Effects of Source and Level of Trace Minerals on Performance, Mineral Excretion, Intestine and Bone Development, and Immune Response in Commercial Turkeys

by

Lihong Li

Dissertation submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Animal and Poultry Sciences

Approved by:

Dr. A. P. McElroy, Chairperson

Dr. C. Novak, Co-chairperson

Dr. R. A. Dalloul

Dr. E. J. Smith

Dr. J. Pierce

Dr. F. W. Pierson

January 23, 2009

Blacksburg, Virginia

Keywords: trace minerals, turkeys, coccidia, performance, excreta, immune response

Effects of Source and Level of Trace Minerals on Performance, Mineral Excretion, Intestine and Bone Development, and Immune Response in Commercial Turkeys

Lihong Li

(ABSTRACT)

To compare the effect of a standard commercial trace mineral dietary program to low levels of organic minerals on turkey performance, mineral excretion, bone strength, and carcass yield, day-old Hybrid poults (n=1,224) were randomly distributed to one of four treatments with 9 replicates. Experimental treatments consisted of: standard inorganic (SI) with a commercial supplementation program (Mn, Zn, Cu, Se), reduced inorganic (RI) with 10% level of SI, and two organic regimens of Bioplex®/Sel-Plex® (at the same level of RI during period 1 and 2 and at 2/3 of RI for period 3, 4, 5, and 6, or at the same level of RI for entire trial). Body weight (BW), body weight gain (BWG), feed conversion ratio (FCR), and feed intake (FI) were evaluated and fresh excreta were collected at d 28, 49, 70, 84, 105 and 133. Tibias and femurs were collected at d 49, 84 and 133. Trace mineral concentration in litter and carcass yield were determined at d 133. Overall, there was no significant effect on BW, cumulative BWG, FCR, or FI due to treatments ($P < 0.05$). The contents of Mn and Zn in excreta and litter were significantly reduced ($P < 0.05$) in Bioplex®/Sel-Plex® or RI diet compared to SI during the study. Cu excretion was significantly reduced at d 84 and 133. Tibias from the SI treatment had increased bone strength at d 49. Carcass yield at processing was significantly improved ($P < 0.05$) by feeding Bioplex®/Sel-Plex® treatments compared to the SI diet.

To investigate the effect of organic or inorganic Zn combined with other trace minerals on turkey performance, immune response, and intestinal development, a 2 by 4 factorial design was utilized with coccidia vaccinated and non-vaccinated and 4 dietary

treatments varying in level and source of Zn with Mn, Cu, and Se. A total of 2,376 day-old Hybrid turkeys were assigned to one of the combinations with 9 replicates of each. Dietary treatments consisted of: 1) standard inorganic (SI), Zn (150 ppm) with Mn (165 ppm), Cu (10 ppm), and Se (0.2 ppm); 2) reduced inorganic (RI), Zn, Mn, and Cu at 10% of SI, and Se at 0.2ppm; 3) organic 1 (O1), at the same level of RI; 4) organic 2 (O2), Zn (30 ppm) with the same level of Mn, Cu, and Se as O1. Body weight, BWG, FI and FCR were determined weekly. Bursa, thymus, and spleen were weighed, and duodenum and jejunum were collected at d 7, 14, 28, and 42. Peripheral blood was collected for T-lymphocyte populations on d 21, 28, and 42. Cumulative FI was influenced by vaccination (P=0.003). Cumulative BWG and BW were significantly decreased by vaccination except on d 14. Cumulative BWG increased in poults fed RI compared with those fed O2 (P=0.03). Poults fed O2 had significantly decreased BW when compared with RI after d 28. Cumulative FCR was not affected by diet and vaccination. Vaccination increased spleen weight on d 7 and thymus weight on d 42 ($P < 0.05$). The birds fed O2 had increased thymus weight when compared with those fed SI at d 7 ($P < 0.05$). The vaccinated poults had higher numbers of CD4+ T-cells than non-vaccinated birds on d 28 and d 42 ($P < 0.05$), and an interaction between diet and vaccination was observed ($P < 0.05$). Compared to non-vaccinated poults, CD4+/CD8+ ratio was significantly increased in vaccinated poults on d 42 ($P = 0.0475$). The villus height in vaccinated birds was significantly increased in the jejunum ($P = 0.0012$), but diets did not affect intestinal morphology. In summary, using low levels of organic or inorganic trace minerals is adequate to maintain turkey performance and immune response and decreased trace minerals excretion.

Acknowledgements

I would like to thank my husband Xiaobo Guo for his support during my Ph. D degree. I also give my deepest appreciation to my parents in China. They provided tremendous physical and spiritual support to me in the past four years.

I especially would like to thank my committee members, Dr. Audrey McElroy, Dr. Curtis Novak, Dr. Rami Dalloul, Dr. Edward Smith, Dr. James Pierce, and Dr. Bill Pierson for their encouragement and guidance. They have put a lot of time and effort into the design and execution of this project. I would like to give a special thanks to Dr. Novak and Dr. McElroy. They have given me much support and instruction regarding my experimental design and assisted with the wonderful revision of my dissertation.

Barbara Self has provided me with a lot of assistance during my research. She also gave me great suggestions to solve problems, from research to language and culture. Christa Honaker and Mary Berger provided me with help in sample collection and preparation of samples for histology. I would like to thank Curt Porterfield and Dale Shumate at the Turkey Research farm for their hard work and kindness. My labmates, Adriana Barri, Carrie Walk, Jennifer Sottosanti, and Robert VanWyhe, Catalina Troche, Xiaolun Sun, Tina Herfel, Amy Parsons, were of great help on sampling days and with lab work. They made sampling an enjoyable process with the humor and music. I would also like to thank Dr. Robert Gogal, Melissa Makris, and Richard Kerr for their help with lymphocyte isolation and flow cytometry.

I am grateful to the executers of the John Lee Pratt Fellowship for providing me with the opportunity to study animal nutrition and pursue my Ph. D degree at Virginia Tech. I also want to thank Alltech for their financial support, specifically Dr. Pierce.

Abbreviations used in this dissertation

Zn: Zinc

Fe: Iron

ZIP: Zinc-and-iron-regulated protein

CDF/ZnT: Cation diffusion facilitator

DMT: Divalent metal transporter

Cu: Copper

IL: Interleukin

IFN: Interferon

TNF: Tumour necrosis factor

AEC: Abdominal exudates cells

MTF: Metal-responsive transcription factor

MT: Metallothionein

Mn: Manganese

SOD: Superoxide dismutase

Se: Selenium

GSH-Px: Glutathione peroxidase

SS: Inorganic sodium selenite

SY: Se-enriched yeast

GALT: Gut-associated lymphoid tissue

CT: Cecal tonsils

PP: Peyer's patches

LP: Lamina propria

M cells: Microfold cells

NK: Natural killer cells

BW: Body weight

BWG: Body weight gain

FCR: Feed conversion ratio

FI: Feed intake

Table of Contents

List of Tables

List of Figures

Chapter I

Introduction

Trace minerals are necessary nutrients for all animals since they are involved in general metabolism, immune system responses, growth, bone mineralization, and defense against oxidative stress. For example, Manganese is an essential trace mineral for normal bone formation, enzyme function, and amino acid metabolism in poultry (Scott *et al.,* 1976), especially in fast-growing commercial poultry where stress in is placed on maximizing performance and integrity of bone structure. Selenium is essential for proper function of antioxidant defenses, regulation of cell growth, maintenance of fertility, and enhancement of immune response (Papp *et al.,* 2007). Zinc is necessary for normal host defense and immune system ontogeny, and it is crucial for the development of normal humoral and cellular immune responses (Rink and Gabriel, 2000).

Since the studies to determine requirements of trace minerals for poultry were conducted several decades ago with birds of limited growth and production potential compared to the birds today, there is now a tendency to over-formulate to ensure the need of trace minerals for intense performance is met (Leeson, 2005). However, the use of excessive levels of inorganic trace minerals may result in environmental concerns, because of increased concentrations of unabsorbed trace minerals in the excreta. Trace minerals can be difficult to remove from soil because they tend to bind to the soil components. Minerals may then enter water supplies and then accumulate to toxic levels in plants and animal. Therefore, interest in using organic minerals has increased because of the potential for higher bioavailability, more efficient absorption, and lower inclusion level compared to inorganic minerals (Ao *et al.,* 2006). A number of animal studies have

shown that the bioavailability of mineral-amino acid chelates is better than the bioavailability of other forms of minerals, such as oxide or sulfate (Kincaid *et al.,* 1997).

The intestinal mucosa of chickens is the main site for enteric pathogen invasion. Colonization of the intestinal mucosa by a pathogen may result in tissue damage and initiate a host immune response. Therefore, the intestinal mucosa is one of the most important barriers against diseases. Cocccidiosis in chickens provides an excellent model for studying the effect of trace minerals on the repair of wounded epithelial cells, since coccidial species damage specific areas of the gastrointestinal tract. Coccidiosis, an enteric infection caused by various species of *Eimeria*, is of economic importance in the commercial turkey industry due to inefficient feed utilization and impaired growth rate. It was reported that 46% of the cost of coccidiosis in the United Kingdom is due to reduced weight gain, and 34% of the cost is due to poor feed conversion (Williams, 1999). The total cost of coccidiosis in the United States estimated by Williams (1999) was at least $1 billion in 1995. Allen and Fetterer (2002) reported the annual cost for the American broiler industry was about $450 million. It has been reported that coccidiosis contributed to Zn-deficiency in infected chicks (Southern and Baker, 1983a). Coccidia infection is also one of the important predisposing factors for necrotic enteritis, which causes significant decrease in performance and mortality in poultry.

Specific aims for the present research include:

1. Evaluate the effect of using commercially available organic trace minerals as compared to traditional inorganic mineral levels on performance of commercial turkeys.

2. Compare the effect of source and level of trace minerals on mineral concentration in excreta and litter.
3. Determine the effect of source and level of trace minerals on the response of the immune system following the use of a commercial turkey coccidial vaccine.

Chapter II

Literature Review

Trace Minerals

Minerals are an inorganic part of feeds. Based on the relative amounts required in the diet, they can be divided into two groups, macro-minerals and micro- or trace minerals. Macro-minerals usually are expressed as a percentage of the diet. Compared with the requirements of macro-minerals, the requirements of trace minerals are expressed as parts per million (ppm) or milligrams per kilogram (mg/kg) of diet. Trace minerals in poultry diets include copper (Cu), iron (Fe), manganese (Mn), selenium (Se), zinc (Zn), and iodine (I). Although cobalt is also required, it should not usually be considered to be supplied as a trace mineral during formulation because it is a part of vitamin B_{12} (NRC, 1994).

Although the daily requirements of trace minerals are expressed in milligrams, they still play a vital role in various body functions. Trace minerals are involved in the functions of enzymes, hormones, vitamins, and metalloenzymes, which play important roles in metabolism, reproduction, bone development, and immune responses of livestock. There are many factors that influence the absorption and utilization of minerals, such as mineral source, inclusion levels, interaction with other minerals, as well as age, nutritional, and physiological status of animals. Adequate trace mineral concentrations and balance among minerals are important, since excessive intake of one or more minerals may interfere with and reduce the bioavailability or utilization of one or more other elements (Kohlmeier, 2003).

Trace minerals are required by animals for maintenance, growth, and reproduction.

A deficiency can cause characteristic disease symptoms. An inadequate supply of one or more specific trace minerals, such as Fe deficiency, causes anaemia. Thus, having accurate estimates of dietary trace minerals requirements of animals is important. Most estimates of requirements for minerals are based on the minimum level required to overcome a deficiency symptom and not necessarily to promote productivity or to enhance immunity (Mullan and D'Souza, 2005). The National Research Council (NRC) plays an important role in establishing nutrient requirements for animals. Since 1953, NRC requirements have been considered as the minimal dietary concentration of a nutrient required to support normal performance (Cromwell and Kirk-Baer, 2005). Althought the last, 9th Revised Edition, of Nutrient Requirements of Poultry by the NRC was published in 1994, it is often criticized as not representing the needs of modern strains of commercial poultry.

The use of mineral supplements when they are not needed is not only a waste of money, but also may cause environmental issues. In commercial production, to meet the requirements for rapid growth rate of broilers and turkeys and egg output of layers, the producers often supplement with excess trace minerals as the "safety factors". Excess Zn intake will result in reduced absorption in the intestine and increased fecal excretion. Research has shown that Zn excretion in manure increased linearly as dietary Zn levels increased (Kim and Patterson, 2004). Poultry meat production (broilers and turkeys) in the United States has significantly increased in the past 40-60 years, resulting in more waste products. These wastes include excess minerals that can accumulate in soils. The accumulated trace minerals are toxic to plants and animals, and they may enter water resources with soil erosion or water infiltration. Therefore, the producers have to face the

pressure to reduce the impact on the environment caused by excess mineral excretion in poultry waste.

Inorganic salts, such as sulphates, carbonates, chlorides and oxides are common sources of minerals used in mineral supplements to meet the animals' needs. Free ions from these salts can form complexes with other dietary constituents in the digestive tract, resulting in low absorption and availability to the animal. For example, the sulphates were usually assumed to be 70% bioavailable (Leeson, 2005). However, organic minerals are usually chelates of protein/amino acids containing minerals, whose consistent bioavailability equates more closely to that of amino acids, i.e. 90-95% (Leeson, 2005). Therefore, interest in using organic minerals has increased because of reported potential of higher bioavailability.

Zinc

Animal studies in the 1930s first documented the essential requirement of Zn for normal growth and health in rats (Todd *et al.,* 1933). Subsequently, Zn has been found to be necessary for health in many other species. Zn is one of the most important trace elements, because of its' significant role in homeostasis, immune responses, oxidative stress, apoptosis, and aging (Stefanidou *et al.,* 2006). Zn is essential for the activation of numerous genes, is a cofactor of more than 300 enzymes, and plays an important role in function of the immune system and disease resistance. Zn deficiency will cause not only decreased lymphocyte concentrations, but also depressed T and B lymphocyte function (Fraker *et al.,* 1977; Dowd *et al.,* 1986).

Absorption is considered the process of influx into the enterocytes, movement through the basolateral membrane, and transportation into the portal circulation (Krebs,

2000). The gastrointestinal tract is the predominant absorption site of exogenous dietary Zn. In humans, the absorption of Zn is in the proximal small bowel, either the distal duodenum or proximal jejunum (Lee *et al.,* 1989; Krebs *et al.,* 1998). In other monogastric animals, the greatest absorption is in the duodenum (Naveh *et al.,* 1988). The route of excretion of unabsorbed Zn is via feces and urine (Krebs, 2000).

Zn must be absorbed from the intestinal lumen and then transported across the baso-lateral membrane into the portal blood (Gaither and Eide, 2001). There are two Zn transporter families, ZIP (zinc-and-iron-regulated protein) and CDF/ZnT (cation diffusion facilitator), that play an important role in Zn transport. ZIP family transporters are mainly involved in cellular Zn uptake, i.e. Zn influx into the cytosol from the outside of cells or from the lumen of intracellular compartments (Kambe *et al.,* 2004). Transporters in the ZnT family mediate Zn efflux out of cells or into cytosolic compartments, which controls free Zn buildup and eliminates potential toxic effects to the cells (Harris, 2002).

Transport of Zn is across the brush border from the intestinal lumen, which appears to be a carrier-mediated process. Kohlmeier (2003) reviewed the possible mechanisms of Zn absorption. The main uptake mechanism of Zn from the human intestinal lumen is with Zn-and Fe-regulated protein (ZIP4), which is expressed on the lumen side of enterocytes throughout the small and large intestine (Wang *et al.,* 2002). The ZIP transporter family can also mobilize other ions, such as, Fe, Mn and cadmium. The proton-coupled divalent metal transporter 1 (DMT1) is a minor uptake route (Kohlmeier, 2003). It also transports Fe, Cu, and other divalent metal ions that may compete with Zn (McMahon and Cousins, 1998a). When Zn is complexed with small peptides, the hydrogen ion/peptide co-transporter (PepT1) is a possible alternative way of

Zn uptake (Tacnet *et al.,* 1993).

Zn is exported from enterocytes into portal blood by Zn transporters 1 (ZnT-1) and 2 (ZnT-2) (Kohlmeier, 2003). A study showed that ZnT-1, ZnT-2, and ZnT-4 had unique patterns of distribution, and their regulation reflects a spectrum of sensitivity to Zn in rats (Liuzzi *et al.,* 2001). ZnT-1, a ubiquitously expressed protein found in the villi of the proximal small bowel, is upregulated when Zn intake is high (McMahon and Cousins, 1998b; Tako *et al.,* 2005). The research indicated that ZnT-1 acts mainly as a Zn exporter and may play a role in Zn homeostasis as a mechanism for Zn acquisition and elimination under conditions of excess Zn (McMahon and Cousins, 1998b). Dietary Zn regulates the expression of ZnT-1 and ZnT-2, while ZnT-4 mRNA expression was not changed by dietary Zn levels (Harris, 2002). Cragg *et al.* (2005) reported that, in response to variations in dietary Zn intake, expression of plasma membrane Zn transporters in the human intestine was regulated to contribute to maintenance of Zn status.

Absorption studies in animals indicated an inverse relationship between percentage absorption and dietary Zn intake (Coppen and Davies, 1987). However, at extremely high Zn intakes, absorption was not affected and excretion was the primarily way to regulate Zn homeostasis (Krebs, 2000). McClung and Bobilya (1999) reported that bovine pulmonary artery endothelial cells increased Zn uptake potential during Zn deficiency, while Zn uptake was not altered when there was an excess. Therefore, one of the most important factors affecting Zn absorption is the dietary intake levels. While adequate levels of Zn must be maintained for growth, excessive Zn concentrations should be avoided. The excess Zn competes with other metal ions for enzyme binding sites and transporter proteins. In addition, excess dietary Zn will cause environmental issues with

the excretion of non-absorbed Zn.

There are primarily two inorganic sources of Zn available for commercial use in poultry, Zn oxide (ZnO) and Zn sulfate monohydrate ($ZnSO_4H_2O$). Although ZnO has less bioavailability relative to $ZnSO_4$ (Sandoval *et al.,* 1997; 1998), they constitute 80-90% of Zn supplemented in the feed industry (Sandoval *et al.,* 1997; Edwards and Baker, 2000). Because $ZnSO_4H_2O$ has the characteristic of being highly water soluble, it allows reactive metal ions to form free-radicals, leading to the breakdown of vitamins (Batal *et al.,* 2001). There are defined organic trace minerals available, which include metal (specific amino acid) complexes, metal- amino acid complexes, metal-amino acid chelates, metal proteinates, metal polysaccharide complexes, and metal propionates (AAFCO, 2005). One of the characteristics considered important to the physiological function of organic chelated and complexed metals is the degree to which the organic ligands remain bound to the metal under physiological pH conditions (Wapnir, 1990).

Bioavailability was defined as the degree to which an ingested nutrient in a particular source is absorbed in a form that can be utilized in metabolism by the normal animal (Baker and Ammerman, 1995). Some researchers have reported greater bioavailability for organic Zn sources than those observed for inorganic forms, including ZnO and $ZnSO_4$ (Spears, 1989; Wedekind *et al.,* 1992). Pigs fed proteinated forms of Zn and Cu had higher liver Zn and Cu concentrations than did pigs fed sulfate forms of these metals, which indicates a higher utilization of Zn and Cu from the proteinated compared with the sulfate sources (Schiavon *et al.,* 2000). Although research suggests that the level of Zn recommended by the NRC for weanling pigs is sufficient for optimal growth performance and immune responses (van Heugten *et al.,* 2003), to ensure that piglets

have adequate Zn, the addition of 2,000 to 3,000 ppm Zn as ZnO is a common recommendation of the swine feed industry. This increase in dietary Zn will cause a linear increase of Zn excretion in the manure (Mohanna and Nys, 1999; Case and Carlson, 2002; Kim and Patterson, 2004; Skrivan *et al.,* 2005). Therefore, considering the danger of Zn accumulation in the soil, organic forms of the element in decreased supplemental level have been used with increasing frequency by the feed industry.

A number of animal studies have shown that the bioavailability of one type of Zn-amino acid chelate or another had been better than what was obtained from other forms of Zn such as oxide or sulfate (Kincaid *et al.,* 1997; Spears and Kegley, 2002). However, not all organic trace minerals are created equal (Cheng *et al.,* 2005). Guo *et al.* (2001) showed that the solubility of various commercially available organic Cu sources, including those from the same class of organic Cu, was different. In contrast, comparison of eight commercial organic Zn compounds and reagent-grade $ZnSO_4 7H_2O$, indicated there was generally equal bioavailability values for chicks and lambs (Cao *et al.,* 2000). Ward *et al.* (1993) found no differences between bioavailability of organic and inorganic minerals.

Zn is involved in many metabolic processes and critical for immune function in animals. Adequate levels of dietary Zn have been shown to improve chick humoral (Stahl *et al.,* 1989; Kidd *et al.,* 1993) and cellular (Kidd *et al.,* 1992; 1993) immunity and reduce chick mortality (Virden *et al.,* 2003). The main clinical signals of Zn deficiency are growth retardation, hypogonadism, diarrhea, and increased susceptibility to infectious diseases in animals (Salgueiro *et al.,* 2000). Zn deficiency has also been demonstrated to decrease cellular immunity (Fletcher *et al.,* 1988), impair thymus (Fraker *et al.,* 1977;

Shankar and Prasad, 1998) and spleen (Beach *et al.,* 1982) development. Other studies have shown Zn deficiency induced programmed cell death (apoptosis) in immature T- and B-cells of the immune system (King *et al.,* 2002; Fraker, 2005) to result not only in decreased lymphocyte concentration, but also in depressed T and B lymphocyte function (Fraker *et al.,* 1977; Dowd *et al.,* 1986). Evidence showed that Zn supplementation in breeder diets enhanced immunity of their progeny (Stahl *et al.,* 1989; Kidd *et al.,* 1992; 2000; Virden *et al.,* 2004). Zn is important for proper disease resistance, and its deficiency has resulted in bacteremia (Flinchum *et al.,* 1989; Kidd *et al.,* 1992; 1994a) and parasitic infections (Fraker *et al.,* 1982).

Abnormal T-lymphocyte development is thought to be the primary consequence of Zn deficiency on immune function (Dardenne and Bach, 1993). Zn deficiency will decrease the development, proliferation, and function of T lymphocytes. Zn-deficient children with acrodermatitis enteropathica had reduced numbers of lymphocytes, particularly T lymphocytes in the blood and peripheral lymphoid tissues, and decreased CD4+:CD8+ cell ratios (Shankar and Prasad, 1998). Abnormal development of T-cells was associated with thymic atrophy (Osati-Ashtiani *et al.,* 1998), as the thymus is the site of maturation of pre-T-cells from the bone marrow into helper T cells (CD4+) and cytotoxic T-cells (CD8+). Reductions in thymic size and cellularity were observed mostly in the thymic cortex, where immature thymocytes develop (Shankar and Prasad, 1998). Thus, the impairment in structure and function of the thymus by Zn deficiency would alter the replenishment of the peripheral immune system with T-cells (Fraker and King, 1998), and the T-cell memory response to antigens is reduced. In addition, thymulin is a Zn-dependent peptide hormone produced in the thymus that helps to maintain immune

function by activating T-lymphocytes and enhancing the cytotoxicity of natural killer (NK) cells (Kohlmeier, 2003). As an essential cofactor of many enzymes in proliferation of dividing cells, Zn may also play an important role in promoting the proliferation of lymphocytes and decrease susceptibility to apoptosis (Kohlmeier, 2003). An imbalance between T helper (Th) 1 and Th2 cells in humans was also observed with Zn deficiency when the data were collected during baseline, at the end of the Zn-restricted period, and following Zn repletion, because Zn may be required for regeneration of new CD4+ T cells and maintenance of T cytolytic cells (Beck *et al.,* 1997). The shift of Th1 to Th2 cells results in cell-mediated immune dysfunction.

B-lymphocyte development in the bone marrow is adversely affected by Zn-deficiency (Shankar and Prasad, 1998). Impaired development was most evident in pre-B and immature B-lymphocytes with mature B-lymphocytes less affected (Shankar and Prasad, 1998). Thus, Zn deficiency decreases development of B-lymphocytes in the marrow resulting in fewer mature B-lymphocytes in the spleen. In vitro, the splenic B-lymphocytes were strongly inhibited in Zn-deficient mice (Fraker *et al.,* 1977). B-lymphocyte antibody function is also inhibited by Zn deficiency (DePasquale-Jardieu and Fraker, 1984). Zn is required for the B-lymphocyte mitogenic and cytokine response to lipopolysaccharide (Driessen *et al.,* 1995). Research indicated that ZnO reduced ($P < 0.05$) anaerobic bacteria number, tended to decrease ($P < 0.1$) lactic acid bacterial translocation to the mesenteric lymph node, and tended to increase ($P < 0.1$) intestinal IgA concentration of weaned piglets on day 20 under commercial conditions (Broom *et al.,* 2006). Total IgM and IgG antibody titers for primary and secondary responses to 7% SRBC were significantly increased in broilers receiving supplemental Zn diet at 181

mg/kg under thermoneutral conditions from wk 4 to 7 (Bartlett and Smith, 2003).

Cytokines are the key messengers of immunologic cells that regulate multiple aspects of leukocyte biology, which can be mediated by corresponding receptors on target cells (Shankar and Prasad, 1998). Cytokines associated with Th1 cells include interleukin (IL)-2, interferon (IFN)-γ, and tumour necrosis factor (TNF)-α, which can promote macrophage activation and production of cytophilic IgG isotypes. Cytokines corresponding to Th2 cell responses include IL-4, IL-5, IL-6, IL-10, and IL-13, which tend to suppress macrophage function and cell-mediated immunity while promoting production of the noncytophilic IgG isotypes and IgE (Romagnani, 1997). Mild Zn deficiency in humans may be accompanied by an imbalance of Th1 and Th2 cell function, which causes impaired resistance to infection. The cytokines IL-1, IL-2, IL-4, and IFN-γ have been reported to be suppressed during Zn deficiency (Shankar and Prasad, 1998). Zn supplementation induced the release of IL-1, IL-6, TNF-α, soluble IL-2 receptor, and IFN-γ by human peripheral blood mononuclear cells (Rink and Gabriel, 2000). Bao *et al.* (2003) reported that Zn stimulates the gene expression of IL-2 and IFN-γ in D1.1 cells (Th1 human malignant lymphoblastoid cell line).

The macrophage, an important cell in both innate and acquired immunity, is adversely affected by Zn deficiency, which can impair cytokine production, and phagocytosis (Shankar and Prasad, 1998). Sephadex stimulation to evaluate macrophage phagocytic ability in abdominal exudate cells (AEC) indicated that numbers of AEC, macrophages in AEC, phagocytic macrophages, and internalized opsonized and unopsonized SRBC were increased by feeding high level Zn at 181 mg/kg in broilers (Bartlett and Smith, 2003).

