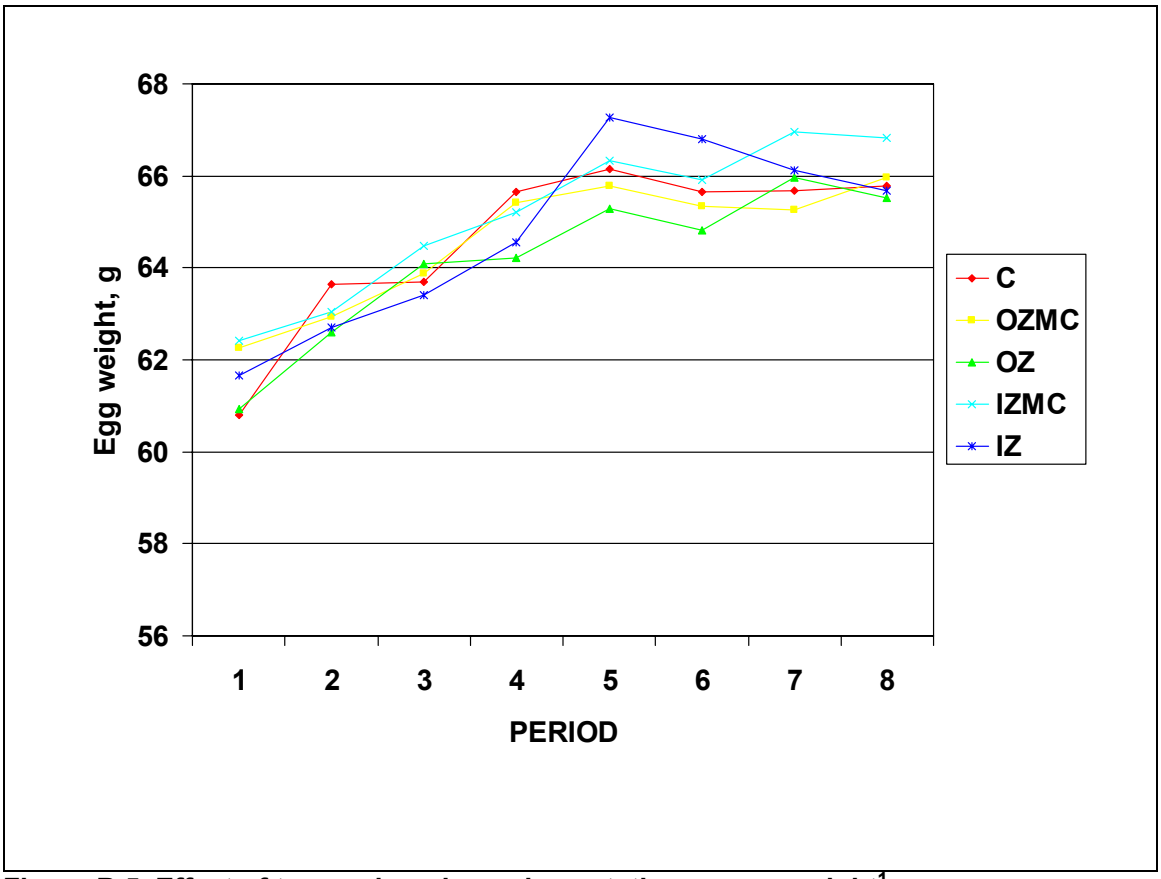

Figure B.5. Effect of trace mineral supplementation on egg weight[1]

[1]Data are means of 12 replications of 16 hens (four cages of four hens each) per replication. C = corn soybean meal (C-SBM) control; OZMC = C-SBM with organic Zn, Mn, and Cu; OZ = C-SBM with organic Zn; IZMC = C-SBM with inorganic Zn, Mn, and Cu; OZ = C-SBM with inorganic Zn.