Studies in turkeys have indicated a beneficial effect of Zn methionine (ZnMet) complex on immune function. Supplemental Zn in the form of Zn-Met increased substrate adherence potential of Sephadex-elicited AEC, increased phagocytic capacity of macrophages, and enhanced macrophage tumorcidal activity in young turkeys (Ferket and Qureshi, 1992). Kidd *et al.* (1994b) observed increased macrophage recruitment and adherence and improved ability to clear systemic *Escherichia coli* in turkeys fed 40 ppm of supplemental ZnMet. The ability of dietary Zn-Met to enhance mononuclear-phagocytic function against *Salmonella arizona* and *S. enteritidis* was investigated in young turkeys (Kidd *et al.,* 1994a). The results showed that cutaneous basophil hypersensitivity to phytohemagglutinin-P, clearance of *S. arizona* from the spleen, and *in vitro* phagocytosis of *S. enteritidis* by Sephadex-elicited AEC were improved when 30 or 45 ppm Zn from ZnMet was supplemented to diets of young turkeys (Kidd *et al.,* 1994a).

Zn is required for structural integrity of nearly 2,000 transcription factors. Zn affects the structure of chromatin, the template function of DNA, and the activity of transcription factors and of RNA polymerases, which determines both the types of mRNA transcripts synthesized and the rate of transcription (Falchuk, 1998). Zn deficiency caused an impaired DNA synthesis *in vitro* and *in vivo* (Chesters, 1992).

Zn has a role in composition and structure of chromatin. Falchuk (1998) summarized the control mechanism of chromatin under Zn deficiency and sufficiency. With Zn sufficient cells, the chromatin consists of principal molecules and genomic DNA with standard histone and non-histone proteins. While, with Zn deficient cells, the composition and structure of chromatin is altered. Because of increased acetylation, the

histones bind more tightly to DNA. Although the amount of basic proteins relative to DNA is the same, a set of arginine rich polypeptides replace histones. These polypeptides and the modified histones affect the structure and function of chromatin. As a result, the DNA of chromatin in Zn deficient cells has a reduced efficiency to serve as a template for RNA polymerase.

Zn plays an important role in the regulation of transcription and replication of DNA by Zn finger proteins (Ho, 2004), which are involved in cell proliferation, differentiation, division, and nuclear transcription factor activation (Coleman, 1992). Zn finger is a protein domain of transcription factors that can bind to DNA. Zn ions are essential for the structure and function of Zn finger proteins. On the other hand, Zn is a cofactor of many enzymes participating in the synthesis of DNA and RNA, such as DNA polymerase and DNA-dependent RNA polymerases. Zn also plays a critical role in DNA integrity. Zn deficiency results in oxidative DNA damage, DNA strand breaks, and DNA fragmentation because of base and nucleotide excision repairs associated with Zn finger or Zn-associated proteins (Ho, 2004).

Metal-responsive transcription factor (MTF-1) is an important Zn sensory transcriptional factor (Vasto *et al.,* 2007). MTF-1 responds to an increase in intracellular Zn by binding to multiple metal response elements in the promoter region of metallothionein (MT) genes, thus regulating Zn homoeostasis in the body (Cousins, 1998). Zn deficiency also causes quantitative and qualitative changes in the transcription process, i.e. decreased RNA synthesis associated with change in the types of messenger RNA. Zn influenced the synthesis of three classes of RNA through its regulatory role with RNA polymerases. Taylor and Blackshear (1995) reported that Zn influences the

stability of mRNA by inhibiting its turnover.

Zn deficiency is detrimental to embryonic development and progeny of experimental animals by suppressing growth, increasing congenital malformations of nearly all organ systems (Falchuk and Montorzi, 2001), and damaging epidermal cells. A study showed increased intestinal crypt-cell production, reduced duration of mitosis in the distal intestinal segment, and improved epithelial cell restitution in Zn-fed mice (Cario *et al.,* 2000). Feeding pharmacological levels of Zn for 14d to newly weaned pigs improved gut morphology by increasing villous height and reducing crypt depth in the duodenum and jejunum, thus potentially increasing the absorptive capacity of the small intestine and consequently improving growth (Carlson *et al.,* 1998). Tako *et al.* (2005) reported significant increases ($P < 0.05$) in the biochemical activity of the brush-border enzymes and transporters and in jejunal villus surface area from day of hatch (96 h post-ZnMet injection), which suggested that ZnMet administration into the prenatal intestine via injection into the amniotic fluid enhanced intestinal development and functionality.

Copper

Cu is important for the function of proteins, such as superoxide dismutase (SOD), ceruloplasmin, and cytochrome c oxidase (Rucker *et al.,* 1998). Cu is involved in nutrient metabolism, development of soft tissues and bone, and antioxidant defense against free radicals (Kohlmeier, 2003). There are two oxidation states of Cu in living matter, cuprous (Cu1+) and cupric (Cu2+) (Arredondo and Nunez, 2005). Usually, Cu2+ is the predominant form in the body, since Cu1+ is easily oxidized to Cu2+ by the presence of oxygen or other electron acceptors (Sharp, 2004).

Cu is absorbed mainly in the small intestine and predominantly excreted in feces, with a minor loss from skin, urine and other secretions (Kohlmeier, 2003). Levels of Cu in the body are balanced by Cu absorption and excretion with effective mechanisms (Linder and Hazegh-Azam, 1996). The balance between absorption and excretion of Cu is tightly regulated to maintain a relatively constant body Cu content (Turnlund *et al.,* 1989; 1998).

There are two possible Cu transport mechanisms in intestinal cells, Cu transporter 1 (Ctr1) (Lee *et al.,* 2000) and DMT1 (Gunshin *et al.,* 1997). Ctr1 is the default intestinal Cu transporter (Sharp, 2003). It was confirmed that Cu uptake is increased by the presence of Ctr1 in mammalian cells (Arredondo and Nunez, 2005). Therefore, Ctr1 might be a transport protein for the facilitated diffusion of Cu in the lumen. Cu is also absorbed by the proton-coupled DMT1 across the brush border (Kohlmeier, 2003). Export of Cu across the basolateral membrane depends on the Cu-transporting ATPase 7A, which means transporters are linked to the hydrolysis of ATP (Kohlmeier, 2003).

Within the mucosal cells, most absorbed Cu is bound to MTs. The MTs have high affinity for Cu, and their expression is increased under high Cu conditions (Sone *et al.,* 1987). Therefore, inflow of Cu may be driven by MT binding under increased Cu conditions. Further translocation of Cu is restricted by binding to MT in the gut mucosa cells (Cousins, 1985), which is good for the animals' health because the redox caused by free Cu can result in damaging oxidation reactions (DiSilvestro, 2005).

The balance among dietary absorption, distribution and utilization, and biliary secretion of excess Cu is very important for the homeostasis of Cu in the body (Sharp, 2004). The liver is the main redistribution organ of Cu, and there are three ways for the

liver to adjust Cu homeostasis. The liver can store Cu with binding to the protein MT (DiSilvestro, 2005). Secondly, the liver also could control the excretion of excess Cu into the bile (Sharp, 2004). In addition, it can secrete Cu back into the blood with incorporation into the protein ceruloplasmin, which transports Cu to other tissues (DiSilvestro, 2005).

Cu is a very important trace mineral in poultry nutrition. It has been used as a growth promoter due to its antimicrobial properties (Miles *et al.,* 1998). Cu deficiency resulted in hypercholesterolaemia (Kelvay *et al.,* 1984) and anaemia in laying hens (Baumgartner *et al.,* 1978). The withdrawal of Fe and Cu in the diet during the last 3 weeks before slaughter decreased feed efficiency in broilers (Ruiz *et al.,* 2000). It was reported that dietary Cu in excess of nutritional requirements reduced cholesterol content in the meat of broilers, because Cu might depress the activity of a certain enzyme in the cholesterol synthesis pathway (Konjufca *et al.,* 1997). Skrivan *et al.* (2000) reported that Cu supplementation (200 mg/kg) was a more efficient method of meat cholesterol reduction than the substitution of fat with oil. The substitution of rapeseed oil for lard decreased the concentration of cholesterol in breast muscles by 13%, while Cu supplementation further reduced the cholesterol content by 25%. Balevi and Coskun (2004) reported that supplemental Cu at 150 mg/kg decreased yolk cholesterol concentrations without any effect on production performance.

There have been several Cu compounds tested in chickens, including Cu sulphate ($CuSO_4$), Cu oxide (CuO), Cu chloride ($CuCl_2$), Cu carbonate ($CuCO_3$), Cu citrate ($Cu_3(C_6H_5O_7)_2$), Cu acetate (Cu ($CH_3CO_2)_2$), Cu oxychloride ($CuCl_2{\cdot}3Cu(OH)_2$), tribasic Cu chloride ($Cu_2(OH)_3Cl$), and Cu lysinate (Cu LYS). Different effects on birds between

different sources of Cu because of different bioavailability were reported. The $CuSO_4$, easily dissolved in both water and acidic solvents, is the most common source of Cu in commercial animal production (Guo *et al.*, 2001). Ewing *et al.* (1998) reported that birds fed diets with $Cu_3(C_6H_5O_7)_2$ had increased broiler BWG compared to the birds fed either $CuSO_4$ or $CuCl_2 \cdot 3Cu(OH)_2$ at 125 mg/kg or 63 mg/kg of Cu feeding, respectively, during a 42-d or 56-d experiment period. Zanetti *et al.* (1991) reported that average daily food intake, daily BWG, and FCR were similar between $CuCO_3$ and Cu $(CH_3CO_2)_2$ in broilers. $Cu_2(OH)_3Cl$ is a commercial Cu source currently available for the poultry industry. It has been shown that there is a higher concentration of Cu in $Cu_2(OH)_3Cl$ compared to that in $CuSO_4$ (58 vs. 25% Cu) (Liu *et al.*, 2005). Other studies reported that Cu source ($Cu_2(OH)_3Cl$ or $CuSO_4$) and dietary level influenced broiler performance and intestinal physiology with rearing on fresh vs. recycled litter (Arias and Koutsos, 2006). Because of lower hygroscopy and solubility in water, $Cu_2(OH)_3Cl$ is a less reactive and destructive form of Cu relative to $CuSO_4$. The organic source of Cu is Cu LYS, a chelated compound. The relative bioavailability of Cu in Cu LYS complex is reportedly 115 or 120% greater compared to in $CuSO_4$ (Baker *et al.*, 1991; Aoyagi and Baker, 1993).

Although the nutritional recommendation for chickens is about 8 mg/kg (NRC, 1994), Cu supplement in the diet is usually very high (100 to 300 mg/kg) to serve as a growth promoter and prophylactic agent. However, there are several side effects with over-supplementation of Cu. It was reported that birds given 600 mg/kg Cu from $CuSO_4$ or $Cu_2(OH)_3Cl$ had significantly ($P < 0.0001$) lower FI, reduced growth, and poorer FCR (Miles, 1998). Liu *et al.* (2005) showed that $CuSO_4$ at 260 mg/kg of Cu began depressing bird performance after being fed for 12 wk. Under controlled experimental conditions,

starter feeds containing in excess of 200 mg/kg Cu (added as $CuSO_4$) induced proventriculitis in broiler chicks, which lead to delayed feed passage and engorgement of the upper gastrointestinal tract (Wideman *et al.,* 1996). One of the other side effects is the environmental issue associated with increased feed excretion of Cu caused by over-supplementation. It was shown that Cu excretion was decreased by 35% as supplementation was reduced from 12 to 4 mg/kg (Dozier *et al.,* 2003).

Copper has been shown to play a role in the development and maintenance of the immune system, with altering several aspects of neutrophil, monocyte, and T-cell function in the immune system (Percival, 1998). However, only limited understanding exists about the exact mechanisms triggered by inadequate trace minerals nutrition. The number of circulating neutrophils is reduced in Cu-deficient animals (Koller *et al.*, 1987) and humans (Heresi *et al.*, 1985), and the ability to generate superoxide anion and kill ingested microorganisms is also reduced (Percival, 1998). Higuchi *et al* (1991) observed anti-neutrophil antibodies in the serum of Cu-deficient patients, which might indicate a mechanism of neutrophil loss. Cu-deficiency also diminished the total mononuclear cells yield, the relative percentage and absolute number of T-cells and the CD4+ and CD8+ T-subsets (Bala *et al.*, 1991), and antibody production from rat spleen cells (Koller *et al.*, 1987). Cu status affects the synthesis and secretion of cytokines, which modulate the activities of immune system (Munoz *et al.*, 2007). It was reported that Cu deficiency attenuates both IL-2 mRNA expression and protein secretion in activated Jurkat human T cell lines (Hopkins and Failla, 1997) by inhibiting transcription of the IL-2 gene (Hopkins and Failla, 1999).

Manganese

Mn is an essential trace mineral for normal bone formation, enzyme function, and amino acid metabolism in poultry (Scott *et al.,* 1976), especially in fast-growing commercial poultry with more stress for optimal performance and bone structure. Mn is essential for a variety of enzymatic and cellular processes in humans (Keen *et al.,* 1999). It is the cofactor for many enzymes, including oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases (Aschner and Aschner, 2005). These enzymes contribute to the metabolism of amino acids, lipids, proteins, and carbohydrates. Mn also plays an important role in redox processes. As a redox-active cofactor of Mn superoxide dismutase (Mn-SOD), Mn is important in detoxification of free radical forms of oxygen (Guerinot, 2000). A deficiency of Mn results in impaired growth, poor bone formation and skeletal defects (Keen *et al.,* 1999).

Mn is widely distributed throughout the body in relatively low concentrations with a few exceptions. Around 25% of total body Mn is found in the skeleton. Bones, liver, pancreas and kidney have relatively high levels of Mn. Liver storage capacity of Mn is limited and is at a rate proportional to dietary supply (up to a certain level). It is absorbed in the small intestine, and excretion of Mn is mainly in the feces via bile (95-98%). The excretion in urine is very low and affected little by intake levels of Mn (Roth, 2006). The balance between absorption and excretion is maintained by the rate of transport across enterocytes in the intestinal wall and by efficient removal within the liver (Papavasiliou *et al.,* 1966). Although exposure to high oral, parenteral, or ambient air concentrations of Mn can cause increased levels of Mn in tissues, the concentration of Mn in the diet still influences both the absorption of Mn from the gastrointestinal tract

and its excretion in bile (Aschner and Aschner, 2005). With high intake levels of Mn, the body has to maintain homeostasis by reduced gastrointestinal tract absorption, enhanced liver metabolism, and increased biliary and pancreatic excretion (Malecki *et al.,* 1996; Finley and Davis, 1999).

The absorption of Mn is mediated by DMT1 in the small intestine, which results in competition between Mn and Fe (Gunshin *et al.,* 1997), since Mn and Fe share the same absorption pathways. Additionally, export across the basolateral membrane may occur by apotransferrin receptor that is likely to mediate Fe transfer into blood (Kohlmeier, 2003). After absorption in the lumen, about half of Mn is bound to transferrin, and most of the remainder is bound to alpha-2-macroglobulin as divalent ions (Scheuhammer and Cherian, 1985). Only about 5% of total plasma Mn is carried by albumin (Kohlmeier, 2003). When bound Mn enters the liver, it may be excreted in bile, or transported to other tissues (DiSilvestro, 2005). Biliary secretion is the main pathway for Mn elimination (Malecki *et al.,* 1996). Pancreatic excretion of Mn is only a small part of the excretion (Davis *et al.,* 1993). A small fraction of the Mn in bile may be reabsorbed by the body (Schroeder *et al.,* 1996).

Mn activates glycosyl transferases, which are important in the formation of mucopolysaccharides (Leach, 1976). Mucopolysaccharides are components of proteoglycans (Leach, 1976). Proteoglycans in the eggshell matrix may contribute to the formation of eggshell structure and texture (Nys *et al.,* 2001). Leach and Gross (1983) reported that hens with Mn-deficiency produced eggs with thinner shells and abnormalities in eggshell ultrastructure. However, Mabe *et al.* (2003) observed that the elastic properties of the eggshells are not significantly changed with the supplementation

of Mn, Cu, and Zn. On the other hand, they reported that the breaking strength and fracture resistance are increased in older groups of birds (60 and 69 wk) with Mn, Cu, and Zn supplemented diets. Research has shown that Mn-SOD is the primary antioxidant enzyme to protect cells from oxidative stress by catalyzing dismutation of superoxide to H_2O_2 and O_2 (Leach, 1976). Lu *et al.* (2007) reported that dietary Mn affected heart, breast and leg muscle Mn-SOD gene transcription. They also observed that Mn might decrease abdominal fat deposition mainly by decreasing lipoprotein lipase activity in abdominal fat.

In immunity, Mn has been shown to play an important role in antibody production (Fletcher *et al.,* 1988). Additionally, Mn is a cofactor for SOD function, which is vital for macrophage and heterophil integrity (Cook-Mills and Fraker, 1993). Supplementation of Zn and Mn in the diet increased relative bursa and thymus weight (Virden *et al.,* 2004). Virden *et al.* (2003) reported that broiler breeders fed Zn and Mn had reduced mortality in floor pens. However, Collins and Moran (1999) showed that the supplementation of inorganic Mn and/or Zn did not improve live performance or processing yields of male broilers.

It was reported that organic Mn sources were more bioavailable than inorganic sources in some studies. The absorption of Mn from organic sources was higher than that of inorganic Mn in both vitro and vivo experiments (Ji *et al.,* 2006a & b). They also reported that methionine was more effective than glycine in facilitating Mn absorption.

Selenium

Se is essential for proper function of antioxidant activity, regulation of cell growth, maintenance of fertility, and enhancement of immune response. Chicks reared on diets

supplemented with Se at levels exceeding 0.25 ppm showed improvement in BWG, feed consumption and FCR (Colnago *et al.,* 1984). Se is a component of the antioxidant enzyme glutathione peroxidase (GSH-Px), which protects cells by destroying the precursors and products of free radicals. Free radicals and peroxides play a significant role in the pathogenesis of various diseases and are thought to participate in damaging oxidative tissues and increasing stress (Guemouri *et al.,* 1991). The protective effect against lipid peroxidative tissue damage by the cellular Se-containing GSH-Px, is attributable mainly to its reduction of H_2O_2 and thereby the decreased formation of hydroxyl radicals, which are prime initiators of lipid peroxidation (Bozkaya *et al.,* 2001).

Absorption of Se is mainly in the duodenum with the greatest absorption in the lower small intestine (Wolffram *et al.,* 1985). Se salts (selenite and selenate) are almost as well absorbed from the intestine as selenoamino acids (Thomson and Stewart, 1973). Selenite uptake might be mediated by the formation of selenodicysteine and selenodiglutathione, which can then be transported across the apical enterocyte membrane by the corresponding amino acid and peptide transporters (Vendeland *et al.,* 1994). It was reported that dietary Se was retained more as the broilers got older and utilized more efficiently when Se concentration was low in the diet (Yoon *et al.,* 2007). The primary excretion routes of Se from the body are by urine, feces and exhalation. Exhalation is a major route only when Se intake is at toxic levels.

Unlike other metal elements, which interact with proteins as cofactors, Se is incorporated into polypeptide chains as part of the amino acid selenocysteine (Sec) (Papp *et al.,* 2007). The group of proteins with Se is called selenoproteins, which are the primary forms to perform a variety of physiological roles of Se (Holben and Smith, 1999).

When Se is limited, the synthesis of selenoproteins is reduced (Driscoll and Copeland, 2003). GSH-Px is one of the most important selenoproteins. It is part of the body antioxidant defense network against peroxidative damage by reducing hydrogen peroxide and free fatty acid hydroperoxides (DiSilvestro, 2005).

Studies showed that variation of SOD and GSH-Px, is important for protection of biological molecules in the cell (Bozkaya *et al.,* 2001). The decrease of the antioxidative enzymes SOD and GSH-Px leads to decreased defense of erythrocytes against free radicals (DiSilvestro, 2005). Se is a structural component of the active site of GSH-Px and Se-GSH-Px catalyzes the reduction of hydrogen and lipid peroxides via oxidation of glutathione. Thus, Se-deficiency would lead to low Se-GSH-Px in rats and in turn would lead to accumulation of lipid peroxides (Sakaguchi *et al.,* 2000). Deficiency of Se is associated with a decrease in GSH-Px activity and increased lipid peroxidation (Molina and Garcia, 1997). Deficiency can also cause oxidative stress in poultry (Surai, 2002).

Se is important for the immune response. It protects the host from oxidative stress generated by the microbicidal effects of macrophages and during inflammatory reactions (Wintergerst *et al.*, 2007). Supplementation of 0.15 and 0.3 ppm Se in the diet (having 0.19 ppm Se) of lambs significantly improves their immune response and antioxidant status (Kumar *et al.*, 2008). Both cellular and humoral responses can be impaired by Se deficiency (Spallholz *et al.*, 1990). Se supplementation has immune-stimulant effects, including an enhancement of activated cell proliferation (Rivera *et al.*, 2003). Selenoprotein deficiency lead to oxidant hyperproduction in T cells and thereby suppressed T cell proliferation in response to T cell receptor stimulation (Shrimali *et al.*, 2008). The mechanism may be related to the ability of Se to up-regulate the expression of

growth-regulatory cytokine IL-2 receptors on the surface of activated lymphocytes and NK cells, thereby facilitating the interaction with IL-2 (Rivera *et al.*, 2003).

Se deficiency decreased titers of IgM and IgG, impaired neutrophil chemotaxis, and decreased antibody production by lymphocytes (Wintergerst *et al.*, 2007). A positive effect of adding Se was observed in trials evaluating infections with E. coli and infectious bursal disease virus in chickens (Larsen *et al.*, 1997). The dietary addition of Se between 0.1 and 0.8 mg/kg resulted in an antibody titer increase to the pathogens (Larsen *et al.*, 1997). Chicks receiving supplements of 200mg vitamin E/kg and 0.2mg Se/kg produced significantly higher haemagglutination inhibition antibody titers 10 d after vaccination with Newcastle disease virus (Singh *et al.,* 2006). Therefore, adequate Se might be necessary for competent immune response against infection. However, excess supplementation may have an adverse effect on certain immunological functions. Ueno *et al.* (2008) reported that only low levels of Se were essential for T-cell mitogenesis even in Se-insufficient splenic cells.

Inorganic sodium selenite (Na_2SeO_3; SS) has been used as common Se source in animal diets for many years. However, with the approval of an organic source of Se (Se-enriched yeast, SY), the interest of studying SY in poultry diets has increased. The Se-enriched yeast is produced by growing the yeast *Saccharomyces cerevisiae* in a high-Se medium (Payne *et al.,* 2005). Research indicated that the majority of the Se in SY is selenomethionine, a Se analog of methionine (Kelly and Power, 1995). Selenomethionine and SY supplements increase egg Se content more than SS when fed to hens (Swanson, 1987). Compared to hens fed SS, the hens fed SY had a more significant increase in the Se concentrations in egg, spleen, and breast muscle (Pan *et al.,* 2007).

Yoon *et al.* (2007) reported that the source of Se did not affect growth performance of broiler chickens. However, they suggested that organic Se was more bioavailable than SS based on the data of Se retention in the body and blood Se relative to Se intake. Roch *et al.* (2000) reported that organic Se showed protective effects against cold-induced ascites and oxidative stress in broilers. Supplementation with organic Se alone enhanced antioxidant defense against suboptimal environments (Ozkan *et al.,* 2007).

There is a synergistic relationship between Se and vitamin E, and Se has a sparing effect on vitamin E. They can participate in similar nutritional and biochemical relationships. They can protect against oxidative damage and enhance humoral and cell-mediated immune responses. Combined dietary supplementation levels of vitamin E and Se, 150 IU/kg and 0.1 ppm, respectively, may be required for better health, disease protection, and overall growth performance (Swain *et al.,* 2000). Supplementation with inorganic Se associated with vitamin E significantly increased GSH-Px activity in broilers (Ozkan *et al.,* 2007). Panda and Rao (1994) reported that there was an enhancement of the immune response following Se and vitamin E supplementation in chicks infected with infectious bursal disease virus. Vitamin E and Se have also been shown to have synergistic effects on immune response against Newcastle disease virus (Singh *et al.,* 2006). Nutritional deficiencies of vitamin E or Se or both impair immune function in young chicks (Swain *et al.,* 2000).

Interaction of Trace Minerals

There are close relationships among the trace minerals, including synergism and antagonism. Synergism means the trace minerals can work together to produce a result, which is not obtainable by any of the trace mineral on their own. Antagonism indicates

that the excess of one or more trace minerals can interfere with the uptake or metabolism of another mineral. A mineral can interact with and affect the requirement for itself or other elements. Therefore, the balance of trace minerals is important to get the maximum benefit of their use for animals.

There are close relationships in absorption between Cu and Zn. It was reported that excess Zn intake decreased Cu absorption (Kohlmeier, 2003). Storey and Greger (1987) reported that intake of excess Zn decreased soft tissue Cu concentrations. The antagonism between Cu and Zn was also observed in that Cu supplementation significantly increased the percentage of Zn associated with large complexes and decreased the percentage of Zn associated with small complexes (Pang and Applegate, 2007). The antagonisms between Zn and Fe and Cu and Fe are another important issue. The chemically similar elements may share a pathway for absorption, thus resulting in competition for uptake into the gastrointestinal tract. It was observed that intake of excess Zn reduced tissue Fe concentrations in birds (Stevenson *et al.,* 1987). Stahl *et al.* (1989) reported that mobilization of stored Fe was affected by excess Zn supplementation. The uptake of Fe is influenced by Cu. One of the reasons may be competition for transport into enterocytes by DMT1 at the intestinal level. Deficiency of Cu alters Fe metabolism by an effect on the ferrioxidase activity of ceruloplasmin, which is essential for Fe release from tissues (Sharp, 2004). Stahl *et al.* (1989) found that ingestion of excess Zn caused decreased Cu concentrations in livers and pancreases, and decreased Fe concentrations in tibia. There is also competitive absorption between Mn and Fe in the gastrointestinal tract (Gunshin *et al.,* 1997).

On the other hand, synergism of trace minerals is also important. For example, Cu,

Zn, and Se are linked together to defend against oxidative stress. Klotz *et al.* (2003) reviewed the cellular defense against oxidative stress with the application of Cu, Zn and Se. Cu and Zn are necessary cofactors to catalyze the dismutation of superoxide to oxygen and hydrogen peroxide by forming the CuZn–SOD. Then selenoenzyme GSH-Px reduces the level of hydrogen peroxide subsequently.

Trace Minerals and Environmental Impacts

Over the last 10 years, the global production of poultry meat and eggs has had a tremendous increase. In 1993, nearly 48 million tonnes of poultry meat and 38 million tonnes eggs were produced globally (Leeson and Summers, 2005). However, the production was increased to 80 million tonnes and 57 million tonnes in 2005, respectively (Leeson and Summers, 2005). It was reported that over 13 billion kg of poultry manure and/or litter are produced each year in the United States (Moore, 1998). Overcash *et al*. (1983) reported that a unit value of 17.4 kg (dry basis) manure produced daily per 1000 kg live weight, for meat-type birds. The most commonly utilized practice of manures and bedding material involves land application as the final step (Williams *et al.*, 1999). Consequently, environmental parameters impacted by waste by-products from the production of poultry meat and eggs are of increasing importance worldwide.

Trace minerals such as Cu, Mn, Se and Zn are added to poultry feed to improve animal performance and to prevent diseases (Colnago *et al.*, 1984; Fletcher *et al.*, 1988; Kidd *et al.*, 1992; Ruiz *et al.*, 2000). However, these trace minerals are often added to the diet at levels that exceed the nutritional requirements of the birds, which result in a wide range of these minerals in poultry waste (Williams *et al.*, 1999). Kingery *et al.* (1993) found Cu and Zn levels were elevated in heavily fertilized soils with poultry litter.

Concentrations of Zn, Fe, and Cu in herbage correlated significantly with the supply of these elements by hen excreta into soil (Skrivan *et al.*, 2005). Elevated levels of trace metals in the soil will result in increased uptake by plants, which will be consumed by animals or man (Moore, 1998). Cu toxicity for some plants has been reported at total soil concentrations of 150-300 mg/kg; Zn toxicity problems for peanuts and cotton have been shown in soils containing Zn concentrations ranging from 8.5 to 1700 mg/kg (Williams *et al.*, 1999).

Gastrointestinal Defense Mechanisms

The intestinal mucosa of chickens is one of the potential gateways of pathogen invasion. The colonization of the intestinal mucosa by a pathogen may result in tissue damage and initiate a host immune response to eliminate the noxious agent, thereby causing an inflammatory response. Therefore, the intestinal tract not only performs digestive and absorptive functions, but the intestinal mucosa is one of the most active defense barriers against the continuous challenge of food antigens and pathogenic microorganisms in the intestinal lumen (Schat and Myers, 1991).

Intestinal Mucus Layer

Interspersed between intestinal epithelial cells are goblet cells that produce mucus. Gastrointestinal mucus forms a continous layer *in vivo* that can be further divided into two strata: a loosely adherent layer removable by suction and a layer firmly attached to the mucosa (Atuma *et al.,* 2001). It provides a protective barrier between the underlying epithelium and the lumen containing noxious agents, destructive hydrolases, and microorganisms (Forstner and Forstner, 1994). Therefore, the mucus layer is one of the

first lines of defense against foreign bacteria and other pathogens.

Mucus consists of about 95% water and 5% mucin glycoproteins, with electrolytes, various cellular and serum proteins, lipids, and nucleic acids (Strous and Dekker, 1992). The mucin glycoproteins, synthesized and secreted by goblet cells, are classified into two groups: neutral and acidic mucins (Forstner and Forstner, 1994). The acidic mucins are subdivided into sulfated and sialylated mucin types (Fontaine *et al.,* 1996). It was suggested that acidic mucins have protective function against bacterial translocation (Fontaine *et al.,* 1996). Strous and Dekker (1992) observed that the presence of acidic mucins were important for gel formation in the protective response against invading enteric pathogens. Uni *et al.* (2003) reported that the acidic mucins were present 3 d before hatch. Thus, the presence of acidic mucins during early development may be an important barrier to invading pathogens since the acquired immune system is not fully developed (Cebra, 1999).

The physical integrity of the mucosa is one of the important factors of mucosal barrier function (Bourlioux *et al.,* 2003). There are millions of villi lined with epithelial cells which undergo continuous renewal. Any process disrupting the orderly migration and renewal of the epithelial cells (infections, toxins, etc.) can interfere with the barrier function (Schat and Myers, 1991). Numerous microvilli, which are extensions of the plasma membrane of the epithelial cells, are necessary for the final digestion and absorption of the nutrients. There is a balance between the degradation of luminal mucins by bacteria and secretions of new mucin by goblet cells (Uni *et al.,* 2003). Goblet cells differentiate from the endodermal stem cells at the base of the crypt and then migrate to the villus tip as they mature (Dunsford *et al.,* 1991). Eventually they are sloughed into the

lumen. The whole process takes approximately 2 to 3 d in poultry (Uni *et al.,* 2000). The migration of goblet cells accompanies the changes in chemical composition of the mucins. Immature goblet cells in the crypts produce neutral mucins containing little sialic acid, while the mature goblet cells along the villi increase in production of sialylated mucins (Specian and Oliver, 1991). In a study with piglets, it was shown that migration of goblet cells in the small intestine was associated with increased sulphated mucins (Brown *et al.,* 1988). Mucins are secreted from the apical surface of goblet cells by two different processes, baseline secretion and compound exocytosis (Deplancke and Gaskins, 2001). Baseline secretion is the constitutive release of newly synthesized mucin granules which move along the periphery of the apical granule mass (Deplancke and Gaskins, 2001). Compound exocytosis is an accelerated secretory process, which causes the acute release of stored mucin granules (Deplancke and Gaskins, 2001). Compound exocytosis occurs in response to stimuli in the lumen.

Intestinal Immunity

The gut-associated lymphoid tissue (GALT) is the largest mass of lymphoid tissue, representing an important element of the total immunologic capacity of the host (Isolauri *et al.,* 2001). The chicken GALT includes organized lymphoid structures such as the bursa of Fabricius, cecal tonsils (CT), Peyer's patches (PP), Meckel's diverticulum, and lymphocyte aggregates scattered along the intra-epithelium and lamina propria (LP) of the gastrointestinal tract (Lillehoj and Trout, 1996). Bar-Shira *et al.* (2003) reported that GALT immune maturation occurs in two stages by observing mRNA expression of chicken IL-2 and IFN-γ. The primary stage was during the first week post-hatch, and the second stage was during the second week.

The CT are discrete lymphoid nodules located at the proximal ends of the ceca near the ileocolonic junction (Befus *et al.,* 1980), which have macrophages and many plasma cells producing IgM, IgG, and IgA. The PP are lymphoid aggregates in the intestine located in the ileum anterior to the ileocecal junction, which possess a morphologically distinct lymphoepithelium with microfold (M) cells, follicles, a B-cell-dependent subepithelial zone, a T-cell-dependent central zone, and no goblet cells (Befus *et al.,* 1980). Intestinal epithelial cells and M cells provide the first point of contact with intestinal bacteria. The M cells in the follicle-associated epithelium transport foreign macromolecules and microorganisms to antigen-presenting cells within and under the epithelial barrier (Neutra *et al.,* 2001). The GALT is largely represented by PP, which are the major inductive sites for IgA responses to pathogenic microorganisms and ingested antigens in the gastrointestinal tract (Lillehoj and Trout, 1996). Following antigen presentation, B and T-cell lymphoblasts leave the PP via the efferent lymphatics, passing through the mesenteric lymph nodes, and entering the systemic circulation via the thoracic duct (Muir, 1998). Meckel's diverticulum is the remnant of the yolk sac on the small intestine, which persists for the lifetime of the bird (Lillehoj and Trout, 1996). It contains germinal centers with B cells and macrophages (Befus *et al.,* 1980).

Intestinal lymphocytes generally reside in two anatomic compartments: epithelium and LP (Schat and Meyers, 1991). The LP of 1-day-old chicks or poults contains little stroma (capilliaries, lacteals, and reticular and muscle fibers) and few lymphocytes. The LP contains IgM- and IgA-positive B-cells and plasma cells and CD4+ T-cells. The intraepithelial lymphocytes are located between the epithelial cells and basement membrane.

The response of the mucosal immune system to an antigen at the mucosal surface involves stimulation of lymphocytes and the local secretion of IgA (Muir, 1998). IgA is now recognized as the main immunoglobulin class secreted by antibody-forming cells in the intestine (Lamm, 1976). One of the main functions of secretory IgA is immune exclusion, where binding of secretory IgA to antigen interferes with pathogen attachment and colonization to the mucosal surface by direct blocking (Williams and Gibbons, 1972). T cell help required for the induction of a humoral immune response at mucosal surfaces involves direct cell-cell contact as well as signals from soluble T-cell derived cytokines (Muir, 1998). Based on their typical cytokine profile, CD4+ T cells are divided into two groups (Mosmann and Coffman, 1989). Th1 cells, which produce IFN-γ and IL-2, are especially important for cell-mediated immunity, but may also provide help to B cells. T helper 2 cells, which preferentially produce IL-4, IL-5, IL-6, and IL-10, provide help to B-cells for production of all major Ig isotypes, including IgA (Romagnani, 1997).

The intestinal immune system immediately recognizes bacteria and rapidly responds to challenges by activation of innate immune cells followed by cascades to trigger acquired immunity. Bourlioux *et al.* (2003) summarized the immune response mechanisms after an antigen is ingested. The mechanisms include: 1) capture of intraluminal antigens by M cells in PP and presentation to specialized cells; 2) circulation of sensitized B- and T blast cells that release mature specific IgA and T cells throughout the gastrointestinal system; 3) involvement of IgA and mature intestinal T cells, which are active against the enteropathic microorganisms.

Coccidia

Protozoa are one of the most common parasites in poultry and other birds, and

some can cause moderate or even severe disease. The most common protozoal parasites in birds are coccidia, specifically those of the genus *Eimeria* (Jeurissen *et al.,* 1996). Infection results in damage to the intestinal tract, which interferes with the digestive process and/or nutrient absorption, dehydration, feed intake, and blood loss. Birds are also more likely to get sick from secondary bacterial infections during an active coccidia infection.

Coccidiosis, caused by protozoan parasites of the genus *Eimeria*, is one of the most important diseases of commercial poultry operations. Based on the compartmentalised model developed by Williams (1999), the total cost of coccidiosis in chickens in the worldwide and the United States was estimated to have been at least $3 billion and $1 billion in 1995, respectively, of which 98.1% involved broilers. Around 80.6% is due to effects of mortality and decreased weight gain and feed conversion, and 17.5% is due to the cost of chemoprophylaxis and therapy. However, Allen and Fetterer (2002) reported the annual cost for the American broiler industry was about $450 million. Therefore, coccidiosis is a major parasitic disease responsible for substantial economic losses in poultry industry due to mortality, inefficient feed utilization, and impaired growth rate of broilers and reduced egg production by layers (Lillehoj *et al.,* 2004)

Life Cycle

Eimeria exhibit complex life-cycle stages, including both inside and outside of the host, intracellular and extracellular stages, and asexual and sexual reproduction (Lillehoj, 1998). Its life cycle includes three distinctive phases: sporogony, merogony (schizogony), and gametogony. It takes about four to six days to complete the whole life cycle, from oocyst ingestion to release out of the host in the fecal contents.

Sporogony is the process of a one-celled zygote within the oocyst undergoing a series of divisions to form sporozoites, which are contained within sporocysts. The oocysts "sporulate" or become infective if moisture, temperature, and oxygen become conducive to growth. Sporulated oocysts have four sporocysts, and each sporocyst contains two sporozoites.

The life cycle of *Eimeria* begins when the birds ingest the active oocysts, which are composed of a capsule with a thick wall protecting the parasites. With the help of grinding action in the gizzard and secretion of bile salt and trypsin in the gut, the oocysts' outer wall is destroyed, which results in the release of sporozoites. Then sporozoites invade villus epithelial cells in specific regions of the digestive tract. During infection, sporozoites are first seen within intestinal intraepithelial lymphocytes, primarily CD8+ cells and macrophages shortly after invasion, and later are found developing inside epithelial cells (Trout and Lillehoj, 1996).

After invading the villus or crypt cells, the reproductive process occurs, which includes a rapid increase in the merozoite stage of the parasite. The sporozoites invade the intestinal epithelium and form trophozoites, which is then followed by nuclear divisions to form immature meronts (schizonts) (Lillehoj and Trout, 1996). During this process, one sporozoite will release about 1,000 merozoites into the gut. Sometimes this process will happen 2 to 4 times before merozoites differentiate into the sexual stages (Yun *et al.,* 2000). When the schizonts produced by trophozoites mature and rupture, there are more merozoites released, which will cause widespread infection. These penetrate the enterocytes and begin a phase of multiplicative asexual development (schizogony or merogony) consisting of several (often three or four) cycles whose

number is usually finite and characteristic of the species (Rose, 1987).

The cessation of asexual development is followed by the formation of gametes (gametogony). Gametogony is the phase of sexual reproduction, i.e. merozoites produced by schizonts develop into sexual forms (gametocytes), including micro- (male) and macro- (female) gametocytes. These micro- and macro-gametes will fuse to form a zygote, which is considered an immature oocyst. Once the zygotes develop into oocysts, they will be released from the intestinal mucosa and are shed in the feces (McDougald and Reid, 1997). These new oocysts can infect other birds, damage the gut, cause poor bird performance, and possibly allow bacteria to invade and cause secondary infections.

Eimeria Species

There are many different types of coccidia that can infect almost all livestock. However, coccidiosis has species-specific character, which means that each species of coccidia only infects one species of livestock. Additionally, immunity to one species of *Eimeria* does not protect against infection by another species. The location and appearance of lesions from infection with each species is specific. It is also helpful in identifying species to observe the size of oocysts and appearance of developmental stages in smears with a microscope (McDougald, 1998). Immunovariability also exists between different isolates within *Eimeria* species that can result in differential host response in the intestinal immune response (Morris *et al.*, 2004).

In the poultry industry, nine species of the genus *Eimeria* have been described in chickens; however, in recent years, only seven of these species are known to be ubiquitous and cause pathogenic effects: *E. acervulina*, *E. maxima*, *E. tenella*, *E. necatrix*, *E. mitis*, *E. brunetti*, *and E. praecox* (McDougald and Reid, 1997). Some of these (e.g., *E.*

mitis and E. praecox) only reduce growth rate and feed utilization (McDougald and Reid, 1997), whereas three (*E. tenella*, *E. maxima*, *and E. acervulina*) may cause heavy mortality in addition to decreased performance parameters.

For turkeys, there are seven different types of *Eimeria* described in United States, which are different from the species prevalent in chickens (*E. adenoeides*, *E. dispersa*, *E. gallopavonis*, *E. innocua*, *E. meleagridis*, *E. meleagrimitis*, and *E. subrotunda*). Only four (*E. adenoeides*, *E. dispersa*, *E. gallopavonis*, and *E. meleagrimitis*) are economically important to the commercial turkey industry (McDougald and Reid, 1997). Typical signs of coccidiosis in turkeys are watery or mucoid diarrhea, ruffled feathers, anorexia, and general signs of morbidity. Although all ages of turkeys are susceptible to *Eimeria* infection, birds older than 6 to 8 wk are considered more resistant to the disease. The prevalence of particular *Eimeria* species depends on many factors. Many of them involve basic characteristics of *Eimeria* biology, including ubiquity of species, survival of oocysts in various climatic and sanitary conditions, inherent susceptibility of different breeds of chickens, age of birds, and so on (Williams, 1999).

The description of the most common and most pathogenic species of coccidia in turkeys was given by McDougald and Reid (1997). *E. adenoeides* appear primarily in the ceca with gross lesions but extend to the lower small intestine and cloaca. Cecal contents are often hardened into a core because of mucosal debris. The cecal and/or intestinal wall is often swollen and edematous. *E. dispersa* appear in the small intestine, principally the midgut region, but some infections may occur in the cecal necks. The lesions of *E. gallopavonis* are restricted to the area posterior to the yolk sac diverticulum and tend to be most severe in the lower small intestine and large intestine. *E. meleagrimitis* invade

primarily the upper-intestine but may spread throughout the small intestine in heavy infections. *E. meleagridis* is considered to have little pathogenicity (McDougald and Reid, 1997). However, Matsler and Chapman (2006) recently reported that an inoculum of 2×10^5 oocysts was found to cause a significant reduction in weight gain from days 0 to 3 and 0 to 6 after infection, which suggested that the significance of this species of *Eimeria* as a pathogen of turkeys should be reassessed.

Host Immune Response

By *in situ* investigations, Jeurissen *et al.* (1996) reported that there are three stages at which to inhibit *Eimeria* infection and development, including: 1) during the searching by a sporozoite for a site to penetrate; 2) during sporozoite entry into the villus epithelium and exposure to intra-epithelial lymphocytes; and 3) during passage through the LP to the crypt epithelium. Infection with *Eimeria* promotes both antibody and cell-mediated immune responses. Although antibodies can be abundantly produced locally, they can not access and act on these intracellular pathogens (Lillehoj *et al.,* 2004). Therefore, antibody-mediated responses play a minor role in protective immunity against coccidiosis. Cell-mediated immune responses are the major host immune response during *Eimeria* infection, and it also can provide protection against reinfection (Lillehoj and Trout, 1996; Lillehoj, 1998; Lillehoj and Lillehoj, 2000; Lillehoj *et al.,* 2004). T lymphocytes, NK cells, and macrophages are involved in the avian cellular immune response to *Eimeria* infection (Lillehoj and Trout, 1996).

There are two types of T lymphocytes: cytotoxic T lymphocytes (CD8+), which recognize foreign antigens in the context of major histocompatibity complex (MHC) class I molecules; and T helper cells (CD4+), which recognize antigens in association with

MHC class II molecules. Studies have shown that CD4+ and CD8+ lymphocytes have different contributions to immunity during coccidiosis infection.

CD4+ lymphocytes are critical to control the parasite replication during primary infection and CD8+ lymphocytes increase during secondary exposure in the intestinal epithelium and LP (Lillehoj and Trout, 1996). There are significantly different results in the number of oocysts in CD4+ cell-depleted chickens after infection with *E. acerulina* and *E. tenella* (Trout and Lillehoj, 1996). The reason for these results was still unclear. This research also showed that CD8+ lymphocytes are necessary for the development of protective immunity to coccidia. Trout and Lillehoj (1995) reported significantly increased numbers of CD8+ cells in the intestine after secondary infection with *E. acervulina*. In contrast, fewer CD8+ cells were seen after primary infection. However, Hong *et al.* (2006) reported that the intestinal lymphocyte subsets, CD3+, CD4+, and CD8+, were increased after primary *E. maxima* inoculation. Only CD4+ cells were increased during both primary and secondary infections. In mice experiments, the results showed that CD4+ cells are more effective than CD8+ in conferring protective immunity to *E. vermiformis* both *in vivo* (Rose *et al.,* 1992) and *in vitro* (Rose *et al.,* 1988). Therefore, in *Eimeria* infections, the scope of host immunity depends upon the species of the parasite and the intestinal site of development (Lillehoj, 1998).

In addition to their potential role in protection against *Eimeria* infection, intestinal lymphocytes have been implicated in the transport of sporozoites (Trout and Lillehoj, 1993). When studying the interactions of sporozoite and host after *E. acervulina* infection, most sporozoites were seen inside CD8+ lymphocytes, and occasionally seen in CD4+ lymphocytes (Trout and Lillehoj, 1995). These reports indicate that CD8+ lymphocytes

were the primarily transporter of sporozoites.

In summary, acquired immunity to coccidiosis may involve immune mechanisms that both reduce the number of intracellular sporozoites and inhibit the natural progression of parasite development.

Cytokines are essential effector molecules of innate and adaptive immunity. According to their role in immune response, current cytokines are classified by the Th1 and Th2 paradigm (Mosmann *et al.,* 1986; Degen *et al.,* 2005). Th1 cells, which secrete IFN-γ, TNF-α, IL-1β, IL-2, IL-6, IL-12, IL- 15, IL-16, IL-17, and IL-18, are primarily responsible for cell-mediated immunity and delayed type hypersensitivity. Conversely, Th2 cells drive humoral immunity by producing IL-3, IL-4, IL-5, IL-6, IL-13, and transforming growth factor (TGF)-β4 (Lillehoj *et al.,* 2001).

In general, the expression of pro-inflammatory, Th1 and Th2, cytokines, and chemokines can significantly increase after primary infection but can remain relatively unchanged following secondary infection (Hong *et al.,* 2006). Hong *et al.* (2006) reported that the pro-inflammatory and Th1 cytokines, IFN-γ, IL-1β, IL-6, IL-12, IL-15, IL-17, and IL-18, were increased following primary infection with *E. maxima*. Furthermore, mRNA levels of the Th2 cytokines, IL-3 and IL-13, were up-regulated between 34- to 8,800- fold. However, only the expression level of the Th2 cytokine IL-13 was increased after secondary infection. In experiments with *E. acervulina* and *E. tenella* infections, transcripts encoding the Th1 and Th1 regulatory cytokines, IFN-γ and IL-12, had a similar increasing trend compared with those during primary infection with *E. maxima* (Hong *et al.,* 2006). During secondary infection, most of Th1 cytokines mRNA levels were relatively unchanged, except for IL-12, which was increased after *E. acervulina* and

decreased after *E. tenella* infection. IL-1β is a very important pro-inflammatory cytokine that is secreted by many different cell types, with stimulated macrophages being the major producer. Lymphocytes from *Eimeria*-infected chickens produced a higher level of IL-1β protein compared to lymphocytes cells from non-infected birds (Byrnes *et al.,* 1993). The study indicated that 7 days after *E. tenella* infection, mRNA expression of the pro-inflammatory cytokine IL-1β was increased 80-fold (Laurent *et al.,* 2001). IFN-γ is also a major cytokine in mediating resistance to many different parasites and has been shown to inhibit *E. tenella* development *in vitro* (Lillehoj and Choi, 1998). It has also been shown to decrease the production of oocyst and improve body weight gain following *E. acervulina* infection (Lillehoj and Choi, 1998; Lillehoj *et al.,* 2004). The mechanism of IFN-γ activity in *Eimeria* infections is still unclear. The only function of IFN- γ that has been well studied in hosts infected with *Eimeria* is its ability to activate macrophages and other inflammatory cells and then increase capacity of these cells to produce reactive intermediates (Ovington *et al.,* 1995).

Natural killer cells are capable of spontaneous cytotoxicity against a wide variety of target cells. The activity of NK cells increased in the early stages of coccidia infection, which suggested that NK cells play a role in the control of parasite proliferation (Lillehoj, 1989). Coinciding with parasite elimination, there was higher level of NK cell activity in splenic and intraepithelial lymphocytes than in non-infected animals. Thus, the intraepithelial lymphocytes, NK cells, may be involved in defense against infection by coccidia. Research has suggested that macrophages possibly modulate the host response to *Eimeria* infection by producing mediators, such as, TNF (Lillehoj and Trout, 1996).

Vaccines

Since the late 1920s, researchers in the USA have undertaken experiments on immunization of chickens against select *Eimeria*, but it was not until the 1950s that the first commercial vaccines (live oocysts of several *Eimeria* species) for chickens were introduced (Chapman *et al.,* 2005). After the 1950s, anticoccidial drugs in the feed were widely used to control coccidiosis in broilers and other poultry. Although these drugs have for the most part effectively controlled coccidiosis over all these years, resistance has developed and their continued usage is questioned (Chapman, 1997). These pressures caused the European Union to ban several anticoccidial drugs. Many poultry producers are investigating vaccination with live *Eimeria* commercial vaccines as an alternative and the only major practical method for controlling coccidiosis if chemotherapy should threaten to fail or be banned (Chapman *et al.,* 2005). Live *Eimeria* vaccines are the most efficient at producing long-lasting protective immunity, mainly because they can most closely replicate natural intestinal infection (Hong *et al.,* 2006).

By continuous research over the past several decades, the developed vaccines include: recombinant vaccines, live vaccines, and the use of cytokines as adjuvants (Dalloul and Lillehoj, 2005, 2006). Now, only live vaccines are currently available commercially. The production of a live attenuated *Eimeria* vaccine is presently accomplished in any of three ways: by passage through embryonated chicken eggs, by gamma irradiation, or by selection for precocity (Williams, 1998). The best method of attenuation is by selection for precocity discovered by Jeffers (1975). ‘‘Precocious’’ refers to a line of *E. tenella* in the chicken that was selected for a reduced prepatent period (time from inoculation of oocysts to production of new oocysts in the feces), or early

development. The reduced reproductive potential results in attenuation of virulence, maintenance of immunogenicity, and genetically controlled stability. Now the term has been used to refer to lines of other *Eimeria* species selected for early development (Shirley and Millard, 1986).

Because of species-specific parasites, a vaccine for chickens should ideally contain all seven species (Shirley and Millard, 1986; Williams, 1998). According to research, the use of a vaccine may control clinical coccidiosis in broilers and achieve performances at least equal to anticoccidial drugs (Williams *et al.,* 1999). For turkeys, the vaccines contain some or all of the *Eimeria* species, *E. adenoeides*, *E. dispersa*, *E. gallopavonis*, and *E. meleagrimitis*. Usually *E. meleagridis* is considered to have little pathogenicity (Levine, 1985). Therefore, it has not been included in commercial vaccines of turkeys. Recently, a lot of work on has been done on *E. meleagridis*. A precocious line of *E. meleagridis* was developed, which keeps the character of loss of pathogenicity and retains immunogenicity (Matsler and Chapman, 2007). By comparing the acquisition of immunity in an experiment with cage and floor-pen birds, Chapman *et al.* (2005) reported that if live oocysts of *Eimeria* species are used to vaccinate day-old chicks, reinfection by oocysts present in the litter is necessary to establish protective immunity. Therefore, the success of vaccination is mostly dependant on environmental and management factors which affect numbers of infective oocysts in commercial poultry houses (Chapman *et al.,* 2005).

Statement of Hypothesis and Objective

Based on the research of trace minerals and immune response, it was hypothesized that:

1. Supplemental organic trace minerals in the diet may result in similar turkey performance when compared to traditional trace mineral levels, while reduced inorganic trace minerals may decrease the performance.
2. Using organic or inorganic trace minerals at low levels would reduce environmental impacts with decreased mineral content in excreta and litter.
3. Immune response and performance may be improved with the application of organic trace minerals following coccidial vaccination.

Therefore, the objective of this project was to evaluate the effect of various levels and combinations of a commercial organic mineral complex (Cu, Zn, Mn and Se) on the performance, mineral concentration in excreta of commercial tom turkeys, and immune response in commercial turkeys as compared to traditional inorganic trace mineral levels following vaccination with coccidia.