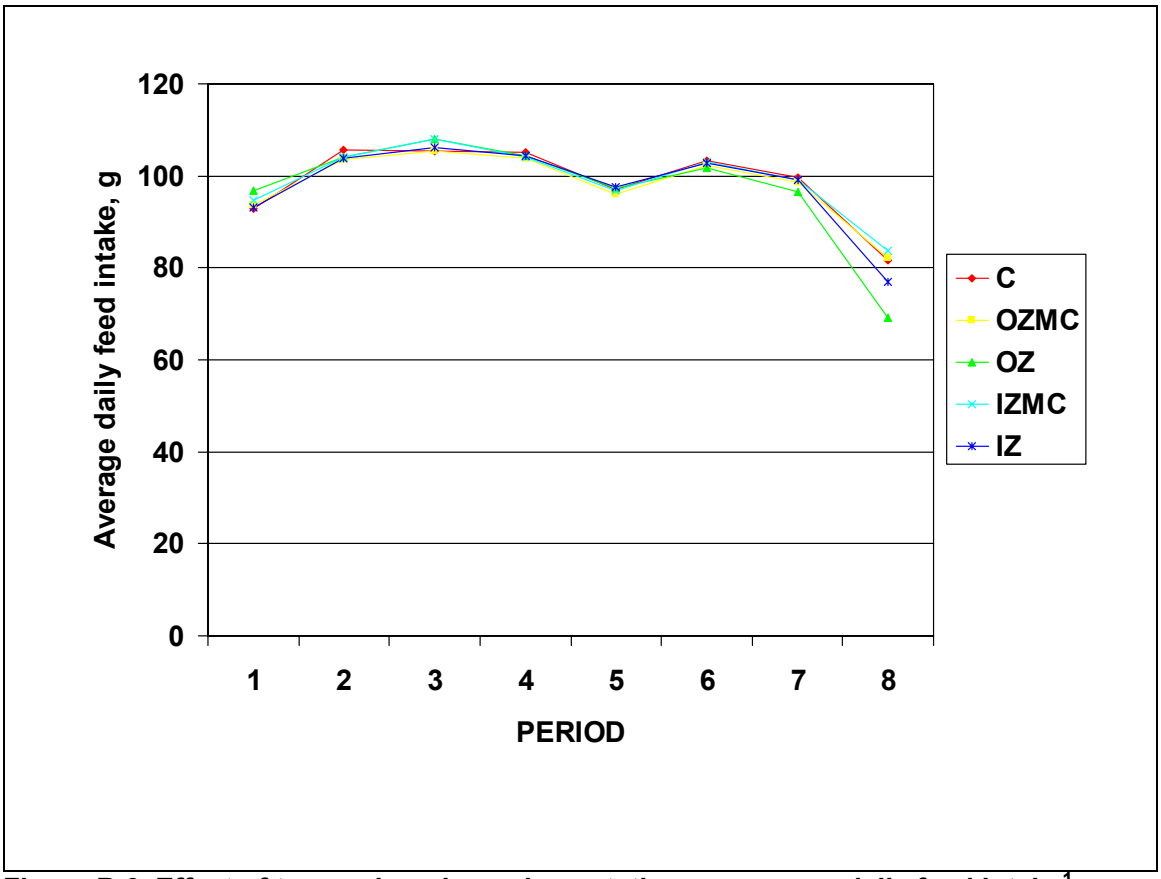

Figure B.6. Effect of trace mineral supplementation on average daily feed intake[1]

[1]Data are means of 12 replications of 16 hens (four cages of four hens each) per replication. C = corn soybean meal (C-SBM) control; OZMC = C-SBM with organic Zn, Mn, and Cu; OZ = C-SBM with organic Zn; IZMC = C-SBM with inorganic Zn, Mn, and Cu; OZ = C-SBM with inorganic Zn.