Chapter III. Replacement of Inorganic with Organic Trace Minerals in Commercial Turkey Diets from 0 to 19 Weeks of Age

Replacement of Inorganic with Organic Trace Minerals in Commercial Turkey Diets from 0 to 19 Weeks of Age

Abstract: A study was conducted to compare the effectiveness of low levels of organic minerals to a standard commercial inorganic trace mineral dietary program on tom turkey performance, mineral content in excreta and litter, bone strength, and carcass yield from 0 to 19 wks of age. Day-old Hybrid poults (n=1,224) were randomly assigned to one of four dietary treatments (9 replicates/treatment). Experimental treatments consisted of: 1) standard inorganic industry (SI) with commercial inorganic supplementation program (Mn, Zn, Cu, and Se at 165ppm, 112.5ppm, 10ppm and 0.2ppm, respectively); 2) reduced inorganic (RI) with inorganic trace minerals at 10% of SI; 3) Bioplex®/Sel-Plex® Pak (O1) with Mn, Zn, Cu, Se at 16.5ppm, 11.25ppm, 1ppm and 0.2ppm during starter (0 to 49 days) and inclusion of Mn, Zn, Cu, Se at 11ppm, 7.5ppm, 0.7ppm and 0.2ppm during grower and finisher (49 to 133 days), respectively; 4) Bioplex®/Sel-Plex® Pak (O2) with inclusion of Mn, Zn, Cu, and Se at 16.5ppm, 11.25ppm, 1ppm and 0.2ppm through the full trial (0 to 133 days). Bioplex®/Sel-Plex® treatments supplied organic trace minerals at or below the level of RI. Body weight (BW), body weight gain (BWG), feed conversion ratio (FCR), and feed intake (FI) were recorded, and fresh excreta were collected on d 28, 49, 70, 84, 105, and 133. Tibia and femur were collected at d 49, 84 and 133 for measurement of bone breaking strengths. Litter trace mineral concentration and carcass yield were determined at d 133. Overall, there was no significant effect on BW ($P = 0.78$), cumulative BWG ($P = 0.78$), FCR ($P = 0.74$) or FI ($P = 0.81$) due to treatments. Excreta trace mineral content (Mn and Zn) was reduced ($P < 0.05$) with the feeding treatments Bioplex®/Sel-Plex® or RI compared to SI throughout the trial. Similarly, Cu in excreta was significantly reduced at 84 and 133 of age with these treatments. Mn and Zn

concentration in litter was also reduced when feeding RI or Bioplex®/Sel-Plex® treatments as compared to SI. Femurs and tibias from the RI and Bioplex®/Sel-Plex® treatments had reduced bone strength in early stages of growth (d 49). Carcass yield (cold carcass, whole boneless breast, and breast fillets) at processing was improved ($P < 0.05$) by feeding Bioplex®/Sel-Plex® treatments compared to SI. In summary, the use of a feeding program with inorganic trace minerals at 10% of SI (standard industry) or a Bioplex®/Sel-Plex® organic mineral program in the diet of tom turkeys was adequate for growth and performance. The use of organic minerals also increased carcass yield at slaughter and decreased Mn and Zn content in litter and excreta.

Key words: organic trace minerals, turkey, performance, excreta, carcass yield

Introduction

Trace minerals play vital roles in animal and human normal growth and health because of their significant roles in homeostasis, immune response, antioxidant defense, and reproduction. They are essential for the activation of numerous genes, cofactors of enzymes, and participants in numerous metabolic pathways within the body.

Since the studies to determine requirements of commercial poultry for trace minerals were conducted several decades ago with birds of limited growth or production potential compared to the birds today, there is now a tendency in the commercial poultry industry to over-formulate (provide excess supplementation) to ensure the extra need of trace minerals for increased performance is met (Leeson, 2005). However, most of the excess trace minerals are not absorbed by birds, and the concentration of minerals excreted in the manure will be increased (Williams *et al.,* 1999), which evokes environmental concerns. It was reported that excess Zn intake will result in reduced absorption and increased excretion, and Zn excretion in the manure increased linearly as dietary Zn levels increased (Kim and Patterson, 2004). Therefore, poultry producers have to face the challenge of reducing the impact on the environment while maintaining or improving bird and economic performance. Basic strategies to reduce excess mineral burden from intensive animal production include reducing the total amounts of minerals in the feeds, minimizing their concentration in the manure, and improving the efficiency of use of minerals by the animals (Williams *et al.,* 1999). As a benefit to the industry, organic minerals have been reported to have potentially higher bioavailability, more efficient absorption, and lower excretion levels than inorganic mineral sources (Hahn and Baker, 1993; Case and Carlson, 2002).

Organic minerals are usually chelates of protein and amino acids containing bonded minerals, whose bioavailability is more close to that of amino acids (Leeson, 2005). A number of animal studies have shown that the bioavailability of mineral-amino acid chelates was better than the bioavailability of other forms of minerals, such as oxide or sulfate. Pigs fed proteinated forms of Zn and Cu had higher liver Zn and Cu concentrations than did pigs fed sulfated forms of these metals, which indicates a higher utilization of Zn and Cu of the proteinated form compared with the sulfated sources (Schiavon *et al.,* 2000). The absorption of chelated organic Mn was much higher than inorganic Mn in the small intestinal of broilers, which might be attributable to different absorption modes for organic and inorganic Mn (Ji *et al.,* 2006a). Se from organic sources was more bioavailable than an inorganic source, as evidenced by Se retention and blood Se relative to Se intake (Yoon *et al.,* 2007).

Bioplex® organic trace minerals are forms of chelated mineral proteinate with higher bioavailability. Bioplex® minerals may meet the higher mineral requirements of modern commercial poultry for maximum performance efficiency while reducing excretion levels. Therefore, Bioplex® could be beneficial to both the animal and the environment. A study indicated that the relative bioavailability value of Bioplex® Zn was 183% that of Zn sulfate based on body weight gain (BWG) data and 157% that of Zn sulfate based on the total tibia Zn content (Ao *et al.,* 2006). Recently, Sel-Plex® has been explored as an alternative to inorganic Se supplementation. It is synthesized by growing a specific strain of yeast to produce selenoproteins, which enhance health and performance due to higher bioavailability. The results indicated that Sel-Plex® Se increased tissue Se concentration, with no affect on growth performance (Payne and Southern, 2005).

Trace minerals interact with each other to perform efficient physiological and nutritional functions. The interactions begin at the absorptive level and continue through metabolism (Surai, 2000). However, it is unclear which dietary inclusion levels and combinations are optimally beneficial for poultry (Rebel *et al.,* 2004) while reducing mineral excretion. Thus, the aim of the present study was to evaluate the effect of a commercial organic minerals complex (Cu, Zn, Mn and Se) on the performance and mineral loss in excreta of commercial tom turkeys from 0 to 19 wks of age as compared to traditional or reduced inorganic trace mineral dietary levels.

Materials and Methods

Birds and Diets

A total of 1,224 day-old, male, Hybrid turkey poults were weighed and randomly divided into 36 floor pens with 34 poults per pen. Poults were distributed to 4 treatments, which were assigned within a block (n=9) to minimize any effect of environmental variance in the research building. A corn-soybean meal basal diet was formulated to meet or exceed NRC (1994) recommendations (Table 3.1) for feeding periods consisting of a period 1 (0 to 28 d of age), period 2 (28 to 49 d of age), period 3 (49 to 70 d of age), period 4 (70 to 84 d of age), period 5 (84 to 105 d of age), and period 6 (105 to 133 d of age). The only difference between the treatments was the level and source (organic or inorganic) of trace minerals (Mn, Zn, Cu, Se). Treatments consisted of 1) standard inorganic (SI): a commercial diet with inorganic trace mineral inclusion of Mn, Zn, Cu, and Se at 165ppm, 112.5ppm, 10ppm and 0.2ppm from MnO, ZnO, $CuSO_4$, and Na_2SeO_3, respectively; 2) reduced inorganic (RI): 10% of SI inorganic trace mineral levels; 3)

Bioplex®[1]/Sel-Plex®[2] Pak (O1): organic trace mineral inclusion of Mn, Zn, Cu, and Se at 16.5ppm, 11.25ppm, 1ppm and 0.2ppm during starter (0 to 49 days) and inclusion of Mn, Zn, Cu, and Se at 11ppm, 7.5ppm, 0.7ppm and 0.2ppm during grower and finisher (49 to 133 days), respectively; 4) Bioplex®/Sel-Plex® Pak (O2): inclusion of Mn, Zn, Cu, and Se at 16.5ppm, 11.25ppm, 1ppm and 0.2ppm for the full trial (0 to 133 days), respectively. All diets were formulated to be isocaloric and isonitrogenous. The starter 1 diets were provided in mash form, and the starter 2, grower, and finisher diets were provided in pelleted form. Feed and water were available *ad libitum* throughout the experiment. The lighting was similar to commercial conditions with 24L: 0D from d 0 to d 7, 20L: 4D from d 8 to d 126, and 24L: 0D from d 127 to d 133. Temperature was initially set at 34˚C with adjustments made according to recommended management protocols. The experimental protocol was approved by the Virginia Tech Animal Care Committee (ACC#05-024-APSAC).

Performance Evaluation

At each dietary period change (d 28, 49, 70, 84, and 105) and at the end of the experiment (d 133), turkeys were weighed by pen (28 and 49 days of age) or individually (70, 84, 105 and 133 days of age). Mortality was recorded daily. Feed intake (FI), body weight (BW), body weight gain (BWG), and feed conversion ratio (FCR) were adjusted based for mortality and calculated for each dietary period and cumulatively.

[1] Alltech, Lexington, KY
[2] Alltech, Lexington, KY

Mineral Content in Excreta and Litter

On d 28, 49, 70, 84, 105, and 133, fresh excreta samples were collected from 2 birds per pen and pooled by pen. Feathers were carefully removed from excreta. At the end of the experiment, litter samples were randomly collected from 3, 6, 6, and 5 pens for diets 1 to 4, respectively. The excreta and litter samples were stored at -20˚C until analyzed. The concentration of trace minerals (Cu, Fe, Mn, Zn) in excreta and litter were determined by Inductively Coupled Plasma Optical Emission Spectrometer®[3] (ICP-OES) analysis (Ao *et al.,* 2006). Briefly, the samples were homogenized and dried at 60˚C for 72 h and then ashed at 600˚C overnight in a muffle furnace. The samples were then microwave digested with HNO_3 (AOAC, 1995) before ICP-OES analysis.

Bone Strength

At d 49, 84, and 133, 18 birds per treatment (2 birds per pen) were randomly selected and euthanized by cervical dislocation to remove the femurs and tibias (right leg) for bone breaking strength analysis. After removing the surrounding soft tissue, femurs and tibias were sealed in plastic bags and frozen at -20˚C until mechanical testing. On the day of testing, femurs and tibias were thawed at room temperature. The bone length and diameter were measured by a dial caliper. Bone breaking strength test was performed using a Material Testing System®[4]. Bones were kept moist during testing at room temperature. Loaded for testing at the midpoint of the shaft, the bone was subjected to a shear test at a constant loading rate of 5 mm/min, using a 1000 N load cell. Following mechanical testing, bone wall thickness was measured using a caliper. Reported bone

[3] Varian Analytical Instruments, Walnut Creek, CA
[4] MTS Systems, Eden Prairie, MN

wall thickness was an average of 3 measurements per bone.

Carcass Yield

At 133 days of age, all turkeys were transported (2.5 h) to a commercial processing plant to determine carcass yield. After scalding, feather removal, and evisceration, turkeys were placed in the chill tank. After removal from the chill tank, carcasses were drained for 15 min and then weighed (cold carcass). The whole boneless breast and breast without fillet were weighed from 20 turkeys per treatment.

Statistical Analysis

All data were analyzed using the MIXED procedures of SAS (SAS Institute, Cary, NC, 1999) with pen representing the experiment unit. The statistical model was

$$y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij},$$

where yij is the observed dependent variable, μ is the grand mean, α_i is the ith dietary treatment effect, β_j is the jth random block effect, and ε_{ij} is the error for treatment i of block j ~ N (0, σ_ε). Significant treatment differences were established using the LSMEANS statement in SAS ($P \leq 0.05$).

Results

Performance Evaluation

Overall, there was no significant difference in BW (Table 3.2) or BWG (Table 3.3) between treatments during the entire study. Feed intake (Table 3.4) was not significantly different between treatments with the exception of d 0 to 49 d. During this time, birds fed

the SI diet had significantly reduced FI compared to those fed the O1 diet (P = 0.004), with the RI and O2 diets intermediate. Significant differences in FCR were observed during d 28 to 49 (P = 0.01), d 0 to 49 (P = 0.002) and period 4 (70 to 84 d) (P = 0.007) (Table 3.5). During period 2 (28 to 49 d) and cumulative period of 1 and 2 (0 to 49 d) less efficient FCR was observed in poults supplied O1 diet as compared with poults fed the SI diet. However, during period 4, FCR in birds that consumed the O1 diet was improved compared to SI or O2 fed turkeys, while RI fed turkeys were similar to all treatments. Cumulatively (0 to 133 d), there were no differences between treatments for BWG, FI, or FCR. Treatment had no effect on mortality throughout the trial (data not shown).

Mineral Content in Excreta and Litter

Dietary treatment had no effect on Fe concentration in excreta (Table 3.6) or litter (Table 3.7). On d 28, Cu content in excreta from poults on the RI diet was lower than from poults on the O1diet. Excreta from SI birds had the highest Cu concentrations on d 84 and d 133. On d 84, Cu concentration from SI fed turkeys was significantly higher than that of RI (P = 0.008) or O1 (P = 0.007) fed turkeys, but similar when compared to excreta of poults consuming O2 diets. On d 133, birds fed Bioplex®/Sel-Plex® (O1 and O2) treatments had the lowest excreta Cu concentration, and the O1 diet resulted in significantly less excreta Cu compared with excreta from SI fed turkeys (P < 0.05). Birds fed SI had the highest amounts of Mn and Zn present in excreta throughout the trial, which at all samplings was significantly higher than that in excreta from RI or Bioplex®/Sel-Plex® fed turkeys (P < 0.05). At d 84, 105, and 133, the amounts of Mn and Zn in excreta of birds fed O1 or O2 treatments were the lowest compared with those fed SI or RI diets. The mineral content in litter mirrored the concentrations for Mn and Zn in

excreta at d 133 (Table 3.7). The concentration of litter Mn and Zn were lower in pens with birds fed RI, O1, or O2 treatments as compared to those fed the SI diet ($P < 0.05$).

Bone Strength

At d 49, strength of tibias was significantly ($P = 0.002$) decreased in the birds fed O1 diet as compared to the poults fed the SI diet (Table 3.8). At d 84, birds fed the RI diet had significantly increased peak load of the femur compared to those fed the SI ($P = 0.003$) or O2 diet ($P = 0.0005$) (Table 3.9). Birds fed the O2 diet had the lowest femur shear stress which was significantly lower compared to RI fed turkeys ($P = 0.001$). Femur breaking strength was not affected by treatments at d 49 or 133 or in the tibia at d 84 or 133. There was no effect of dietary treatments on femur/tibia length, diameter, or bone wall thickness at d 49, 84, or 133 (data not shown).

CarcassYield

The poults fed the O1 treatment had improved cold carcass ($P = 0.005$), whole boneless breast ($P = 0.004$), and breast fillet weights ($P = 0.0001$) at processing as compared to SI (Table 3.10). The fillet yield was also increased by feeding of the O2 treatment as compared to the SI diet. Carcass yields in birds fed the RI diet were intermediate to other groups.

Discussion

The results of the effect of inorganic and organic minerals on performance (BW, BWG, FI and FCR) in the present experiment were in agreement with previous published literature. Bartlett and Smith (2003) reported that Zn at low (34mg/kg), adequate

(68mg/kg), or high (181mg/kg) supplementation did not significantly influence broiler growth performance during a 7 wk study. In addition, supplementation with 45ppm Zn from Zn-Met had no effect on BWG or feed efficiency in 21 d old turkeys, as compared to supplying a Zn adequate basal diet (130 ppm Zn) (Kidd *et al.,* 1994a). Mohanna and Nys (1999) explained that a total dietary Zn concentration of 45 ppm was necessary for normal growth in chickens. Body weight gain and FCR were also not affected by the source and level of Mn (Lu *et al.,* 2007) or Se (Payne and Southern, 2005; Yoon *et al.,* 2007) in broilers. Similarly, no difference in growth rate, FCR, or mortality of chickens was observed with 4 to 12 ppm of Cu supplementation (Dozier *et al.,* 2003).

Birds fed SI had the highest amounts of Mn and Zn present in excreta throughout the trial when compared to that in RI or Bioplex®/Sel-Plex® fed turkeys. It was reported that source and level of Cu, Fe, Mn, and Zn in the diet influenced excretion of these minerals. Creech *et al.* (2004) reported that reducing dietary concentrations of Zn, Cu, Mn, and Fe of pig diets caused a significant decrease in fecal mineral excretion without negatively affecting pig performance from weaning through development. Reports have shown that Zn excretion increased linearly with dietary Zn supplementation (Mohanna and Nys, 1999). When minerals are supplemented in excess of the animal's requirement, more minerals tend to be excreted as a result of reduced utilization (Skrivan *et al.,* 2005). Therefore, the excess Mn and Zn were excreted from the poults fed the SI diet, which caused more environmental presence.

The amounts of Mn and Zn in excreta of birds with Bioplex®/Sel-Plex® were the lowest compared with those fed RI diet at d 84, 105, and 133. An experiment showed cumulative Zn excretion was decreased in birds fed Zn in the form of an amino acid

complex compared to Zn sulphate supplementation (Burrell *et al.*, 2004). Similarly, organic Mn was more efficiently absorbed than inorganic Mn (MnSO4) in broilers (Ji *et al.,* 2006a; b). This is probably due to different absorption mechanisms for organic and inorganic forms of trace minerals. Inorganic trace minerals are absorbed passively by simple diffusion in the lumen, which causes competitive absorption among numerous minerals. However, organic trace minerals are absorbed actively by amino acid transport mechanisms (Choct *et al.,* 2004). Alternatively, Dibner *et al.* (2007) summarized another possible mechanism. The inorganic sources of minerals will cause dissociation of salt in low pH of the upper gastrointestinal system. When reaching the higher pH of distal gut segments, minerals can ionize and bind to a number of other minerals, nutrients, and non-nutritive components of the digesta, which results in insolubility of these ions. The insoluble forms of minerals will be excreted. Organic minerals could bind to or associate with amino acids, peptides, proteins, or other ligands, which provide stability in the upper gastrointestinal system. The protected trace minerals are delivered to the epithelium of the small intestine without dissociation in the crop, proventriculus, and gizzard. Therefore, using organic trace minerals or lower levels of inorganic trace minerals may be an efficient way of minimizing excretion rate and reducing environmental impact.

In the present experiment, the concentration of Cu in excreta was not significantly different between treatments at the sampling times with the exception of d 133. This may be because of a low level of Cu supplementation in the SI diet. It was reported that excreta of hens fed a diet with 240 ppm of Cu contained 16 times more Cu than that of hens fed a basal diet with 9.2 ppm Cu (Skrivan *et al.,* 2006). In the present trial, turkeys fed organic Cu resulted in a decreased excretion at d 133 as compared to the inorganic

supplementation in SI.

Bone is a dynamic tissue influenced by physiological, nutritional and physical factors (Rath *et al.,* 2000). Availability of trace minerals, particularly the combination of Zn, Cu, and Mn, plays a critical role in early development of bone because of their function in building structural connective tissue (Dibner *et al.,* 2007), and normal thyroid hormone (T_3 and T_4) metabolism. It was reported that removal of the trace mineral premix decreased bone strength during the grower and finisher periods in chickens (Shelton and Southern, 2006). Coexisting deficiencies of iodine, Fe, Se, and Zn can impair thyroid function, which directly affects cartilage growth and maturation (Zimmermann and Kohrle, 2002). The T_3 receptor is thought to require Zn to adopt its biologically active conformation (Oviedo and Ferket, 2005). Zn is also necessary for chondrocyte proliferation and differentiation and the function of vitamin D receptors, which contain two Zn fingers (Lu *et al.*, 2000). Se is involved in transulfation to form cysteine from methionine and in the deionization of thyroid hormones from T_4 to T_3 by 3, 5, 3'-triiodotironina (Oviedo and Ferket, 2005). These processes are necessary for chondrocyte maturation. Cu influences bone formation, skeletal mineralization, and the crosslinking of collagen and elastin (Carlton and Henderson, 1964). Cu-deficiency (< 1 ppm) was shown to decrease collagen crosslink formation and to lower mineralization (Opsahl *et al.*, 1982). Mn is essential for the development of organic matrix formation of bone, maintains bone mineralization, and is a co-factor for several enzymes in bone tissue (Palacios, 2006).

In the present trial, bone breaking strength of the tibia was reduced at d 49 when using low supplementation of trace minerals. It was reported that the levels of trace

minerals deposited in bone increase with the dietary level offered (Oviedo and Ferket, 2005). Lu *et al.* (2007) reported the lowest percentage of leg abnormality was observed with Mn supplemented at 200 ppm as compared to soybean meal-based diet at 21.3 ppm or the basal diet supplemented with Mn at 100 ppm regardless of Mn source. Se-deficiency resulted in a 23% and 21% reduction in bone mineral density of the femur and tibia in growing male rats (Moreno-Reyes *et al.*, 2001). Therefore, it was not surprising to observe the improved tibial bone strength in poults with the SI diet at d 49, since the inclusion of trace minerals in SI diet was the highest compared to other treatments. The source of trace minerals is also important for bone development. It was reported that supplementation of 20 and 40 ppm of Zn and Mn from methionine chelate significantly reduced the incidence of shaky leg and angular defects in half in comparison to inorganic sulfate forms of these minerals in turkeys (Ferket *et al.*, 1992). Banks *et al.* (2004) observed that supplementation with 250 ppm of Cu from Cu lysine resulted in chicks with greater toe and tibia ash weights and percentage as compared with birds supplemented with Cu sulfate. However, no improved effect in tibia and femur was observed with the supplementation of organic trace minerals at d 49 in the present study, which may be due to low inclusion levels in the diet. At d 84, the peak load of femur was significantly decreased with SI and O2 diets compared to RI and O1. The reason for this result was not clear. However, it seems the poults did not need excess trace minerals to maintain bone development after the early stage. There were no differences later in production, but bone integrity may be an issue in the early stage of the production cycle.

Carcass yield was improved in birds fed diets with the organic trace minerals in the present trial, which may be caused by the effect of organic Se. It was reported that

broilers fed diets supplemented with organic Se had significantly improved eviscerated weight, breast yield, and reduced drip loss (Choct *et al.,* 2004). It was explained that organic Se may have enhanced the efficiency of protein deposition or water retention in tissues. Mateo *et al.* (2007) reported that organic Se supplementation reduced drip loss of growing-finishing pigs. However, Payne and Southern (2005) found that the eviscerated weight, chill weight, and breast fillet weight were not affected by the source of Se, although organic Se increased tissue Se concentration.

In summary, using organic trace minerals in turkey diets as compared to standard industry inorganic trace minerals had no negative effect on cumulative growth performance, but did improve carcass yield. Additionally, potential environmental impact of trace minerals in litter (Mn and Zn) was significantly reduced with the use of the organic minerals and reduced levels of inorganic trace minerals. However, data suggest that bone integrity may be an issue early in the production cycle, although no mortality was observed related to bone abnormalities. Therefore, using organic or inorganic trace minerals at low levels may be an effective replacement for higher levels of inorganic trace minerals in commercial turkey diets.

Table 3.1. Composition and nutrient content of basal diets1

Item[2]	Period 1	Period 2	Period 3	Period 4	Period 5	Period 6
Ingredient	----------(%)----------					
Corn	41.67	44.10	53.69	57.72	65.03	65.68
Wheat middlings	5.00	5.00	-	-	-	-
Soybean meal	35.25	32.75	29.15	26.9	18.65	19.7
PBM	7.00	8.00	8.00	8.00	8.00	4.00
Seapreme (65.5)	4.00	3.00	2.00	-	-	-
Limestone	0.70	0.65	0.75	0.80	0.65	0.80
Phosphate (18.5)	1.60	1.45	1.10	0.40	-	-
Phosphate (18)	0.50	0.50	1.00	1.00	1.00	1.00
Fat-(3800)	2.50	3.00	3.00	4.00	5.50	8.00
Rhodimet	0.32	0.33	0.27	0.225	0.245	0.235
Lysine-Liquid	0.45	0.42	0.395	0.295	0.345	0.165
Natuphos	-	-	-	0.005	0.005	0.005
Choline chloride	0.16	0.14	0.105	0.105	0.05	0.045
Allzyme Vegpro	0.05	0.05	0.05	0.05	0.05	0.05
MycoCURB	0.10	0.05	0.05	0.05	-	-
Sodium bicarbonate	0.10	0.10	-	-	-	-
Vitamin E	0.012	-	-	-	-	0.012
Vitamin premix	0.115	0.10	0.075	0.06	0.05	0.025
Salt	0.10	0.075	0.145	0.195	0.23	0.185
Copper sulfate	0.05	0.05	0.05	0.05	0.05	-
Hy D	0.05	0.05	0.035	0.035	0.035	-
BIO-MOS	0.10	0.10	0.05	0.05	0.05	0.05
MTB-100	0.10	-	-	-	-	-
3-Nitro 20	0.025	0.025	0.025	-	-	-
Calculated nutrient content						
ME (kcal/kg)	2,898.7	2,949.3	3,061.7	3,174.0	3,330.4	3,486.8
CP (%)	28.0	27.0	24.4	22.7	19.0	17.5
Fat (%)	5.50	6.00	5.91	6.78	8.40	10.40
Calcium (%)	1.48	1.46	1.50	1.35	1.18	1.00
Available phosphate (%)	1.05	1.02	0.98	0.80	0.69	0.60
Sodium	0.17	0.18	0.20	0.21	0.22	0.18

[1] Period 1= 0 to 28 d of age; period 2= 28 to 49 d of age; period 3= 49 to 70 d of age; period 4= 70 to 84 d of age; period 5= 84 to 105 d of age; period 6= 105 to 133 d of age.

[2] PBM=poultry by-product meal; Rhodimet was from Adisseo (Alpharetta, GA); Natuphos was from BASF (Florham Park, NJ); Allzyme Vegpro was from Alltech (Lexington, KY); MycoCURB was from Renaissance Nutrition (Roaring Spring, PA); Vitamin premix supplied (per kg of diet): vitamin A (retinyl acetate), IU; vitamin D_3, ICU; vitamin K_3 (menadione dimethylpyrimidinol bisulfite), mg; thiamin, mg; riboflavin, mg; niacin, mg; folic acid, mg; biotin, mg; vitamin B12 (cyanocobalamin), µg. Hy D was from DSM (Parsippany, NJ); BIO-MOS and MTB-100 were from Alltech (Lexington, KY); 3-Nitro 20 was from Alpharma Animal Health (Fort Lee, NJ).