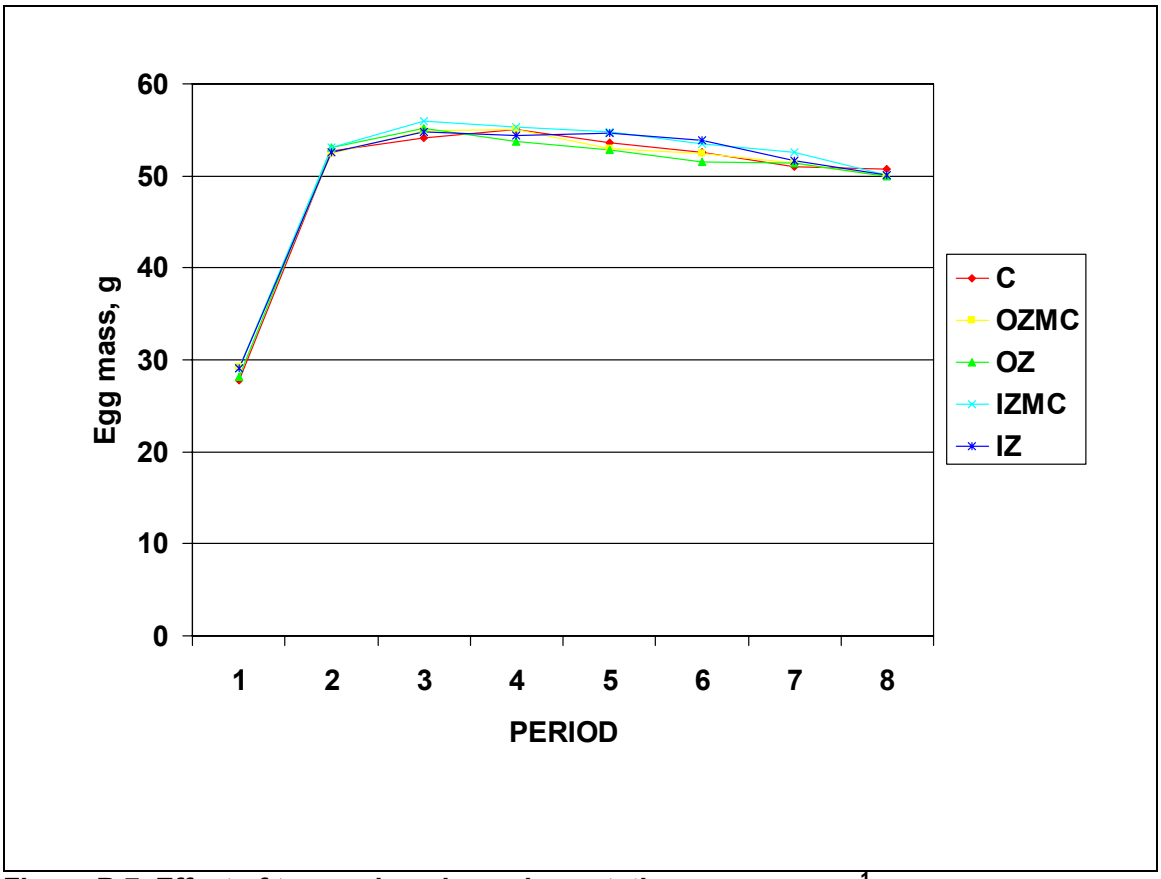

Figure B.7. Effect of trace mineral supplementation on egg mass[1]

[1]Data are means of 12 replications of 16 hens (four cages of four hens each) per replication. C = corn soybean meal (C-SBM) control; OZMC = C-SBM with organic Zn, Mn, and Cu; OZ = C-SBM with organic Zn; IZMC = C-SBM with inorganic Zn, Mn, and Cu; OZ = C-SBM with inorganic Zn.

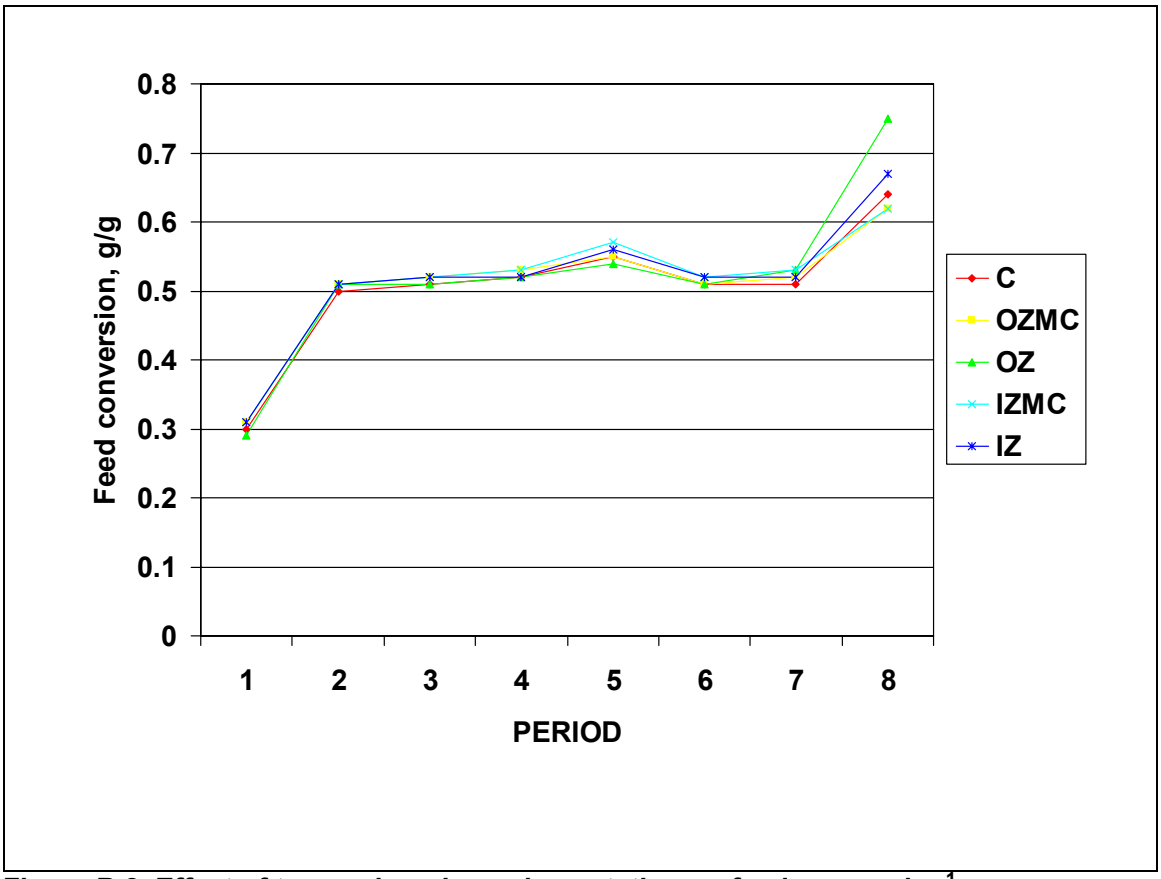

Figure B.8. Effect of trace mineral supplementation on feed conversion[1]

[1]Data are means of 12 replications of 16 hens (four cages of four hens each) per replication. C = corn soybean meal (C-SBM) control; OZMC = C-SBM with organic Zn, Mn, and Cu; OZ = C-SBM with organic Zn; IZMC = C-SBM with inorganic Zn, Mn, and Cu; OZ = C-SBM with inorganic Zn.

VITA

Tanika O'Connor-Dennie was born on October 12, 1979 in Kingston, Jamaica, to Mr. Hopeton O'Connor-Dennie and Mrs. Willa I. Dennie. She graduated from Wolmer's Girls' High School in the spring of 1997 and attended the College of Agriculture, Science, and Education where she obtained her Associate of Science degree in agricultural education and a diploma in education. She then transferred to Louisiana State University in the spring of 2001. In the spring of 2002, she received her Bachelor of Science degree from Louisiana State University in Animal-Dairy-Poultry Science. Tanika was accepted into Louisiana State University Graduate School and began her graduate studies in non-ruminant nutrition in the summer of 2002 under Dr. L. Lee Southern.

Tanika started her graduate career investigating the effects of organic mineral supplementation on egg production in laying hens that had previously gone through first molt. The project lasted eight months and involved egg collection every morning seven days a week and conducting egg specific gravity measures every four weeks. In the summer of 2003, Tanika started investigating the nutrient sparing effects of virginiamycin, looking specifically at nonphytate phosphorous and calcium utilization. Tanika presently is a candidate for the degree of Master of Science for the summer of 2004.