Table 3.2. Effect of organic or inorganic trace mineral supplementation on BW of commercial turkeys (kg/bird)

Age (day)	0	28	49	70	84	105	133
Diet[1]							
SI	0.0533	1.272	3.625	7.066	9.302	12.771	16.674
RI	0.0536	1.297	3.703	7.143	9.463	12.764	16.710
O1	0.0535	1.304	3.676	7.074	9.465	12.966	16.775
O2	0.0538	1.276	3.659	6.972	9.246	12.706	16.892
SEM[2]	0.0002	0.0160	0.0403	0.0718	0.0868	0.1302	0.1614

[1] SI=standard inorganic (a commercial diet with inorganic trace mineral inclusion of Mn, Zn, Cu, Se at 165ppm, 112.5ppm, 10ppm and 0.2ppm from MnO, ZnO, CuSO4 and Na2SeO3, respectively); RI=reduced inorganic (10% of SI inorganic trace mineral levels); O1= Bioplex®/Sel-Plex® Pak inclusion of Mn, Zn, Cu, Se at 16.5ppm, 11.25ppm, 1ppm and 0.2ppm during starter (0-49 days), inclusion of Mn, Zn, Cu, Se at 11ppm, 7.5ppm, 0.7ppm and 0.2ppm during grower and finisher (49-133days); O2= Bioplex®/Sel-Plex® Pak inclusion of Mn, Zn, Cu, Se at 16.5ppm, 11.25ppm, 1ppm and 0.2ppm through the full trial (0-133 days).

[2] All means are an average of 9 pens per treatment.

Table 3.3. Effect of organic or inorganic trace mineral supplementation on BWG of commercial turkeys (kg/bird per period)

	Period 1	Period 2		Period 3	Period 4		Period 5	Period 6	Cumulative
Diet[1]	0 to 28 d	28 to 49 d	0 to 49 d	49 to 70 d	70 to 84 d	0 to 84 d	84 to 105 d	105 to 133 d	0 to 133 d
SI	1.219	2.353	3.572	3.381	2.222	9.249	3.481	3.886	16.621
RI	1.243	2.406	3.649	3.416	2.307	9.409	3.299	3.900	16.657
O1	1.250	2.372	3.622	3.371	2.390	9.411	3.511	3.848	16.722
O2	1.222	2.383	3.605	3.289	2.271	9.193	3.455	4.122	16.838
SEM[2]	0.0160	0.0296	0.0402	0.0494	0.0531	0.0868	0.0707	0.1282	0.1614

[1] SI=standard inorganic (a commercial diet with inorganic trace mineral inclusion of Mn, Zn, Cu, Se at 165ppm, 112.5ppm, 10ppm and 0.2ppm from MnO, ZnO, CuSO4 and Na2SeO3, respectively); RI=reduced inorganic (10% of SI inorganic trace mineral levels); O1= Bioplex®/Sel-Plex® Pak inclusion of Mn, Zn, Cu, Se at 16.5ppm, 11.25ppm, 1ppm and 0.2ppm during starter (0-49 days), inclusion of Mn, Zn, Cu, Se at 11ppm, 7.5ppm, 0.7ppm and 0.2ppm during grower and finisher (49-133days); O2= Bioplex®/Sel-Plex® Pak inclusion of Mn, Zn, Cu, Se at 16.5ppm, 11.25ppm, 1ppm and 0.2ppm through the full trial (0-133 days).

[2] All means are an average of 9 pens per treatment.

Table 3.4. Effect of organic or inorganic trace mineral supplementation on FI (kg/bird per period) of commercial turkeys

	Period 1	Period 2		Period 3	Period 4		Period 5	Period 6	Cumulative
Diet[1]	0 to 28 d	28 to 49 d	0 to 49 d	49 to 70 d	70 to 84 d	0 to 84 d	84 to 105 d	105 to 133 d	0 to 133 d
SI	1.625	4.030	5.655[b]	7.421	6.464	19.540	10.138	10.955	40.721
RI	1.652	4.253	5.905[ab]	7.405	6.284	19.594	9.622	10.526	39.742
O1	1.761	4.348	6.108[a]	7.235	6.279	19.623	10.383	10.270	40.269
O2	1.685	4.245	5.930[ab]	7.412	6.631	19.974	10.075	10.627	40.676
SEM[2]	0.0416	0.0819	0.0993	0.1701	0.1035	0.2727	0.2444	0.4210	0.8283

[a-b] Means within a column without common superscripts are different ($P \leq 0.05$).

[1] SI=standard inorganic (a commercial diet with inorganic trace mineral inclusion of Mn, Zn, Cu, Se at 165ppm, 112.5ppm, 10ppm and 0.2ppm from MnO, ZnO, CuSO4 and Na2SeO3, respectively); RI=reduced inorganic (10% of SI inorganic trace mineral levels); O1= Bioplex®/Sel-Plex® Pak inclusion of Mn, Zn, Cu, Se at 16.5ppm, 11.25ppm, 1ppm and 0.2ppm during starter (0-49 days), inclusion of Mn, Zn, Cu, Se at 11ppm, 7.5ppm, 0.7ppm and 0.2ppm during grower and finisher (49-133days); O2= Bioplex®/Sel-Plex® Pak inclusion of Mn, Zn, Cu, Se at 16.5ppm, 11.25ppm, 1ppm and 0.2ppm through the full trial (0-133 days).

[2] All means are an average of 9 pens per treatment.

Table 3.5. Effect of organic or inorganic trace mineral supplementation on FCR of commercial turkeys

	Period 1	Period 2		Period 3	Period 4		Period 5	Period 6	Cumulative
Diet[1]	0 to 28 d	28 to 49 d	0 to 49 d	49 to 70 d	70 to 84 d	0 to 84 d	84 to 105 d	105 to 133 d	0 to 133 d
SI	1.334	1.712[a]	1.582[a]	2.199	2.932[b]	2.113	2.921	2.822	2.448
RI	1.329	1.769[ab]	1.619[ab]	2.168	2.730[ab]	2.082	2.925	2.727	2.388
O1	1.408	1.836[b]	1.685[b]	2.146	2.630[a]	2.084	2.973	2.677	2.407
O2	1.379	1.779[ab]	1.645[ab]	2.260	2.940[b]	2.175	2.922	2.595	2.415
SEM[2]	0.0273	0.0322	0.0204	0.0564	0.0723	0.0298	0.0615	0.1230	0.0381

[a-b] Means within a column without common superscripts are different ($P \leq 0.05$).

[1] SI=standard inorganic (a commercial diet with inorganic trace mineral inclusion of Mn, Zn, Cu, Se at 165ppm, 112.5ppm, 10ppm and 0.2ppm from MnO, ZnO, CuSO4 and Na2SeO3, respectively); RI=reduced inorganic (10% of SI inorganic trace mineral levels); O1= Bioplex®/Sel-Plex® Pak inclusion of Mn, Zn, Cu, Se at 16.5ppm, 11.25ppm, 1ppm and 0.2ppm during starter (0-49 days), inclusion of Mn, Zn, Cu, Se at 11ppm, 7.5ppm, 0.7ppm and 0.2ppm during grower and finisher (49-133days); O2= Bioplex®/Sel-Plex® Pak inclusion of Mn, Zn, Cu, Se at 16.5ppm, 11.25ppm, 1ppm and 0.2ppm through the full trial (0-133 days).

[2] All means are an average of 9 pens per treatment.

Table 3.6. Effect of organic or inorganic trace mineral supplementation on excreta trace mineral concentration (ppm)

Diet[1]	SI	RI	O1	O2	SEM[2]
Fe					
d 28	719.54	674.99	621.61	630.09	40.88
d 49	829.86	807.53	792.29	790.35	46.70
d 70	960.37	868.91	864.43	875.44	63.98
d 84	561.44	543.16	596.37	583.49	20.00
d 105	496.25	597.78	535.62	520.75	40.73
d 133	474.95	443.79	435.21	427.04	20.20
Cu					
d 28	292.51^{ab}	257.97^{b}	327.85^{a}	306.00^{ab}	15.11
d 49	318.87	338.24	319.61	318.72	22.20
d 70	307.26	315.35	330.89	277.09	20.61
d 84	366.22^{a}	285.13^{b}	282.95^{b}	312.30^{ab}	19.78
d 105	409.22	314.87	431.96	395.37	34.01
d 133	150.79^{a}	82.98^{ab}	46.44^{b}	57.87^{ab}	25.01
Mn					
d 28	389.46^{a}	209.46^{b}	173.08^{b}	208.06^{b}	18.54
d 49	653.46^{a}	153.34^{c}	252.05^{b}	170.98^{c}	14.56
d 70	563.11^{a}	259.13^{b}	301.51^{b}	143.93^{c}	13.32
d 84	616.08^{a}	175.55^{b}	126.44^{c}	131.54^{c}	9.51
d 105	608.87^{a}	216.08^{b}	118.62^{c}	129.16^{c}	10.93
d 133	473.23^{a}	159.21^{b}	114.05^{b}	116.81^{b}	17.23
Zn					
d 28	361.06^{a}	198.08^{b}	190.81^{b}	219.41^{b}	13.91
d 49	454.15^{a}	141.79^{c}	220.22^{b}	160.69^{c}	10.56
d 70	496.00^{a}	257.53^{c}	312.38^{b}	150.81^{d}	12.37
d 84	510.49^{a}	197.67^{b}	144.99^{c}	159.60^{bc}	12.76
d 105	550.90^{a}	255.11^{b}	177.56^{c}	180.74^{c}	13.30
d 133	473.39^{a}	193.34^{b}	164.82^{b}	163.52^{b}	23.67

$^{a-c}$ Means within a row without common superscripts are different ($P \leq 0.05$).

[1] SI=standard inorganic (a commercial diet with inorganic trace mineral inclusion of Mn, Zn, Cu, Se at 165ppm, 112.5ppm, 10ppm and 0.2ppm from MnO, ZnO, CuSO4 and Na2SeO3, respectively); RI=reduced inorganic (10% of SI inorganic trace mineral levels); O1= Bioplex®/Sel-Plex® Pak inclusion of Mn, Zn, Cu, Se at 16.5ppm, 11.25ppm, 1ppm and 0.2ppm during starter (0-49 days), inclusion of Mn, Zn, Cu, Se at 11ppm, 7.5ppm, 0.7ppm and 0.2ppm during grower and finisher (49-133days); O2= Bioplex®/Sel-Plex® Pak inclusion of Mn, Zn, Cu, Se at 16.5ppm, 11.25ppm, 1ppm and 0.2ppm through the full trial (0-133 days).

[2] All means are an average of 9 pens per treatment.

Table 3.7. Effect of organic or inorganic trace mineral supplementation on litter[1] mineral content (ppm)

	Cu	Fe	Mn	Zn
Diet[2]				
SI	303.80 ± 12.28	628.89 ± 25.18	447.41 ± 12.65[a]	407.70 ± 12.01[a]
RI	281.02 ± 7.18	652.12 ± 14.72	195.14 ± 7.39[b]	205. 29 ± 7.02[b]
O1	311.28 ± 8.29	644.17 ± 17.00	173.94 ± 8.54[bc]	193.62 ± 8.10[b]
O2	290.70 ± 9.31	628.89 ± 19.10	139.51 ± 9.59[c]	153.53 ± 9.11[c]

[a-c] Means within a column without common superscripts are different ($P \leq 0.05$).
[1] Values represent the mean of 3, 6, 6 and 5 pens for diets 1-4, respectively.
[2] SI=standard inorganic (a commercial diet with inorganic trace mineral inclusion of Mn, Zn, Cu, Se at 165ppm, 112.5ppm, 10ppm and 0.2ppm from MnO, ZnO, CuSO4 and Na2SeO3, respectively); RI=reduced inorganic (10% of SI trace mineral levels); O1= Bioplex®/Sel-Plex® Pak inclusion of Mn, Zn, Cu, Se at 16.5ppm, 11.25ppm, 1ppm and 0.2ppm during starter (0-49 days), inclusion of Mn, Zn, Cu, Se at 11ppm, 7.5ppm, 0.7ppm and 0.2ppm during grower and finisher (49-133days); O2= Bioplex®/Sel-Plex® Pak inclusion of Mn, Zn, Cu, Se at 16.5ppm, 11.25ppm, 1ppm and 0.2ppm through the full trial (0-133 days).

Table 3.8. Effect of organic or inorganic trace mineral supplementation on bone breaking strengths of tibia in commercial turkeys

Diet[1]	SI	RI	O1	O2	SEM[2]
d 49					
Peak load (N)	872.7	813.1	698.1	695.1	47.03
Fracture deflection (mm)	1.26	1.29	1.53	1.29	0.113
Strength (N/mm)	708.3[a]	651.1[ab]	503.2[b]	574.3[ab]	40.56
Shear stress (N/mm^2)	17769	14706	14343	14437	1199.1
d 84					
Peak load (N)	1042.4	1185.0	1059.4	977.6	86.76
Fracture deflection (mm)	1.58	1.57	1.37	1.32	0.143
Strength (N/mm)	704.3	749.5	813.0	757.4	59.26
Shear stress (N/mm^2)	34712	37732	34629	27933	3474.3
d 133					
Peak load (N)	1249.4	1164.3	1321.1	1249.5	86.92
Fracture deflection (mm)	1.50	1.40	1.47	1.51	0.109
Strength (N/mm)	879.9	909.6	923.5	882.0	81.92
Shear stress (N/mm^2)	46390	37780	42980	42639	4083.3

[a-b] Means within a column without common superscripts are different ($P \leq 0.05$).
[1] SI=standard inorganic (a commercial diet with inorganic trace mineral inclusion of Mn, Zn, Cu, Se at 165ppm, 112.5ppm, 10ppm and 0.2ppm from MnO, ZnO, CuSO4 and Na2SeO3, respectively); RI=reduced inorganic (10% of SI trace mineral levels); O1= Bioplex®/Sel-Plex® Pak inclusion of Mn, Zn, Cu, Se at 16.5ppm, 11.25ppm, 1ppm and 0.2ppm during starter (0-49 days), inclusion of Mn, Zn, Cu, Se at 11ppm, 7.5ppm, 0.7ppm and 0.2ppm during grower and finisher (49-133days); O2= Bioplex®/Sel-Plex® Pak inclusion of Mn, Zn, Cu, Se at 16.5ppm, 11.25ppm, 1ppm and 0.2ppm through the full trial (0-133 days).
[2] All means are an average of 9 pens per treatment.

Table 3.9. Effect of organic or inorganic trace mineral supplementation on bone breaking strengths of femur in commercial turkeys

Diet[1]	SI	RI	O1	O2	SEM[2]
d 49					
Peak load (N)	831.1	756.3	765.5	761.7	22.59
Fracture deflection (mm)	1.39	1.32	1.38	1.30	0.130
Strength (N/mm)	674.3	577.1	583.6	592.7	45.16
Shear stress (N/mm^2)	22570	19412	20402	19772	859.3
d 84					
Peak load (N)	1071.1^{b}	1220.2^{a}	1118.5^{ab}	1006.4^{b}	32.15
Fracture deflection (mm)	2.11	2.36	1.89	2.12	0.128
Strength (N/mm)	526.6	520.8	595.4	498.6	34.32
Shear stress (N/mm^2)	42414^{ab}	49300^{a}	44105^{ab}	37257^{b}	1985.5
d 133					
Peak load (N)	1416.5	1511.7	1516.8	1356.5	86.28
Fracture deflection (mm)	2.43	2.13	2.09	1.84	0.184
Strength (N/mm)	585.5	734.1	775.1	790.7	73.68
Shear stress (N/mm^2)	49131	52539	54791	47704	3805.4

[a-b] Means within a column without common superscripts are different ($P \leq 0.05$).

[1] SI=standard inorganic (a commercial diet with inorganic trace mineral inclusion of Mn, Zn, Cu, Se at 165ppm, 112.5ppm, 10ppm and 0.2ppm from MnO, ZnO, CuSO4 and Na2SeO3, respectively); RI=reduced inorganic (10% of SI trace mineral levels); O1= Bioplex®/Sel-Plex® Pak inclusion of Mn, Zn, Cu, Se at 16.5ppm, 11.25ppm, 1ppm and 0.2ppm during starter (0-49 days), inclusion of Mn, Zn, Cu, Se at 11ppm, 7.5ppm, 0.7ppm and 0.2ppm during grower and finisher (49-133days); O2= Bioplex®/Sel-Plex® Pak inclusion of Mn, Zn, Cu, Se at 16.5ppm, 11.25ppm, 1ppm and 0.2ppm through the full trial (0-133 days).

[2] All means are an average of 9 pens per treatment.

Table 3.10. Effect of organic or inorganic trace mineral supplementation on carcass yield of commercial turkeys (kg)

	Cold carcass	Breast w/o fillets	Fillets
Diet[1]			
SI	13.044[b]	2.966[b]	0.684[c]
RI	13.377[ab]	3.115[ab]	0.710[bc]
O1	14.071[a]	3.342[a]	0.796[a]
O2	13.939[ab]	3.165[ab]	0.762[ab]
SEM[2]	0.2457	0.0873	0.0191

[a-c] Means within a column without common superscripts are different ($P \leq 0.05$).

[1] SI=standard inorganic (a commercial diet with inorganic trace mineral inclusion of Mn, Zn, Cu, Se at 165ppm, 112.5ppm, 10ppm and 0.2ppm from MnO, ZnO, CuSO4 and Na2SeO3, respectively); RI=reduced inorganic (10% of SI trace mineral levels); O1= Bioplex®/Sel-Plex® Pak inclusion of Mn, Zn, Cu, Se at 16.5ppm, 11.25ppm, 1ppm and 0.2ppm during starter (0-49 days), inclusion of Mn, Zn, Cu, Se at 11ppm, 7.5ppm, 0.7ppm and 0.2ppm during grower and finisher (49-133days); O2= Bioplex®/Sel-Plex® Pak inclusion of Mn, Zn, Cu, Se at 16.5ppm, 11.25ppm, 1ppm and 0.2ppm through the full trial (0-133 days).

[2] All means are an average of 20 turkeys at 133 days of age.

Chapter IV. Effect of Source and Level of Zinc Combined with Manganese, Copper, and Selenium on Performance and Immune Response of Young Female Turkeys

Effect of Source and Level of Zinc Combined with Manganese, Copper, and Selenium on Performance and Immune Response of Young Female Turkeys

Abstract: An experiment was conducted to compare the effect of using commercially available organic or inorganic Zn in combination with other trace minerals on performance, immune response, intestinal morphology, and mucin production of commercial turkey hens from 0 to 6 wks of age. A total of 2,376 day old Hybrid Converter turkey hens were randomly distributed to 72 floor pens. A 4 by 2 factorial design was utilized with coccidia vaccinated and non-vaccinated and 4 dietary treatments varying in Zn concentration in organic or inorganic form with other trace minerals. Each combination of vaccination and dietary treatment was replicated 9 times. Dietary treatments consisted of: 1) standard inorganic (SI), inorganic Zn at 150 ppm with inorganic source of Mn (165 ppm), Cu (10 ppm), and Se (0.2 ppm); 2) reduced inorganic (RI), inorganic Zn, Mn, and Cu at 10% of SI, and inorganic Se at 0.2ppm; 3) organic 1 (O1), organic Zn at 15 ppm with organic source of Mn (16.5 ppm), Cu (1 ppm), and Se (0.2 ppm); 4) organic 2 (O2), organic Zn at 30 ppm with the same level and source of other trace minerals as O1. Body weight (BW), body weight gain (BWG), feed intake (FI) and adjusted feed conversion ratio (FCR) were calculated at d7, 14, 21, 28, 35, and 42. Immune organ weights (bursa, thymus, and spleen) and tissue from duodenum and jejunum were collected at d7, 14, 28, and 42. All organ weights were analyzed as a percentage of BW. Peripheral blood was collected to evaluate T-lymphocyte populations on d 21, 28 and 42. Cumulative FI was influenced by vaccination ($P = 0.003$) from 0 to 6 wk of age, and cumulative BWG was significantly ($P = 0.03$) increased in poults fed the RI compared with the O2 diet.Vaccination with a commercial coccidia vaccine significantly ($P < 0.05$) decreased cumulative BWG and BW at d 7, 21, 28, 35, and 42.

Body weight was significantly decreased in poults fed the O2 diet compared with the RI diet at d 28, 35, and 42. Cumulative FCR was not affected by either diet ($P = 0.37$) or vaccination ($P = 0.22$). Vaccination with a live coccidia vaccine significantly increased spleen weight at d 7 ($P = 0.01$) and thymus weight at d 42 ($P = 0.01$). The birds fed the O2 diet had increased ($P < 0.05$) thymus weight compared with those fed the SI diet at d 7. Vaccinated birds had a significantly greater number of CD4+ T-cells than did the non-vaccinated birds at d 28 ($P = 0.008$) and d 42($P = 0.0002$). An interaction between diet and vaccination was observed for CD4+ T-cell numbers at d 28 ($P = 0.03$) and d 42 ($P = 0.01$). There was no difference in CD8+ T-cell numbers during the experiment. The ratio of CD4+ to CD8+ T cells was significantly ($P = 0.05$) increased only in vaccinated poults at d 42 when compared to non-vaccinated. Jejunal villus height in vaccinated birds was significantly increased, while no effect of diet was observed on villus height, crypt depth, or the ratio of villus height to crypt depth in duodenum or jejunum. In conclusion, using low levels of organic or inorganic trace minerals was adequate to maintain turkey performance and immune response.

Key words: trace minerals, turkeys, coccidia, performance, immune response

Introduction

Trace minerals are involved in many metabolic processes and are critical for immune function in animals. For example, animal studies in the 1930s first documented the essential requirement of Zn for normal growth and health in rats (Todd *et al.,* 1933). Subsequently, Zn was found to be deficient in swine, poultry, cattle and humans. Zn is a cofactor of more than 200 enzymes and plays an important role in function of the immune system and disease resistance. It is necessary for normal host defense and immune system ontogeny, and it is crucial for the development of normal humoral and cellular immune responses (Rink and Gabriel, 2000). Zinc deficiency will cause thymic atrophy and result in ineffective responses to bacterial, viral, and parasitic pathogens, subsequently affecting the health and performance of animals (Fletcher *et al.,* 1988).

In commercial production, to ensure they meet trace mineral requirements of broilers and turkeys with rapid growth rates and the egg output of layers, nutritionists often use excess trace minerals. However, the use of high levels of inorganic Zn, which result in excess Zn in excreta, may result in environmental concerns in the future. Interest in using organic minerals has increased because of the reported potential of higher bioavailability, more efficient absorption, and lower inclusion level as compared to inorganic mineral sources (Hahn and Baker, 1993). Research reported organic complexes of Zn provide a more available source of Zn in chicks (Wedekind *et al.,* 1992), although improvements in Zn availability and growth performance in pigs have not been consistently demonstrated (Hill *et al.,* 1986; Swinkels *et al.,* 1996). Others have suggested that organic Zn complexes may be metabolized differently (Spears, 1989; Kidd *et al.,* 1996). Zn methionine (Zn-Met) has been shown to heighten cellular immunity in

poultry as compared to that of Zn sulphate (Ferket and Qureshi, 1992) or Zn oxide (Kidd *et al.,* 1993). In addition, enhancement of immunity by dietary Zn seems to be primarily attributable to components of the cellular immune system. Dietary supplementation with Zn-Met has been shown to improve cellular immunity in progeny of broiler breeders (Kidd *et al.,* 1993), to increase macrophage tumoricidal activity in young turkeys (Ferket and Qureshi, 1992), and to improve progeny survival during an *E. coli* challenge in chickens (Flinchum *et al.,* 1989).

Stress and disease have a direct bearing on the nutrient requirements of animals (Taylor-Pickard, 2005). Under 'optimal level of stress', growth rate, feed efficiency, and productivity are maximized (Gross and Siegel, 1997). Higher levels of stress increase immune defense against bacteria and parasites; however, there is a cost in "resource allocation" of growth and reproduction (Gross and Siegel, 1997). Mullan and D'Souza (2005) suggested studies with disease challenge are a good way to approach determining mineral requirements for modern animals, since there are one or more diseases encountered in commercial industry. Therefore, disease challenge situations may be needed to demonstrate the complete potential of organic or inorganic trace minerals.

Avian coccidiosis is a major parasitic disease of poultry, which results in substantial economic burden with mortality and inefficient feed utilization (Dalloul and Lillehoj, 2005). Beach and Corl (1925) first reported that chickens infected with live coccidia resisted challenge with the same parasite. Therefore, vaccination, which induces strong protective immunity in hosts, is a feasible means to control coccidiosis (Lillehoj *et al.,* 2004).

Previous research has demonstrated that dietary supplementation with as little as

11.25 ppm organic Zn is sufficient to provide similar growth and performance compared to a commercial diet (150 ppm used commercially in an inorganic form). However, in that study there was no immunological challenge. It has not yet been determined what level of Zn supplementation is required to maintain immune system competency when turkeys are under stressful or disease challenge situations. The objective of the current research was to evaluate different levels of organic and inorganic Zn in combination with Mn, Cu, and Se to determine the effects of these minerals on the response of the immune system following the use of a commercial turkey coccidial vaccine.

Materials and Methods

Birds and Diets

A total of 2,376 day old Hybrid Converter female turkeys were obtained on day of hatch and randomly distributed to 72 floor pens with clean pine shavings (n=33 turkeys/pen; density = 0.96 ft^2) where they were reared until 42 d of age. A 4 by 2 factorial treatment design consisted of two vaccine treatments (coccidia vaccinated and non-vaccinated poults) and 4 dietary treatments varying in Zn concentration in an organic or inorganic form. The dietary treatments included: 1) standard inorganic (SI), inorganic Zn (150 ppm) with inorganic sources of Mn (165 ppm), Cu (10 ppm), and Se (0.2 ppm); 2) reduced inorganic (RI), inorganic Zn, Mn, Cu at 10% of SI, and inorganic Se (0.2ppm); 3) organic 1 (O1), organic Zn (15 ppm) with organic sources of Mn (16.5 ppm), Cu (1 ppm), and Se (0.2 ppm); 4) organic 2 (O2), organic Zn (30 ppm) with the same level and source of other organic trace minerals as O1.

Within each of four dietary treatments, there were groups of poults that were

either vaccinated or not vaccinated with a live coccidial vaccine for a total of 8 treatments. Poults vaccinated received Coccivac-T®[5], a commercial product, which contains *E. adenoeides*, *E. meleagrimitis*, *E. gallopavonis*, *and E. dispersa*, prior to placement. Each vaccination and dietary treatment combination was replicated 9 times (pen). A corn-soybean meal (SBM) basal diet was formulated to meet or exceed NRC (1994) recommendations (Table 4.1) for pre-starter (0 to 21 d of age) and starter (21 to 42 d of age) periods. The pre-starter diet was in crumbled form, while the starter was in pelleted form. Feed and water (nipple drinkers) were administered *ad libitum* throughout the trial. The lighting was similar to commercial conditions with 24L: 0D from d 0 to d 7, 20L: 4D from d 8 to d 42. Turkeys were grown using commercially recommended management procedures. The experimental protocol was approved by the Virginia Tech Institutional Animal Care and Use Committee (#07-008-APSC).

Performance Evaluation

On d 0, 7, 14, 21, 28, 35, and 42, turkeys were weighed by pen. Birds were culled based on leg problems, severe clinical morbidity, or delayed growth. Mortality and culls were recorded daily. Feed intake (FI), body weight (BW), body weight gain (BWG), and feed conversion ratio (FCR) were adjusted based on the record of mortality and calculated for weekly, feeding (0 to 21 d and 21 to 42 d), and cumulative (0 to 42 d) periods.

Immune Organs Weight

Nine turkeys from each treatment (1 turkey per replicate) were euthanized to

[5] Schering-Plough, Millsboro, DE

obtain immune organs on d 7, 14, 28 and 42. Bursa, thymus (right side), and spleen were collected and weighed. Organ weights were expressed as a percentage of live BW to evaluate the development of immune organs.

Mineral Content Analysis in Livers

On d 7 and 14, livers were collected from the same bird (1 turkey per replicate) used for immune organ analysis to determine trace mineral concentration. Liver samples were stored at -20˚C until analysis. The samples were homogenized and dried with a Genesis 25SQ EL Freeze Dryer®[6]. The trace minerals concentration (Cu, Mn, Se, Zn) in livers was measured by Inductively Coupled Plasma Optical Emission Spectrometer®[7] (ICP-OES) analysis (Ao *et al.,* 2006). Samples were homogenized and dried at 60˚C for 72 h and then ashed at 600˚C overnight in a muffle furnace. The samples were then microwave digested with HNO_3 (AOAC, 1995) before ICP-OES analysis.

T-lymphocyte Populations

Peripheral blood (5ml) was collected via the jugular vein with a 23-g needle from 6 turkeys per treatment on d 21, 28 and 42. The same turkeys were bled at each collection to evaluate changes over time. The blood was transferred into a sterile heparinized tube and mixed gently. At room temperature, the peripheral blood lymphocytes were removed by a gentle "swirl" technique as described by Gogal *et al.* (1997). Briefly, blood was centrifuged twice at 50 X g for 10 minutes to separate the buffy coat, the lymphocyte rich layer. The buffy coat was then collected with the swirl technique. A 1ml sterile glass pipette was placed into the tube just above the buffy coat

[6] VirTis, Gardiner, NY
[7] Varian Analytical Instruments, Walnut Creek, CA

layer, gently stirred, and the buffy coat volume was aspirated gently. The collected buffy coat layer was dispensed into a plastic conical centrifuge tube and placed on ice. This collection technique was repeated up to 3 times. The buffy coat-plasma suspension was washed with RPMI-1640[8] (5ml) and PBS (5ml) at 250 X g and 7˚C, successively. The final pellet was then resuspended in 5 ml PBS. The number of lymphocytes was measured with a MultisizerTM 3 Coulter Counter®[9]. Once cells were enumerated, the lymphocytes were adjusted to a concentration of 5 X 10^6 cells/ml. Mouse anti-chicken CD4 (CT4-FITC) and mouse anti-chicken CD8 (3-298-R-PE) monoclonal antibodies[10] were added at 1µg/10^6 cells and 0.2µg/10^6 cells, respectively, and incubated for 30 min on ice. The excess antibodies were removed by centrifuging tubes at 250 X g and 8˚C, and the concentration number and ratio of CD4+ and CD8+ T-lymphocytes were determined by flow cytometry (BD FACSARIA™[11]).

Morphology and Mucin Measurements

At d 7, 14, 28, and 42, nine turkeys per treatment (1 turkey per replicate) were euthanized to collect five-centimeter segments from the duodenum (prior to the pancreatic bile ducts) and jejunum (medial portion posterior to the bile ducts and anterior to Meckel's diverticulum) for morphology and mucin measurements. The intestinal segments were rinsed with PBS and fixed in 10% neutral buffered formalin (NBF) before processing. Each segment was cut into five equal sections and placed into a tissue cassette with NBF. Tissues were processed in a Tissue-Tek® VIP[12] to dehydrate, clear,

[8] Fisher Scientific, Suwanee, GA
[9] Beckman coulter, Inc., Fullerton, CA
[10] Southern Biotechnology Associates, Inc, Birmingham, AL
[11] BD Biosciences, San Jose, CA
[12] Sakura Finetek U.S.A., Inc, Torrance, CA

and infiltrate tissues. After processing, tissues were embedded in paraffin with Tissue-Tek® TEC™[13], cut to 5 μm thickness with a microtome, and mounted onto slides. Two duplicate slides were made of each tissue, the slides were stained with hematoxylin and eosin for villus height and crypt depth (Figure 4.1) and alcian blue-periodic acid-Schiff stain (AB/PAS) to identify mucin producing goblet cell in duodenum and jejunum (Figure 4.2). The AB/PAS stain demonstrated the presence of acid and neutral mucins, with acidic mucosubstances stained by alcian blue and neutral mucosubstances stained by PAS. Three of five sections on each slide were selected to evaluate villus height, crypt depth, villus height to crypt depth ratio, and goblet cells measurements. Four measurements per section were obtained (n=12 measurements per bird, 9 birds per treatment). Four villi per section were measured for the areas to count the number of goblet cells. Pictures were obtained to measure villus height, crypt depth, and villus surface area with an Olympus DP 70™ camera[14]. All measurements were made using SigmaScan Pro 5[15]. The mucin producing goblet cells were enumerated and expressed as the number of goblet cells per unit of epithelial area (mm^2).

Statistical Analysis

Body weight, BWG, FI, FCR, immune organ weight, mineral content in liver, and T-lymphocyte populations were analyzed with the MIXED procedures of SAS for a 2×4 factorial completely randomized design. Pens were treated as the experimental unit. Villus heights, crypt depths, villus height to crypt depth ratio, and mucin producing goblet cells were evaluated using the MIXED procedure of a three factorial design with days of

[13] Sakura Finetek U.S.A., Inc, Torrance, CA
[14] Olympus America Inc., Melville, NY
[15] SPSS Inc., Chicago, IL

age, vaccine and diet as factors. For morphology measurements, bird was the experimental unit. Significant treatment differences were established using LSMEANS with Tukey correction. Significance was set at $P<0.05$.

Results

Performance Evaluation

The level and source of Zn combined with Cu, Mn, and Se supplementations had significant effects on BW at d 28, d 35, and d 42 (Table 4.2). At d 28 and 35, poults fed the RI or SI diets had significantly increased BW compared with O2 treatment. However, only the RI diet resulted in significantly improved BW at d 42 as compared to O2 diet ($P = 0.03$). The SI and O1 BW were intermediate to other groups. Birds administered the coccidia vaccine had significant decreases in BW at d 7 ($P=0.04$), d 21 ($P=0.03$), d 28 ($P=0.02$), d 35 ($P=0.0002$), and d 42 ($P=0.02$) when compared to non-vaccinated poults. No significant vaccine and Zn interaction was observed for BW during the entire study.

The effects of supplemental trace mineral levels and source on BWG of turkeys are shown in Table 4.3. Level and source of Zn combined with Cu, Mn, and Se supplementations had significant effects on BWG during the periods of d 21 to 28, d 28 to 35, and d 0 to 42. From d 21 to 28, turkeys fed the RI or SI diets had significantly increased BWG compared with O2 treatment. However, only the SI diet resulted in significantly improved BWG as compared to O2 from d 28 to 35, with RI and O1 intermediate and not different from either SI or O2. Poults administered the coccidia vaccine had significant decreases in BWG from d 0 to 7 ($P=0.04$), d 14 to 21 ($P=0.0002$), d 0 to 21 ($P=0.03$), d 28 to 35 ($P<0.0001$), d 21 to 42 ($P=0.05$), and d 0 to 42 ($P=0.02$) as

compared to non-vaccinated poults. A significant vaccination and Zn interaction was observed only for the period of d 14 to 21 (P=0.04). The turkeys fed the RI diet without coccidiosis vaccination had the highest BWG, while poults on the O2 diet with coccidia vaccination had the lowest BWG. For cumulative BWG, there were significant main effects induced by diet and vaccination. Poults fed the RI diet had significantly increased BWG compared with birds fed O2 (P=0.03), with SI and O1 intermediate in BWG and not different from other treatments. Non-vaccinated poults had better BWG (P=0.02) when compared to the vaccinated birds for the cumulative grow-out.

The data for FI are shown in Table 4.4. The level and source of trace minerals in the diet significantly influenced FI of turkeys from d 21 to d 28 only (P=0.003). Both SI and RI diets resulted in higher FI when compared to the O1 diet. Vaccination significantly decreased FI for all periods except from d 7 to 14 and d 35 to 42. For the cumulative trial period (d 0 to 42), vaccination resulted in significantly decreased FI (P=0.003). There was no interaction between diet and vaccination observed for FI during the entire 6 wk trial.

Feed conversion ratio was affected by the level and source of trace minerals from d 14 to 21 and d 0 to 21 (Table 4.5). From d 14 to 21, feeding of O2 resulted in less efficient FCR when compared to SI or RI with no difference from O1. For the entire pre-starter period (d 0 to 21), again birds fed the O2 diet had less efficient feed conversions when compared to those fed the RI and O1 diet but not when compared to those fed the SI diet. Cumulative FCR did not differ among the groups for main effects or interactions.

Supplementation with different levels and sources of trace minerals did not affect

mortality at any time, but vaccination resulted in significantly higher (P =0.0002) mortality from d 0 to 7 (Figure 4.3). No other differences in mortality were observed.

Immune Organs Weight

Bursa weight as a percent of BW did not differ among the treatments in this study (data not shown). Thymus weight was affected by dietary treatments at d 7 (P=0.05) with turkeys fed the O2 diet having significantly increased thymus weight when compared to the SI diet (Table 4.6). Thymus weight was increased (P=0.01) in vaccinated turkeys when compared to non-vaccinated poults at d 42. Vaccination also resulted in increased spleen weights on d 7 (P=0.01). No interaction between vaccination and Zn was observed for bursa, thymus, or spleen relative weight at any time in the study.

Mineral Content Analysis in Livers

Concentrations of Cu, Mn, Se, and Zn in livers from the poults are shown in Table 4.7. Hepatic concentrations of Cu were fairly stable at d 7 and d 14, and there was no effect of trace mineral supplements or vaccination on Cu concentration. Dietary treatment did have an effect on Mn concentration. The highest liver concentration of Mn was found in poults fed SI, which was significantly higher than RI or O1 diets at both d 7 (P=0.01) and 14 (P=0.005). Concentration of Mn was also significantly affected by vaccination at d 14 (P=0.03) with higher concentration of liver Mn in vaccinated turkeys. No effect of trace mineral supplementation or vaccination on Se content of liver was apparent at d 7, however, poults fed organic Se in O1 or O2 had increased concentrations of Se in liver compared with SI at d 14. Vaccinated birds had significantly lower liver concentration of Zn as compared with unvaccinated birds at d 7 (P=0.04) and higher at d 14 (P=0.01).

There was no interaction of diet and vaccination found in liver trace mineral concentrations at d 7 or 14.

T-lymphocyte Populations

CD8+ lymphocytes were not altered by level and source of trace minerals or vaccination during the study (Table 4.8). However, vaccination increased the percent of CD4+ lymphocytes at d 28 (P=0.008) and d 42 (P=0.0002) and increased the ratio of CD4+ to CD8+ lymphocytes at d 42 (P=0.05). An interaction of vaccination and trace minerals was observed with the percent of CD4+ T-lymphocytes at d 28 (P=0.03) and d 42 (P=0.01). At both d 28 and d 42, the interaction of diet and vaccination was most evident in the O2 fed turkeys as compared with other groups. Vaccination resulted in a significantly increased CD4+ lymphocyte population as compared to the non-vaccinated group in turkeys on the O2 diet. In all other dietary treatments, there were no significant differences in the CD4+ numbers between non-vaccinated and vaccinated turkeys. There was no effect of diet on the numbers of CD4+ lymphocytes, CD8+ lymphocytes, or the ratio of CD4+ to CD8+.

Morphology and Mucin Measurements

Crypt depth and the ratio of villus height to crypt depth in the duodenum were significantly altered by interactions of diet and age and vaccine and age (Table 4.9). A main effect of age was observed for all measurements. Given the normal growth of intestinal tissue early in the life of poults, the main effect of age was expected and not the main focus of this research. Duodenum villus height was increased from d 7 to d 42. There were no significant differences caused by the main effects of diet or vaccine in

duodenum morphology during the study. Crypt depth was significantly affected by the interaction of vaccine and age ($P<0.0001$) (Figure 4.4). On d 7 and d 28, vaccinated poults had deeper crypts than non-vaccinated birds, but the opposite results were seen on d 14 and 42. Interactions of diet and age ($P=0.04$) (Figure 4.5) and vaccine and age ($P<0.0001$) (Figure 4.6) were observed with the ratio of villus height to crypt depth in the duodenum. From d 7 to 28 the villus height to crypt depth ratio was similar for all dietary treatments, but responses diverged at d 42 with the SI and O2 diets resulting in higher ratios than observed with RI and O1 diets. The interaction of vaccination and age on villus height to crypt depth ratio was related to the differences in crypt depth at different ages, as non-vaccinated birds had higher ratios at d 7 and 28 and lower ratios at d 14 and 42.

In the jejunum, villus height and the ratio of villus height to crypt depth significantly increased with age (Table 4.9). Vaccination had a significant effect on villus height and the ratio of villus height to crypt depth, while dietary treatment had no significant effect on jejunum morphology. No significant differences in crypt depth between treatments were observed with the main effects of diet, vaccine, or age. There were multiple significant interactions of vaccine and age in jejunum morphology parameters. The interaction of vaccination and age on jejunum villus height was most evident at d 14 as compared to all other ages (Figure 4.7). At d 14, vaccinated turkeys had increased ($P=0.005$) villus height as compared to non-vaccinated groups, but villus heights were similar between vaccinated and non-vaccinated groups at all other days. Results for the interaction of vaccination and age on jejunum crypt depth showed that on d 7 vaccinated birds had deeper crypts and on d 14 more shallow crypts than

non-vaccinated birds (Figure 4.8). The jejunum crypt depth in non-vaccinated and vaccinated poults was similar at d 28 and 42. The differences in villus height and crypt depth with age resulted in differences in the ratio of these measurements as well (Figure 4.9). The ratios between the non-vaccinated and vaccinated groups were different at d 7 and 14 but similar to each other at d 28 and 42.

A summary of results for mucin producing goblet cell numbers in the duodenum and jejunum is shown in Table 4.10. Main effects of age and also vaccination were seen with goblet cell number. Significant interactions of vaccination and age, diet and age, and vaccination and diet and age were observed for both duodenum and jejunum goblet cell number. The interaction of diet and age on number of goblet cells in the duodenum was predominantly indicated by the response of poults fed the O2 diet (Figure 4.10). The number of goblet cells in the duodenum of birds receiving the SI, RI, and O1 diets was similar at each age, however birds receiving the O2 diet had a lower number of goblet cells than all the other groups at d 28. A significant vaccination and age interaction for goblet cells in the duodenum is depicted in Figure 4.11. At d 7 and 28, there were similar numbers of goblet cells between non-vaccinated and vaccinated birds, but at d 14 and 42, vaccination resulted in an increased ($P=0.004$) number of goblet cells. The three way interaction of diet, vaccination, and age for the number of goblet cells in the duodenum was complex (Figure 4.12). The turkeys from UV-RI and UV-O1 treatments had similar numbers of goblet cells, which increased between d 14 and 28 and slightly decreased from d 28 to 42. Similar goblet cell numbers were measured with the UV-SI and UV-O2 treatments, which decreased slightly from d 7 to 14 and then remained consistent to d 42. In the vaccinated birds from all dietary treatments, results were similar to each other with

the exception of the V-O2 group, which had significantly lower goblet cells than other groups at d 28 with a sharp increase by d 42 to reach similar numbers as other vaccinated groups. As with other interactions of diet and age, the O2 diet appeared to have the most differential response in goblet cell number at the ages sampled in this trial (Figure 4.13). At d 7, 14, and 28 the dietary treatments had similar numbers of goblet cells in the jejunum, but by d 42, jejunum from birds fed the O2 diet had fewer goblet cells. In contrast, birds fed the RI diet appeared to diverge to have more goblet cells at d 42. An interaction of vaccination and age on goblet cell numbers in the jejunum was observed at d 7 with a higher number of goblet cells in vaccinated as compared to non-vaccinated birds (Figure 4.14). Numbers of goblet cells between these groups were similar thereafter to d 42. The three way interaction of diet, vaccine, and age on the number of goblet cells in the jejunum was most evident with the responses of the UV-O2 treatment and the V-SI treatments (Figure 4.15). The V-SI treatment had one of the highest numbers of goblet cells at d 7, but the lowest at d 14. Thereafter, this group had similar numbers of goblet cells as measured in other groups. Most of the non-vaccinated birds had similar goblet cell numbers between dietary treatments from d 7 to d 28 at which time the UV-O2 decreased to the lowest number of goblet cells by d 42.

Discussion

It was not surprising to observe the decreased BWG and FI caused by *Eimeria* infection from vaccination, since it typically causes a disruption of the intestinal mucosa and compromised nutrient absorption (Yun *et al.,* 2000). Additionally, combating the *Eimeria* infection induced an immune response in the intestine. The immune system has a high demand for nutrients, which could also, although to a lesser extent, have contributed

to impaired growth (Morris *et al.,* 2004). It was reported by Turk and Stephens (1966) that nutrient deficiency symptoms were observed in chickens during the early phase of a coccidial infection, and then increased growth rates appeared during the recovery phase of the disease with orally administered ^{65}Zn. They explained that cell destruction during the coccidia infection decreased nutrient absorption, while cellular repair in the recovery phase permitted absorption rates to return toward the rates of uninfected birds. Turk (1981) found that after an early depression of growth rate at d 6 of infection, birds had increased growth rates, and body weights returned to that of the controls by d 35. These results are consistent with the observation in this study.

This study also showed that birds fed RI diet had significantly increased cumulative BWG, improved FCR in pre-starter period, and improved BW at d 28, 35, and 42 compared with those fed O2 diet. Previous studies demonstrated that Zn utilization was diminished by *E. acervulina* infection (Southern and Barker, 1983a; b). This could indicate that Zn deficiency was induced when chicks were fed diets containing marginal Zn levels during an *E. acervulina* infection. One of the main clinical manifestations associated with Zn deficiency is growth retardation. Based on the performance data, inorganic Zn supplemented at 15 ppm was adequate for turkey growth even during *Eimeria* infection.

The difference in performance between O1 and O2 may be caused by the level of Zn in the diet. Although consequences of Zn deficiency have been recognized for many years, the potential consequences of excessive Zn intake must also be considered. It was reported that body weight of chicks supplemented with high Zn (1,000 ppm) was lower ($P < 0.05$) during weeks 1 and 2 when compared to those of chicks fed the control diet

with 60 ppm Zn (Sandoval *et al.*, 1998). Experiments showed that supplementation of 1,500 ppm Zn from reagent grade Zn sulfate in broiler diets resulted in depression in feed consumption and body weight (Sandoval *et al.*, 1997). Chicks given 2,000, 4,000 or 6,000 mg Zn/kg diet from 2 to 6 weeks of age grew poorly, many showed gizzard erosion, and dissecting aneurysms occurred in a few birds receiving 6,000 mg Zn/kg (Dewar *et al.*, 1983). A study indicated that the relative bioavailability value of organic Zn was 183% that of Zn sulfate based on body weight gain (BWG) data and 157% that of Zn sulfate based on total tibia Zn content (Ao *et al.,* 2006). Therefore, the decreased performance in O2 may have been caused by approaching the toxicity levels of Zn.

Unlike chickens, there is commonly higher mortality in the first two weeks with turkeys, an occurence known as "starve-out". Failure of poults to eat or drink is considered to be the cause. This situation may be compounded by vaccination (Leeson and Summers, 2005), and it could have contributed to the elevated mortality during d 0 to 7 in vaccinated birds in this trial.

Not surprisingly, no effect of vaccination was found on bursa weight in this study. Although antibodies can be abundantly produced locally in tissues, they can not access and act on intracellular pathogens (Lillehoj, 2004). Therefore, antibody-mediated responses play a minor role in protective immunity against coccidiosis, while cell-mediated responses are the major host immune responses associated with *Eimeria* infection (Lillehoj and Trout, 1996; Lillehoj *et al.,* 2004). On the other hand, spleens, which have both T- and B-cell areas, and thymus, the site of T-cell development, were stimulated by *Eimeria* infection.

Zinc deficiency reduced immune system function (Rink and Kirchner, 2000) with

decreased cellular immunity (Fletcher *et al.,* 1988) and caused lower weights of the spleen and thymus in mice fed 5ppm Zn when compared to control mice fed with 100ppm Zn (Beach *et al.,* 1982). Since B-lymphocytes are less dependent on Zn for proliferation than T-lymphocytes (Shankar and Prasad, 1998), it is logical that there was no significant effect on bursa weight caused by the level or source of trace minerals.

It has been reported that *E. acervulina* infection increased tissue concentrations of Cu (Southern and Baker, 1982a), Mn (Brown and Southern, 1985), and Fe (Southern and Baker, 1982b). However, tissue concentrations of Zn were decreased by coccidial infection (Southern and Baker, 1983a; Bafundo *et al.,* 1984). Why the effect with Zn is different from the other trace minerals is not known. It was reported that absorption of Zn was decreased during the acute phase of a coccidia infection (6 days post-inoculation) and increased during recovery phases of the infection (Southern and Baker, 1983a). Giraldo and Southern (1988) summarized the possible mechanisms for coccidiosis-induced increase in mineral absorption, which included 1) increased intestinal cell permeability on the mucosal surface caused by coccidial damage (Turk and Stephens, 1967); 2) decreased passage rate of ingesta during coccidiosis infection (Turk, 1974); 3) compensatory gain during the recovery period (Turk, 1974); 4) increased minerals solubility with decreased duodenal pH caused by coccidiosis infection (Fox *et al.,* 1987); and 5) as the result of secondary bacterial infections (Richards *et al.,* 1985). However, another study showed that compensatory gain was not responsible for the increase in liver Cu accumulation in coccidiosis-infected chicks (Giraldo and Southern, 1988), which may be the reason we did not observe an effect of vaccination on Cu concentration in liver.

In this study, poults fed organic Se showed higher Se concentrations in liver as

compared to those from the inorganic source fed birds. Pan *et al.* (2007) observed that both source (inorganic and organic) and level of Se significantly influenced Se concentrations in eggs and blood. It was suggested that organic Se was more bioavaiable than inorganic based on the data of Se retention relative to Se intake. The Se content of breast and thigh meat in broilers was significantly increased by organic dietary Se supplementation (Skrivan *et al.*, 2008). The Se yeast, predominantly selenomethionine, appeared to be preferentially deposited in proteins as evidenced by greater levels in both albumen and breast muscle (Leeson *et al.*, 2008). However, it is still unclear whether deposition of selenomethionine is a direct consequence of the need for Se, or that alternatively Se deposition is a simple consequence of the deposition of the associated methionine or proteins and peptides.

Concentration of Cu and Zn in liver was fairly stable at d 7 and 14, and there was no effect of diet. The liver is important for trace minerals metabolism. Trace minerals absorbed from the lumen are transported to liver and then released into the circulation for transport to the tissues. Previous reports have indicated that the effects of Zn, Fe and Cu in the basal diet on liver concentrations of these minerals were relatively small (Skrivan *et al.,* 2005), although there has been increased Zn concentration in bone and liver with a high dietary level of Zn (Sandoval *et al.,* 1998). It was reported that Mn in tissues had a highly linear relationship between liver Mn concentration and dietary Mn (liver Mn increased as dietary Mn increased) (Black *et al.,* 1984). The same was seen in this study.

Invasion of coccidia into the epithelial cells and the subsequent cellular destruction causes inflammatory responses at the infection site. In experiments with mice, it was shown that CD4+ cells were more effective than CD8+ in conferring protective

immunity to *E. vermiformis* both *in vivo* (Rose *et al.,* 1992) and *in vitro* (Rose *et al.,* 1988). Only CD4+ cells were increased during both primary and secondary infections (Hong *et al.,* 2006), which is in agreement with data of this trial during the primary infection from vaccination. Since *Eimeria* infection occurs locally in the intestine, the lymphocyte subpopulations in peripheral blood may not reflect the specific changes caused by infection. It was reported that dynamic changes in intestinal T-lymphocytes were not adequately represented in peripheral lymph nodes or blood (Smit-McBride, *et al.*, 1998). Flow cytometric analysis demonstrated severe depletion of CD4+CD8- single-positive T cells and CD4+CD8+ double-positive T cells in intestinal lamina propria lymphocytes and intraepithelial lymphocytes during primary simian immunodeficiency virus infection in rhesus macaques which persisted through the entire course of infection. In contrast, CD4+ T-cell depletion was gradual in peripheral lymph nodes and blood (Smit-McBride, *et al.*, 1998). Therefore, further investigation of lymphocyte subpopulations locally in the intestine is needed to investigate dietary impacts on immune responses to *Eimeria*. Zn-deficiency induces an imbalance between Th1 and Th2 cells in humans, as Zn may be required for regeneration of new CD4+ T cells and maintenance of T cytolytic cells (Beck *et al.,* 1997). Zn-deficiency has been shown to cause reduced numbers of lymphocytes, particularly T lymphocytes in the blood and peripheral lymphoid tissues, and decreased CD4+ to CD8+ cell ratios in children with acrodermatitis enteropathica (Shankar and Prasad, 1998). In the present data of lymphocyte subpopulations, there was no main effect of diet observed, suggesting that the supplementation level of Zn was adequate to support the immune response, even at 15ppm.

In the duodenum, data from the present study suggested increased villus height, decreased crypt depth, and increased villus height to crypt depth ratios at d 14 with vaccinated birds. These observed effects may be the outcome of compensation for *Eimeria* infection at d 7 or may be caused by the different challenge species for turkeys or dosages of *Eimeria*. It is established that villus length and crypt depth increase with age in poults (Uni *et al.,* 1995; Iji *et al.,* 2001), which is consistent with the data in the present study. The intestine plays a defensive role in response to damage of epithelial cells by pathogens by increasing the rate of epithelial renewal (Gaskins, 1997) and impacting villus and crypt architecture and activities of many brush border digestive enzymes. Villus height decreased, crypt depth increased, and villus height to crypt depth ratios decreased in coccidia challenged groups compared with non-challenged controls (Morris *et al.,* 2004), which is in agreement with results in the present experiment at d 7 (the time at which the vaccine would result in first infection). They suggested that the increased crypt depths were directly related to increased replacement or turnover of epithelial cells during infection, which means the host's intestine was trying to compensate for the effects of *Eimeria* infection induced villi damage.

There were no differences in the number of goblet cells in either the duodenum or jejunum caused by dietary treatments. It has been reported that intestinal microbiota (Deplancke and Gaskins, 2001) and dietary composition (Smirnov *et al.,* 2004; 2005) can stimulate or influence the release of mucins from goblet cells. Germ-free rodents had a decrease in goblet cell size and number and reduced mucus layer thickness as compared with conventionally raised rodents (Kandori *et al.,* 1996). Klf4 (formerly GKLF) is a Zn-finger transcription factor expressed in the epithelia of the skin, the lungs, the

gastrointestinal tract and several other organs (Garrett-Sinha *et al.*, 1996). Klf4–/– mice demonstrated a 90% decrease in the number of goblet cells in the colon (Katz *et al.*, 2002). Therefore, Zn plays an important role in goblet cells synthesis. No difference in total number of goblet cells indicated that the supplementation level of Zn in the diet was adequate. In the present research, we did not measure the goblet cell mucin profile in the duodenum or jejunum, although AB/PAS staining demonstrated the presence of both acidic and neutral mucins. Past research has found differences in mucin profiles based on management practices (Meslin *et al.*, 1999; Uni *et al.*, 2003) and diet (Sharma *et al.*, 1997).

As observed in the present research, other reports have shown increasing gradients of goblet cell density along the duodenal to ileal axis (Uni *et al.,* 2003; Smirnov *et al.,* 2004). In the duodenum, the number of goblet cells was slightly decreased at d 7 in vaccinated birds, but at d 14, vaccination resulted in an increased number of goblet cells. It was demonstrated that *E. vermiformis* infection in mice resulted in a significantly decreased number of goblet cells in the jejunum and ileum on d 8 and 10 post-infection, and there was recovery of the villous epithelium along with regeneration of the crypt and goblet cells from d 12 post-infection (Linh *et al.,* 2008). Goblet cells returned to nearly normal levels by d 14 post-infection in both the jejunum and ileum. This research also suggested that the effect of *Eimeria* on goblet cell number was only restricted to the site of the parasite infection and was only observed during the time when the parasites were present. *E. meleagrimitis*, one species of coccidia in the vaccine and the most pathogenic of the upper-intestinal coccidia in turkeys is primarily infective in the upper intestine. Thus, the infection of *E. meleagrimitis* may have resulted in the decrease of goblet cells in duodenum at d 7 and recovery with resolution of initial infection at d 14 in the present

study.

Our research showed vaccination increased the number of goblet cells in the duodenum, which indirectly suggested that there would be more release of mucins in the lumen. Parasitic infestation can stimulate the release of mucins from goblet cells (Miller *et al.*, 1981). The mucus layer, secreted from goblet cells, is an important determinant of gut health and disease. It is one of the first lines of defense against pathogens in the GIT, and it is a medium for protection, lubrication and transport between the luminal contents and the epithelial cells (Kindon *et al.,* 1995). It plays an important role in host-pathogen interaction in the intestine (Roskens and Erlandsen, 2002). Tierney *et al.* (2007) observed that *E. tenella* interacted with native chicken intestinal mucin, which in turn inhibited parasite invasion into epithelial cells *in vitro*.

In summary, using low levels of organic or inorganic Zn (15ppm) with other organic trace minerals had no significant effect on BW, cumulative BWG, FI, or FCR compared with organic Zn at 30 ppm or inorganic at 150 ppm. Results suggested that lower levels (15ppm) of organic Zn were actually more beneficial than higher levels (30ppm). There was also no significant effect of dietary Zn source or levels on immune organ weights, lymphocyte subpopulations in peripheral blood, or intestinal morphology. All of the data suggested that the supplementation of Zn at 15 ppm, either in organic or inorganic form, is an adequate level to maintain turkey performance and appropriate immune response, even with the challenge of a commercial turkey coccidia vaccine.

Table 4.1. Composition and nutrient content of basal diets[1]

Item	Pre-starter	Starter
Ingredient	----------(%)----------	
Ground corn fine	39.4119	42.9251
DDGS ethanol	5.0000	5.0000
Canola meal	5.0000	5.0000
Dehulled soymeal	38.5760	34.7683
Feather meal	3.0000	3.0000
Calcium carbonate	1.8531	1.7237
Mono-dical phosphate	2.8253	2.6065
Salt	0.3341	0.3114
AFL-15 MXD ML	3.0329	3.7451
Vitamin A PX 162,500	0.0081	0.0077
Biotin 0.1	0.0300	0.0285
B-12 Conc-300	0.0060	0.0057
Cal Pan 80	0.0154	0.0147
Choline chloride-70	0.1857	0.1714
Vitamin E Supp 227,000	0.0132	0.0126
Vitamin D PX (20)	0.0125	0.0119
Folic acid 2%	0.0125	0.0119
L-lysine HCl 98.5	0.3104	0.3106
Vitamin K MPB 11,640	0.0344	0.0326
Liquid MHA	0.2054	0.1880
Niacin/nictnad 99.5	0.0100	0.0095
Pyridoxine 1	0.0500	0.0475
RIBO Supp 60 g/lb	0.0114	0.0108
Thiamine 1%	0.0490	0.0465
L-threonine	0.0127	0.0100
Calculated nutrient content		
ME (kcal/lb)	1300	1340
CP (%)	27.00	25.50
Calcium (%)	1.40	1.30
Available phosphate (%)	0.75	0.70
Sodium (%)	0.18	0.17
Lysine (%)	1.70	1.60
Methionine (%)	0.66	0.62
Methionine + cystine (%)	1.11	1.04
Threonine (%)	1.00	0.94
Tryptophan (%)	0.27	0.26

[1] Pre-starter= 0 to 21 d of age; starter= 21 to 42 d of age.

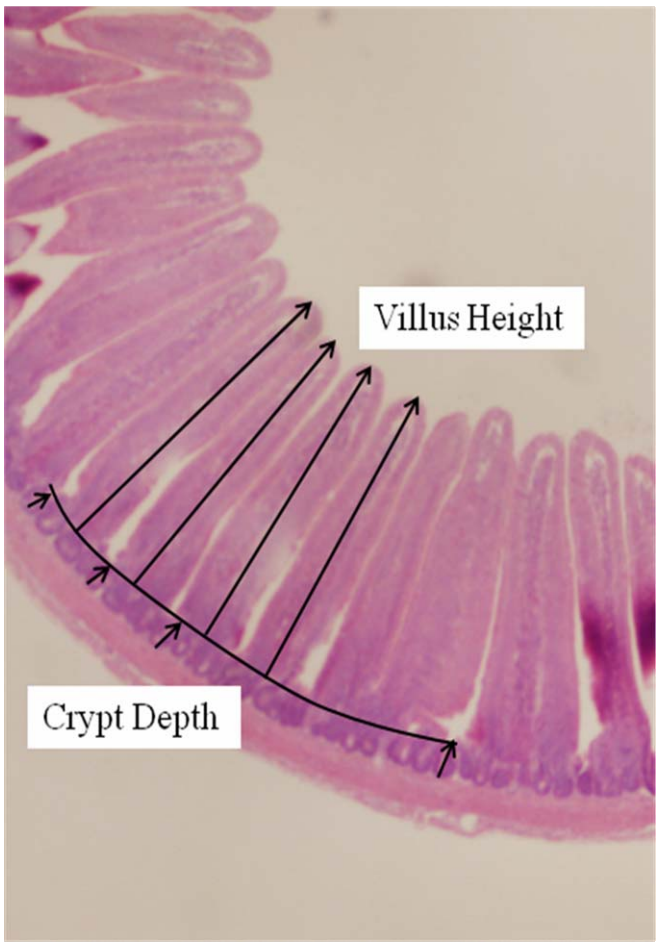

Figure 4.1. Measurement of jejunum villus height and crypt depth with 4X magnification. The longer arrow indicates villus height and the shorter one represent crypts depth.

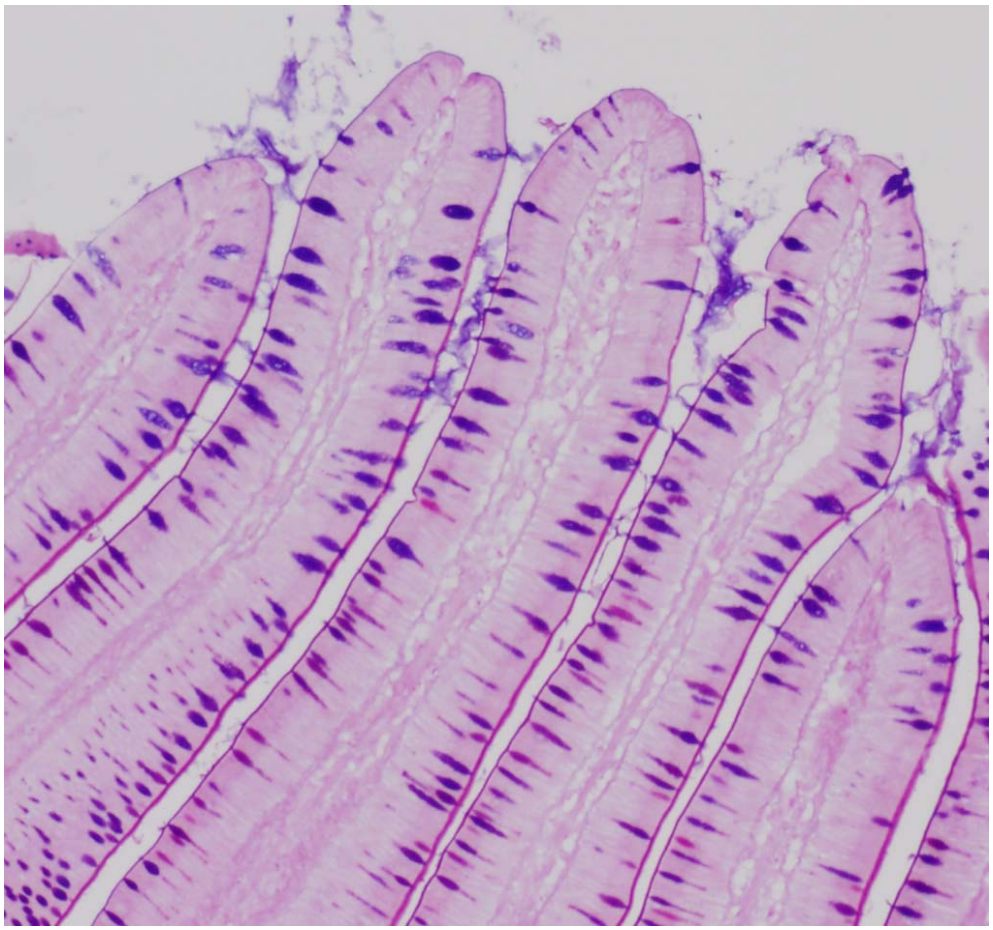

Figure 4.2. The mucins in jejunum were stained by AB/PAS technique 10X magnification.
The blue staining represents acid mucins, magenta represents neutral mucins, and purple indicates a mixture of acidic and neutral mucins.

Table 4.2. Effect of level and source of Zn combined with Cu, Mn, and Se supplementations on BW (kg/bird) of commercial turkeys

Age	d 0	d 7	d 14	d 21	d 28	d 35	d 42
Treatment							
UV-SI	0.0504	0.1601	0.3868	0.7187	1.1936	1.8454	2.5920
V-SI	0.0505	0.1561	0.3804	0.6985	1.1538	1.7687	2.4841
UV-RI	0.0509	0.1602	0.3835	0.7167	1.1862	1.8323	2.5671
V-RI	0.0504	0.1567	0.3858	0.7107	1.1768	1.7922	2.5312
UV-O1	0.0502	0.1618	0.3854	0.7086	1.1672	1.7948	2.5278
V-O1	0.0503	0.1589	0.3873	0.7119	1.1603	1.7726	2.5207
UV-O2	0.0502	0.1563	0.3779	0.7073	1.1492	1.7706	2.4865
V-O2	0.0505	0.1548	0.3771	0.6899	1.1360	1.7360	2.4743
SEM[1]	0.0002	0.0020	0.0041	0.0065	0.0101	0.0154	0.0237
Main effect							
Diet[2]							
SI	0.0505	0.1581	0.3836	0.7086	1.1737[a]	1.8070[a]	2.5380[ab]
RI	0.0506	0.1584	0.3846	0.7137	1.1815[a]	1.8123[a]	2.5492[a]
O1	0.0503	0.1603	0.3863	0.7103	1.1637[ab]	1.7837[ab]	2.5243[ab]
O2	0.0504	0.1555	0.3775	0.6986	1.1426[b]	1.7533[b]	2.4804[b]
Vaccine[3]							
UV	0.0504	0.1596[a]	0.3834	0.7128[a]	1.1741[a]	1.8108[a]	2.5434[a]
V	0.0504	0.1566[b]	0.3826	0.7027[b]	1.1567[b]	1.7674[b]	2.5026[b]
Statistical effects							
Diet	NS	NS	NS	NS	0.002	0.001	0.03
Vaccine	NS	0.04	NS	0.03	0.02	0.0002	0.02
Diet*Vaccine	NS	NS	NS	NS	NS	NS	NS

[a-b] Means within a column without common superscripts are different ($P \leq 0.05$).

[1] All means are an average of 9 pens per treatment.

[2] SI=standard inorganic, inorganic Zn (150 ppm) with inorganic sources of Mn (165 ppm), Cu (10 ppm), and Se (0.2 ppm); RI=reduced inorganic, inorganic Zn, Mn, Cu at 10% of SI, and inorganic Se (0.2ppm); O1= organic 1, organic Zn (15 ppm) with organic sources of Mn (16.5 ppm), Cu (1 ppm), and Se (0.2 ppm); O2= organic 2, organic Zn (30 ppm) with the same level and source of other organic trace minerals as O1.

[3] UV=non-vaccinated; V=vaccinated with Coccivac®-T.

Table 4.3. Effect of level and source of Zn combined with Cu, Mn, and Se supplementations on BWG (kg/bird per period) of commercial turkeys

	Pre-starter				Starter				
	0 to 7 d	7 to 14 d	14 to 21 d	0 to 21 d	21 to 28 d	28 to 35 d	35 to 42 d	21 to 42 d	0 to 42 d
Treatment									
UV-SI	0.110	0.227	0.332^{ab}	0.668	0.475	0.652	0.747	1.873	2.542
V-SI	0.106	0.224	0.318^{bc}	0.648	0.455	0.615	0.715	1.786	2.434
UV-RI	0.109	0.223	0.333^{a}	0.666	0.470	0.646	0.735	1.850	2.516
V-RI	0.106	0.229	0.325^{abc}	0.660	0.466	0.615	0.739	1.821	2.481
UV-O1	0.112	0.224	0.323^{abc}	0.659	0.459	0.628	0.733	1.819	2.478
V-O1	0.109	0.228	0.325^{abc}	0.662	0.448	0.612	0.745	1.809	2.470
UV-O2	0.106	0.222	0.329^{ab}	0.657	0.442	0.621	0.716	1.779	2.436
V-O2	0.104	0.222	0.313^{c}	0.639	0.446	0.600	0.738	1.784	2.424
SEM[1]	0.002	0.003	0.003	0.006	0.006	0.008	0.013	0.021	0.024
Main effect									
Diet[2]									
SI	0.108	0.226	0.325	0.658	0.465^{a}	0.633^{a}	0.731	1.829	2.488^{ab}
RI	0.108	0.226	0.329	0.663	0.468^{a}	0.631^{ab}	0.737	1.836	2.499^{a}
O1	0.110	0.226	0.324	0.660	0.454^{ab}	0.620^{ab}	0.739	1.814	2.474^{ab}
O2	0.105	0.222	0.321	0.648	0.444^{b}	0.611^{b}	0.727	1.782	2.430^{b}
Vaccine[3]									
UV	0.109^{a}	0.224	0.329^{a}	0.662^{a}	0.461	0.637^{a}	0.733	1.831^{a}	2.493^{a}
V	0.106^{b}	0.226	0.320^{b}	0.652^{b}	0.454	0.611^{b}	0.734	1.800^{b}	2.452^{b}
Statistical effects									
Diet	NS	NS	NS	NS	0.0004	0.02	NS	NS	0.03
Vaccine	0.04	NS	0.0002	0.03	NS	<0.0001	NS	0.046	0.02
Diet*Vaccine	NS	NS	0.04	NS	NS	NS	NS	NS	NS

[a-c] Means within a column without common superscripts are different ($P \leq 0.05$).
[1] All means are an average of 9 pens per treatment.
[2] SI=standard inorganic, inorganic Zn (150 ppm) with inorganic sources of Mn (165 ppm), Cu (10 ppm), and Se (0.2 ppm); RI=reduced inorganic, inorganic Zn, Mn, Cu at 10% of SI, and inorganic Se (0.2ppm); O1= organic 1, organic Zn (15 ppm) with organic sources of Mn (16.5 ppm), Cu (1 ppm), and Se (0.2 ppm); O2= organic 2, organic Zn (30 ppm) with the same level and source of other organic trace minerals as O1.
[3] UV=non-vaccinated; V=vaccinatedwith Coccivac®-T.

Table 4.4. Effect of level and source of Zn combined with Cu, Mn, and Se supplementations on FI (kg/bird per period) of commercial turkeys

	Pre-starter				Starter				
	0 to 7 d	7 to 14 d	14 to 21 d	0 to 21 d	21 to 28 d	28 to 35 d	35 to 42 d	21 to 42 d	0 to 42 d
Treatment									
UV-SI	0.143	0.284	0.483	0.910	0.737	1.010	1.232	2.979	3.889
V-SI	0.134	0.287	0.466	0.887	0.711	0.932	1.227	2.871	3.758
UV-RI	0.139	0.284	0.481	0.905	0.730	1.003	1.215	2.948	3.852
V-RI	0.136	0.286	0.476	0.898	0.719	0.992	1.201	2.912	3.810
UV-O1	0.140	0.286	0.476	0.902	0.710	0.972	1.195	2.877	3.779
V-O1	0.132	0.287	0.475	0.893	0.689	0.947	1.193	2.832	3.728
UV-O2	0.135	0.281	0.487	0.902	0.725	0.969	1.193	2.886	3.789
V-O2	0.129	0.285	0.475	0.888	0.690	0.935	1.190	2.814	3.703
SEM[1]	0.004	0.003	0.005	0.008	0.008	0.020	0.017	0.031	0.035
Main effect									
Diet[2]									
SI	0.138	0.286	0.474	0.898	0.724[a]	0.971	1.230	2.925	3.823
RI	0.137	0.285	0.479	0.901	0.724[a]	0.998	1.208	2.930	3.831
O1	0.136	0.286	0.475	0.897	0.699[b]	0.959	1.194	2.855	3.753
O2	0.132	0.283	0.481	0.895	0.707[ab]	0.952	1.191	2.850	3.746
Vaccine[3]									
UV	0.139[a]	0.284	0.482[a]	0.905[a]	0.725[a]	0.988[a]	1.209	2.922[a]	3.827[a]
V	0.133[b]	0.286	0.473[b]	0.892[b]	0.702[b]	0.952[b]	1.203	2.857[b]	3.750[b]
Statistical effects									
Diet	NS	NS	NS	NS	0.003	NS	NS	NS	NS
Vaccine	0.01	NS	0.01	0.03	<0.0001	0.01	NS	0.005	0.003
Diet*Vaccine	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a-c] Means within a column without common superscripts are different ($P \leq 0.05$).
[1] All means are an average of 9 pens per treatment.
[2] SI=standard inorganic, inorganic Zn (150 ppm) with inorganic sources of Mn (165 ppm), Cu (10 ppm), and Se (0.2 ppm); RI=reduced inorganic, inorganic Zn, Mn, Cu at 10% of SI, and inorganic Se (0.2ppm); O1= organic 1, organic Zn (15 ppm) with organic sources of Mn (16.5 ppm), Cu (1 ppm), and Se (0.2 ppm); O2= organic 2, organic Zn (30 ppm) with the same level and source of other organic trace minerals as O1.
[3] UV=non-vaccinated; V=vaccinated with Coccivac®-T.

Table 4.5. Effect of level and source of Zn combined with Cu, Mn, and Se supplementations on FCR of commercial turkeys

	Pre-starter				Starter				
	0 to 7 d	7 to 14 d	14 to 21 d	0 to 21 d	21 to 28 d	28 to 35 d	35 to 42 d	21 to 42 d	0 to 42 d
Treatment									
UV-SI	1.301	1.255	1.456	1.361	1.552	1.550	1.651	1.590	1.530
V-SI	1.269	1.282	1.466	1.370	1.564	1.516	1.720	1.610	1.545
UV-RI	1.278	1.273	1.445	1.359	1.556	1.553	1.654	1.594	1.532
V-RI	1.273	1.248	1.467	1.360	1.542	1.612	1.629	1.600	1.536
UV-O1	1.257	1.279	1.471	1.369	1.549	1.550	1.636	1.582	1.525
V-O1	1.217	1.255	1.462	1.350	1.538	1.548	1.604	1.566	1.510
UV-O2	1.275	1.267	1.478	1.374	1.643	1.560	1.669	1.623	1.556
V-O2	1.236	1.281	1.519	1.389	1.548	1.560	1.615	1.578	1.528
SEM[1]	0.031	0.012	0.014	0.008	0.021	0.031	0.027	0.016	0.012
Main effect									
Diet[2]									
SI	1.285	1.268	1.461[a]	1.365[ab]	1.558	1.533	1.686	1.600	1.538
RI	1.275	1.260	1.456[a]	1.359[a]	1.549	1.583	1.642	1.597	1.534
O1	1.237	1.267	1.467[ab]	1.360[a]	1.543	1.549	1.620	1.574	1.517
O2	1.255	1.274	1.499[b]	1.382[b]	1.596	1.560	1.642	1.601	1.542
Vaccine[3]									
UV	1.278	1.268	1.463	1.366	1.575	1.553	1.653	1.597	1.536
V	1.249	1.266	1.478	1.367	1.548	1.559	1.642	1.589	1.530
Statistical effects									
Diet	NS	NS	0.02	0.02	NS	NS	NS	NS	NS
Vaccine	NS	NS	NS	NS	NS	NS	NS	NS	NS
Diet*Vaccine	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a-c] Means within a column without common superscripts are different ($P \leq 0.05$).
[1] All means are an average of 9 pens per treatment.
[2] SI=standard inorganic, inorganic Zn (150 ppm) with inorganic sources of Mn (165 ppm), Cu (10 ppm), and Se (0.2 ppm); RI=reduced inorganic, inorganic Zn, Mn, Cu at 10% of SI, and inorganic Se (0.2ppm); O1= organic 1, organic Zn (15 ppm) with organic sources of Mn (16.5 ppm), Cu (1 ppm), and Se (0.2 ppm); O2= organic 2, organic Zn (30 ppm) with the same level and source of other organic trace minerals as O1.
[3] UV=non-vaccinated; V=vaccinated with Coccivac®-T.

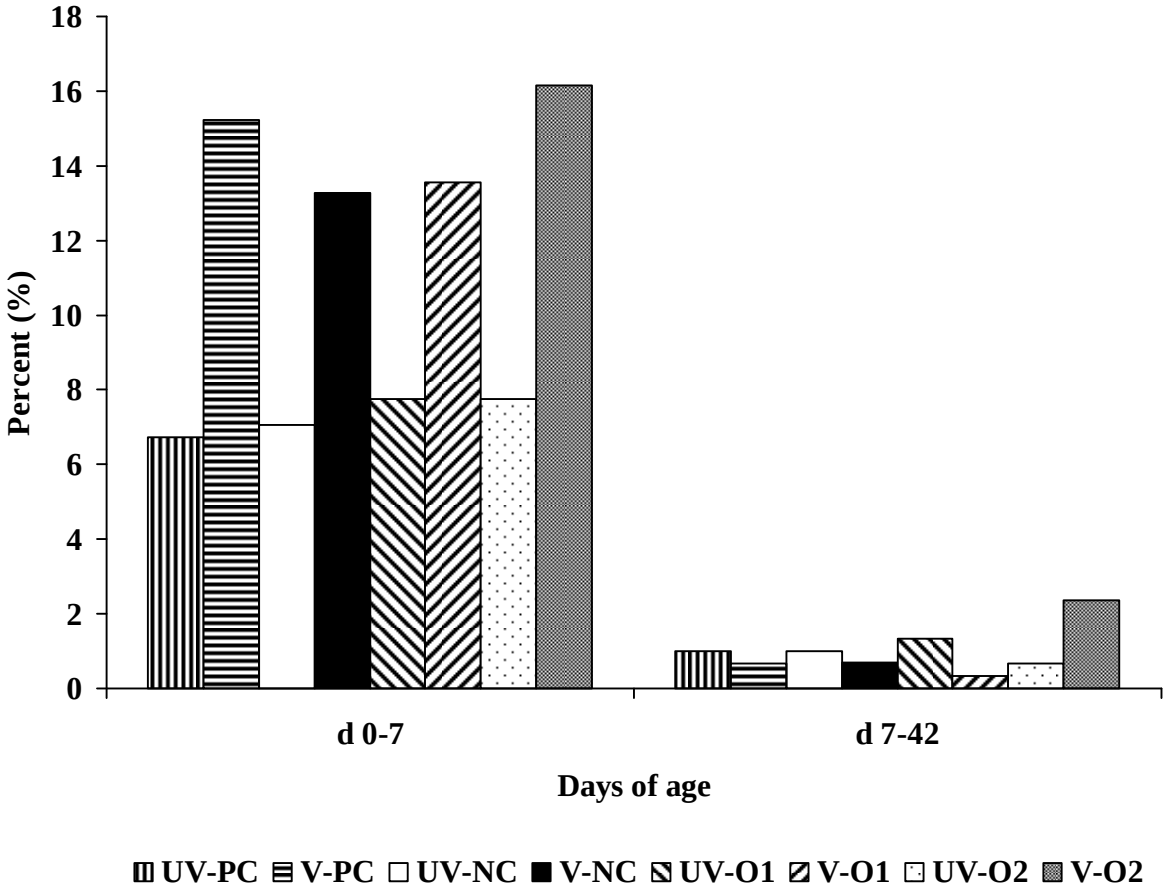

Figure 4.3. Effects of level and source of Zn combined with Cu, Mn, and Se supplementations on percent mortality.

SI=standard inorganic, inorganic Zn (150 ppm) with inorganic sources of Mn (165 ppm), Cu (10 ppm), and Se (0.2 ppm); RI=reduced inorganic, inorganic Zn, Mn, Cu at 10% of SI, and inorganic Se (0.2ppm); O1= organic 1, organic Zn (15 ppm) with organic sources of Mn (16.5 ppm), Cu (1 ppm), and Se (0.2 ppm); O2= organic 2, organic Zn (30 ppm) with the same level and source of other organic trace minerals as O1. UV=non-vaccinated; V=vaccinated with Coccivac®-T. Main effects of vaccine were significant (P =0.0002) from d 0 to 7.

Table 4.6. Effect of level and source of Zn combined with Cu, Mn, and Se supplementations on immune organ weight (as % of BW) of commercial turkeys

	Thymus (%) (right side)				Spleen (%)			
	d 7	d 14	d 28	d 42	d 7	d 14	d 28	d 42
Treatment								
UV-SI	0.059	0.098	0.063	0.052	0.065	0.086	0.132	0.111
V-SI	0.066	0.082	0.070	0.047	0.079	0.107	0.117	0.112
UV-RI	0.073	0.088	0.071	0.048	0.060	0.105	0.128	0.124
V-RI	0.074	0.101	0.079	0.071	0.069	0.092	0.125	0.112
UV-O1	0.064	0.084	0.068	0.041	0.070	0.085	0.115	0.119
V-O1	0.071	0.101	0.079	0.057	0.092	0.104	0.132	0.110
UV-O2	0.082	0.090	0.077	0.050	0.061	0.094	0.130	0.113
V-O2	0.082	0.105	0.074	0.065	0.068	0.091	0.124	0.117
SEM[1]	0.007	0.009	0.009	0.007	0.007	0.008	0.009	0.007
Main effect								
Diet[2]								
SI	0.062[b]	0.090	0.066	0.050	0.072	0.097	0.124	0.111
RI	0.074[ab]	0.094	0.075	0.059	0.065	0.098	0.127	0.118
O1	0.068[ab]	0.092	0.074	0.049	0.081	0.094	0.123	0.114
O2	0.082[a]	0.097	0.075	0.058	0.065	0.092	0.127	0.115
Vaccine[3]								
UV	0.070	0.090	0.070	0.048[b]	0.064[b]	0.092	0.126	0.117
V	0.073	0.097	0.075	0.060[a]	0.077[a]	0.098	0.124	0.113
Statistical								
effects								
Diet	0.045	NS	NS	NS	NS	NS	NS	NS
Vaccine	NS	NS	NS	0.010	0.01	NS	NS	NS
Diet*Vaccine	NS	NS	NS	NS	NS	NS	NS	NS

[a-c] Means within a column without common superscripts are different ($P \leq 0.05$).

[1] All means are an average of 9 pens per treatment.

[2] SI=standard inorganic, inorganic Zn (150 ppm) with inorganic sources of Mn (165 ppm), Cu (10 ppm), and Se (0.2 ppm); RI=reduced inorganic, inorganic Zn, Mn, Cu at 10% of SI, and inorganic Se (0.2ppm); O1= organic 1, organic Zn (15 ppm) with organic sources of Mn (16.5 ppm), Cu (1 ppm), and Se (0.2 ppm); O2= organic 2, organic Zn (30 ppm) with the same level and source of other organic trace minerals as O1.

[3] UV=non-vaccinated; V=vaccinated with Coccivac®-T.

Table 4.7. Effect of level and source of Zn combined with Cu, Mn, and Se supplementations on trace minerals content in liver (μg/g) of commercial turkeys

	d 7				d 14			
	Cu	Mn	Se	Zn	Cu	Mn	Se	Zn
Treatment								
UV-SI	16.805	11.948	1.785	92.279	23.289	12.841	2.685	84.252
V-SI	14.864	12.448	1.814	83.226	23.712	15.093	2.312	92.586
UV-RI	15.496	10.973	2.060	108.130	18.917	12.425	3.051	87.893
V-RI	17.108	10.557	1.547	78.468	20.947	12.251	2.544	89.839
UV-O1	14.704	10.368	2.130	84.311	22.824	11.821	3.245	88.064
V-O1	14.699	10.850	1.966	77.747	19.164	12.464	3.315	97.439
UV-O2	14.224	11.140	2.191	76.556	18.428	12.763	3.317	87.386
V-O2	18.698	10.640	2.164	80.856	29.475	13.567	3.347	89.876
SEM[1]	1.182	0.511	0.197	6.934	3.913	0.545	0.186	3.102
Main effect								
Diet[2]								
SI	15.834	12.198[a]	1.780	87.753	23.500	13.967[a]	2.499[c]	88.419
RI	16.302	10.765[b]	1.803	93.299	19.932	12.338[b]	2.799[bc]	88.866
O1	14.702	10.609[b]	2.048	81.029	20.994	12.142[b]	3.280[ab]	92.751
O2	16.461	10.890[ab]	2.177	78.706	23.951	13.165[ab]	3.332[a]	88.631
Vaccine[3]								
UV	15.307	11.107	2.041	90.319[a]	20.865	12.462[b]	3.074	86.899[b]
V	16.342	11.124	1.873	80.074[b]	23.324	13.344[a]	2.879	92.435[a]
Statistical								
effects								
Diet	NS	0.01	NS	NS	NS	0.005	<.0001	NS
Vaccine	NS	NS	NS	0.04	NS	0.03	NS	0.01
Diet*Vaccine	NS	NS	NS	NS	NS	NS	NS	NS

[a-c] Means within a column without common superscripts are different ($P \leq 0.05$).

[1] All means are an average of 9 pens per treatment.

[2] SI=standard inorganic, inorganic Zn (150 ppm) with inorganic sources of Mn (165 ppm), Cu (10 ppm), and Se (0.2 ppm); RI=reduced inorganic, inorganic Zn, Mn, Cu at 10% of SI, and inorganic Se (0.2ppm); O1= organic 1, organic Zn (15 ppm) with organic sources of Mn (16.5 ppm), Cu (1 ppm), and Se (0.2 ppm); O2= organic 2, organic Zn (30 ppm) with the same level and source of other organic trace minerals as O1.

[3] UV=non-vaccinated; V=vaccinated with Coccivac®-T.

Table 4.8. Effect of level and source of Zn combined with Cu, Mn, and Se supplementations on lymphocyte subpopulation of commercial turkeys

	d 21			d 28			d 42		
	CD4+	CD8+	Ratio[4]	CD4+	CD8+	Ratio	CD4+	CD8+	Ratio
Treatment									
UV-SI	9.778	7.437	1.356	10.743^{ab}	4.292	2.609	5.175^{abc}	5.443	1.085
V-SI	9.714	5.118	1.805	8.005^{b}	3.802	2.231	5.110^{bc}	5.897	0.968
UV-RI	8.687	7.580	1.119	12.322^{ab}	5.347	2.420	5.037^{bc}	5.100	1.094
V-RI	10.700	7.177	1.570	15.534^{ab}	4.920	3.227	6.437^{abc}	4.525	1.435
UV-O1	8.056	5.138	1.667	9.417^{ab}	4.642	2.515	4.327^{bc}	4.657	1.023
V-O1	10.592	7.293	1.420	14.858^{ab}	5.408	2.864	8.185^{ab}	7.730	1.109
UV-O2	8.684	9.270	0.957	9.027^{b}	4.488	2.098	2.738^{c}	3.692	0.828
V-O2	8.173	5.378	1.534	18.108^{a}	6.496	2.951	10.030^{a}	6.782	1.495
SEM[1]	1.810	0.976	0.256	1.904	0.753	0.455	1.044	1.171	0.170
Main effect									
Diet[2]									
SI	9.746	6.277	1.580	9.374	4.047	2.420	5.143	5.670	1.026
RI	9.693	7.378	1.345	13.928	5.133	2.824	5.737	4.813	1.264
O1	9.324	6.216	1.543	12.138	5.025	2.690	6.256	6.193	1.066
O2	8.428	7.324	1.245	13.567	5.492	2.525	6.384	5.237	1.162
Vaccine[3]									
UV	8.801	7.356	1.275	10.377^{b}	4.692	2.411	4.319^{b}	4.723	1.007^{b}
V	9.795	6.241	1.582	14.126^{a}	5.157	2.818	7.440^{a}	6.233	1.252^{a}
Statistical effects									
Diet	NS	NS	NS	NS	NS	NS	NS	NS	NS
Vaccine	NS	NS	NS	0.008	NS	NS	0.0002	NS	0.048
Diet*Vaccine	NS	NS	NS	0.03	NS	NS	0.01	NS	NS

[a-c] Means within a column without common superscripts are different ($P \leq 0.05$).
[1] All means are an average of 9 pens per treatment.
[2] SI=standard inorganic, inorganic Zn (150 ppm) with inorganic sources of Mn (165 ppm), Cu (10 ppm), and Se (0.2 ppm); RI=reduced inorganic, inorganic Zn, Mn, Cu at 10% of SI, and inorganic Se (0.2ppm); O1= organic 1, organic Zn (15 ppm) with organic sources of Mn (16.5 ppm), Cu (1 ppm), and Se (0.2 ppm); O2= organic 2, organic Zn (30 ppm) with the same level and source of other organic trace minerals as O1.
[3] UV=non-vaccinated; V=vaccinated with Coccivac®-T.
[4] Ratio= CD4+/CD8+.

Table 4.9. Effect of level and source of Zn combined with Cu, Mn, and Se supplementations on turkey intestinal morphology of commercial turkeys

	Duodenum			Jejunum		
	Villus (mm)	Crypt (mm)	Ratio[6]	Villus (mm)	Crypt (mm)	Ratio
Diet[1]						
SI	1.858	0.057	34.408	1.107	0.061	18.698
RI	1.829	0.056	33.776	1.077	0.060	18.407
O1	1.838	0.057	33.125	1.111	0.059	19.100
O2	1.846	0.056	34.308	1.114	0.060	18.857
SEM[2]	0.017	0.001	0.670	0.018	0.001	0.404
P	0.6952	0.9193	0.5040	0.4725	0.5524	0.6806
Vaccine[3]						
UV	1.841	0.056	33.735	1.072	0.060	18.107
V	1.844	0.056	34.073	1.132	0.060	19.424
SEM[4]	0.013	0.001	0.474	0.013	0.001	0.286
P	0.8520	0.8293	0.6146	0.0012	0.6633	0.0013
Age						
d 7	1.358	0.059	23.677	0.773	0.059	13.448
d 14	1.703	0.056	31.236	1.052	0.059	18.719
d 28	2.081	0.057	37.107	1.218	0.061	20.127
d 42	2.230	0.053	43.597	1.366	0.061	22.771
SEM[5]	0.018	0.001	0.670	0.018	0.002	0.404
P	<0.0001	0.0024	<0.0001	<0.0001	0.1951	<0.0001
Interaction						
Diet*Vaccine	0.7602	0.2398	0.1923	0.3023	0.7866	0.7843
Diet*Age	0.2692	0.0920	0.0416	0.3994	0.4206	0.1398
Vaccine*Age	0.1884	<0.0001	<0.0001	0.0047	<0.0001	<0.0001
Diet*Vaccine*Age	0.5747	0.5882	0.1651	0.9718	0.6934	0.7818

[1] SI=standard inorganic, inorganic Zn (150 ppm) with inorganic sources of Mn (165 ppm), Cu (10 ppm), and Se (0.2 ppm); RI=reduced inorganic, inorganic Zn, Mn, Cu at 10% of SI, and inorganic Se (0.2ppm); O1= organic 1, organic Zn (15 ppm) with organic sources of Mn (16.5 ppm), Cu (1 ppm), and Se (0.2 ppm); O2= organic 2, organic Zn (30 ppm) with the same level and source of other organic trace minerals as O1.
[2] Means are an average of 18 pens per treatment.
[3] UV=non-vaccinated; V=vaccinated with Coccivac®-T.
[4] Means are an average of 36 pens per treatment.
[5] Means are an average of 72 pens per treatment.
[6] Ratio=Villus: crypt.

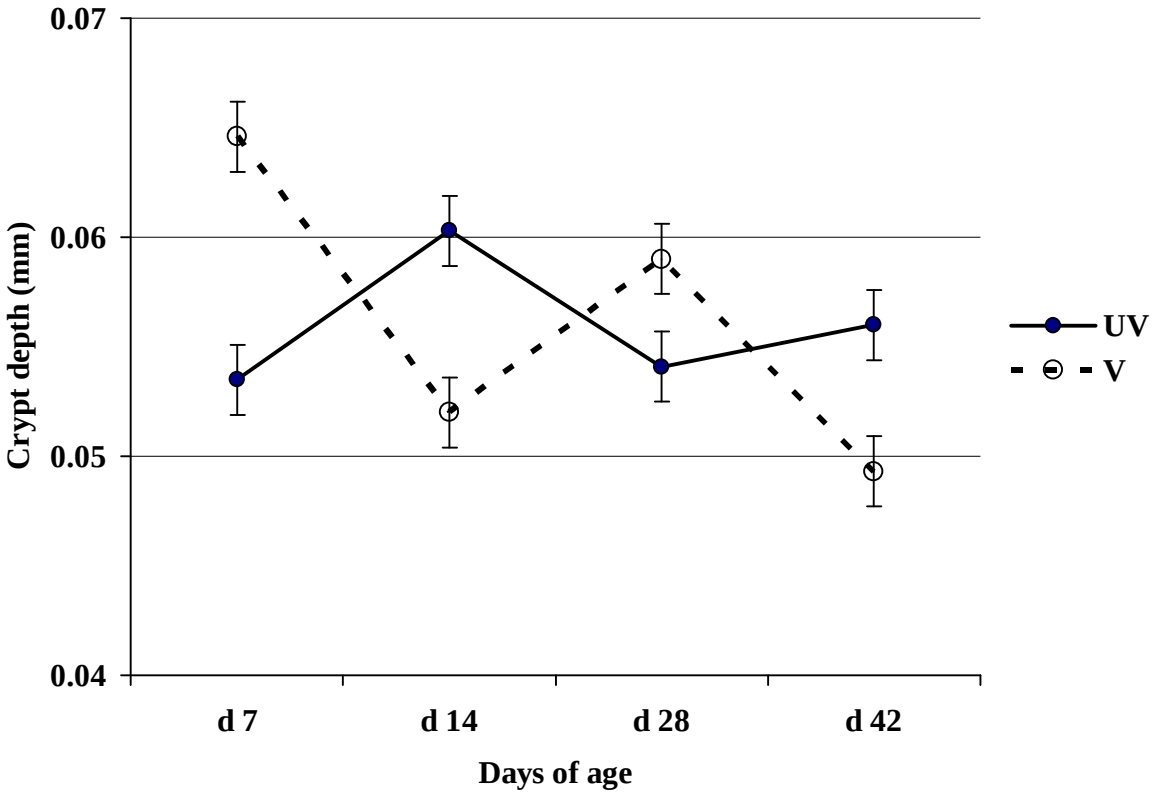

Figure 4.4. Interaction of vaccination and age on duodenum crypt depth. UV=non-vaccinated; V=vaccinated with Coccivac®-T. Interaction (P<0.0001) of vaccination and age was significant. Error bars indicate SEM.

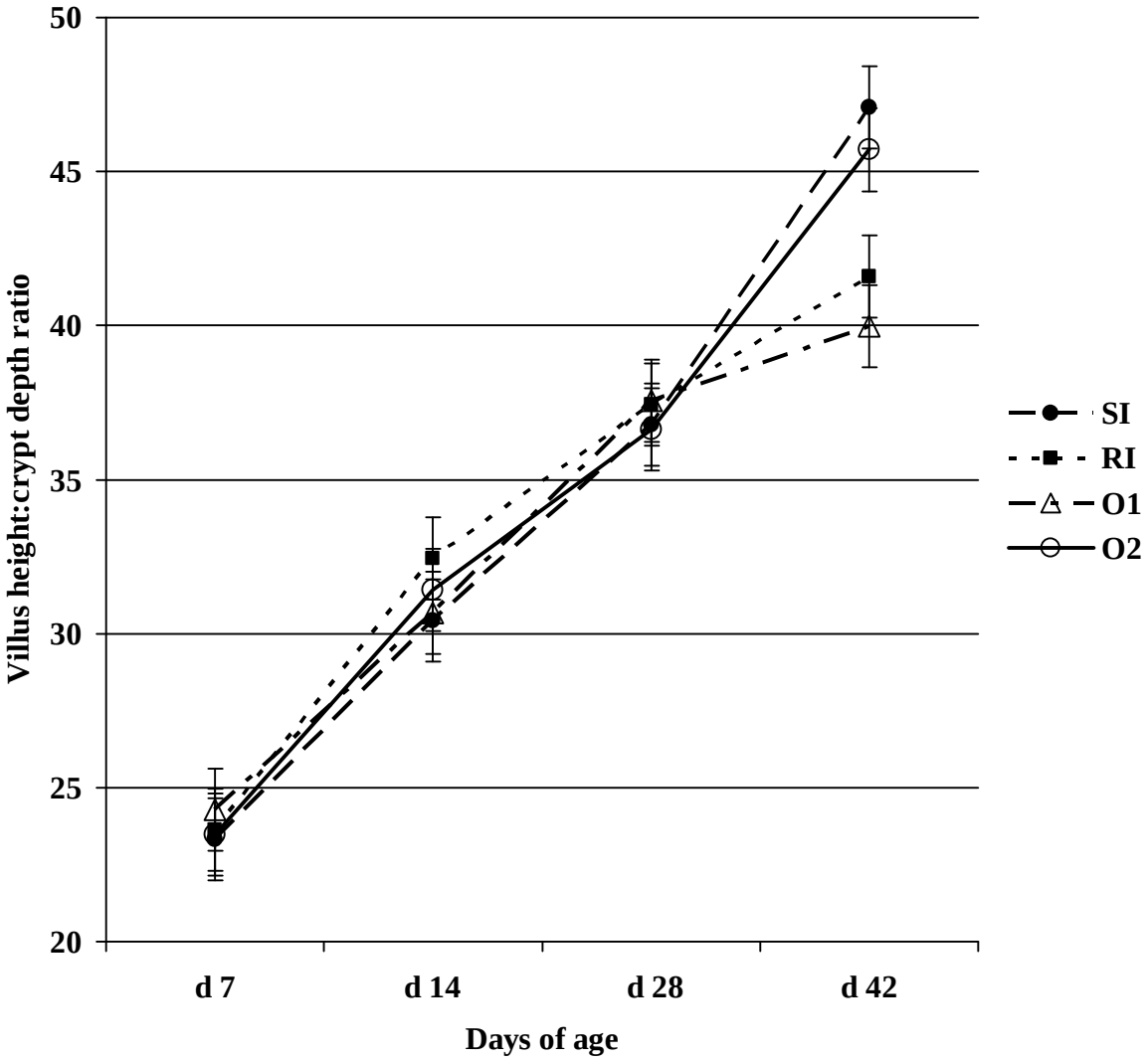

Figure 4.5. Interaction of diet and age on duodenum villus height to crypt depth ratio. SI=standard inorganic, inorganic Zn (150 ppm) with inorganic sources of Mn (165 ppm), Cu (10 ppm), and Se (0.2 ppm); RI=reduced inorganic, inorganic Zn, Mn, Cu at 10% of SI, and inorganic Se (0.2ppm); O1= organic 1, organic Zn (15 ppm) with organic sources of Mn (16.5 ppm), Cu (1 ppm), and Se (0.2 ppm); O2= organic 2, organic Zn (30 ppm) with the same level and source of other organic trace minerals as O1. Interaction (P =0.0416) of diet and age was significant. Error bars indicate SEM and n=18 pens at each day.

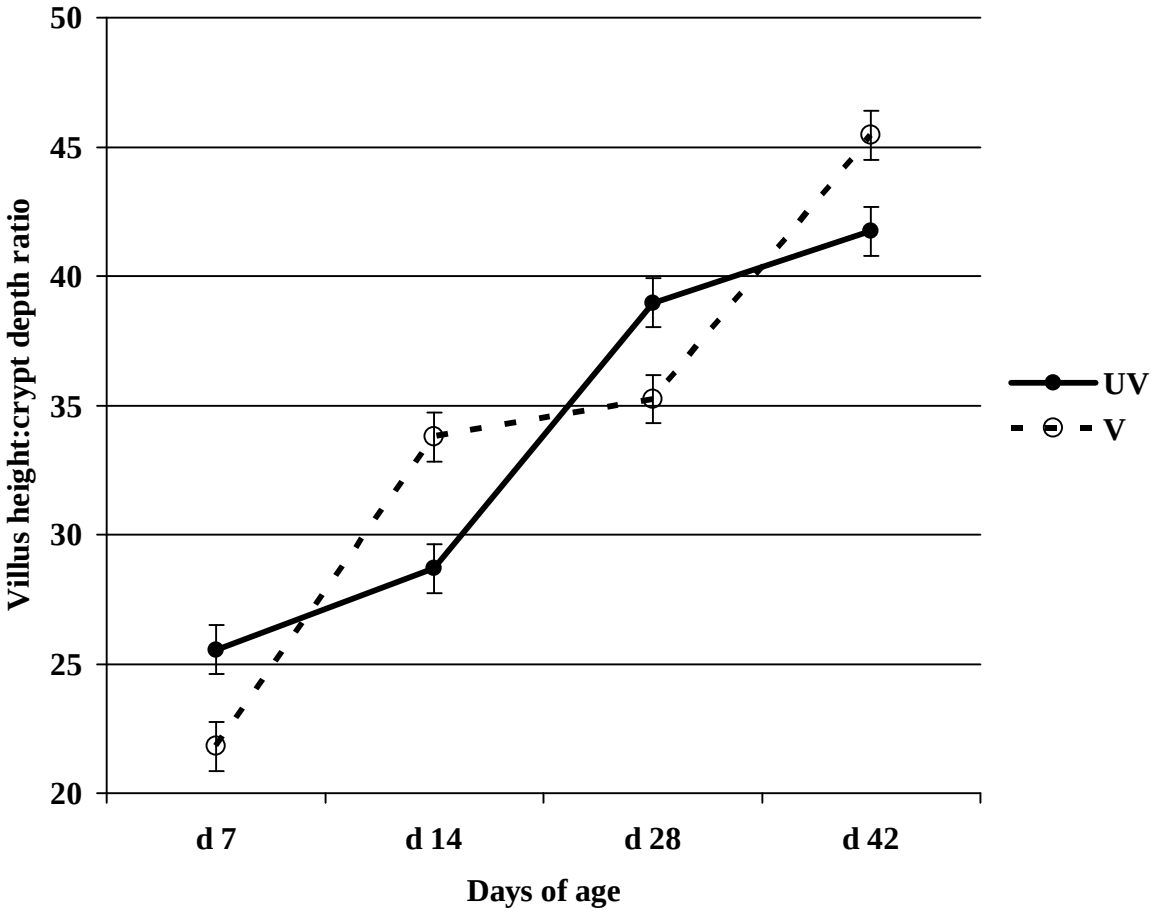

Figure 4.6. Interaction of vaccination and age on duodenum villus height to crypt depth ratio.
UV=non-vaccinated; V=vaccinated with Coccivac®-T. Interaction ($P<0.0001$) of vaccination and age was significant. Error bars indicate SEM.

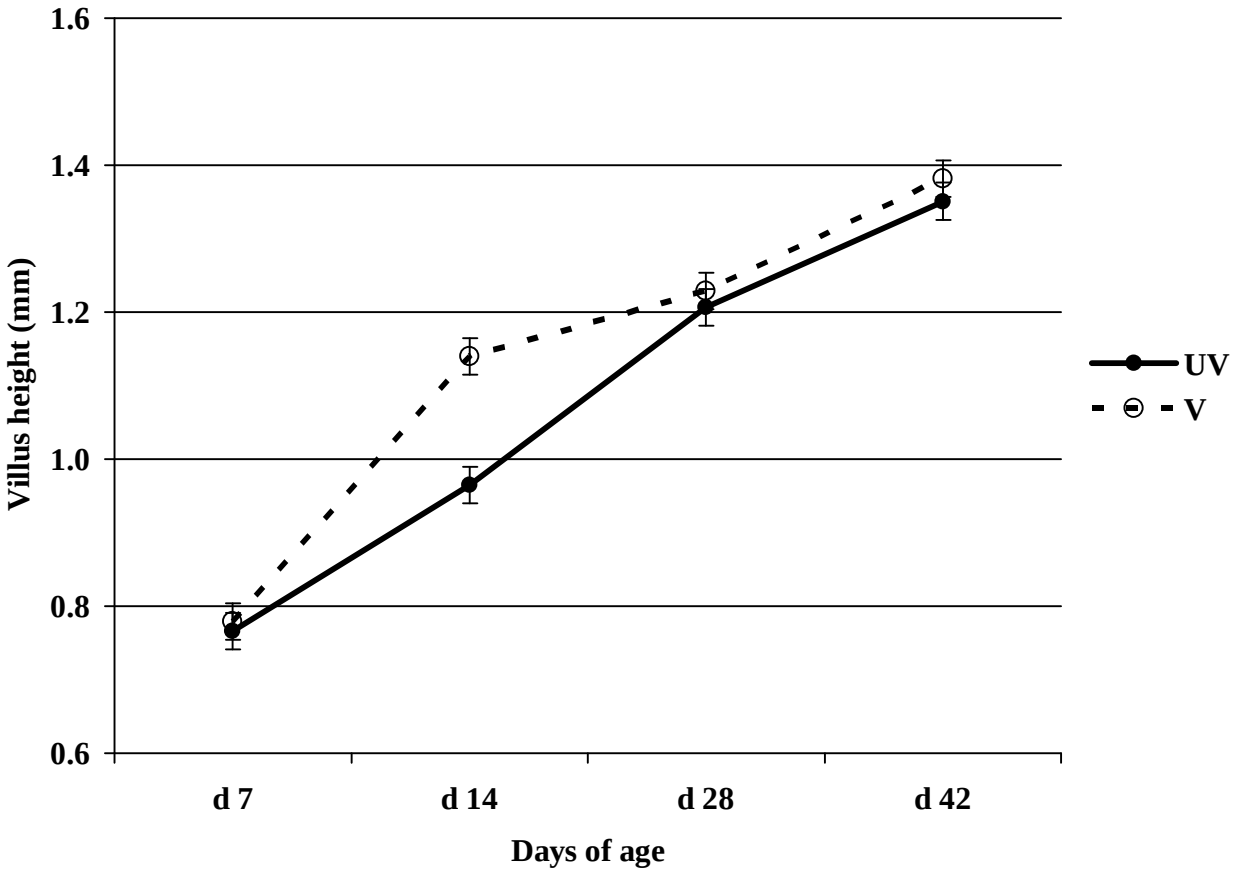

Figure 4.7. Interaction of vaccination and age for jejunum villus height. UV=non-vaccinated; V=vaccinated with Coccivac®-T. Interaction (P =0.0047) of vaccination and age was significant. Error bars indicate SEM.

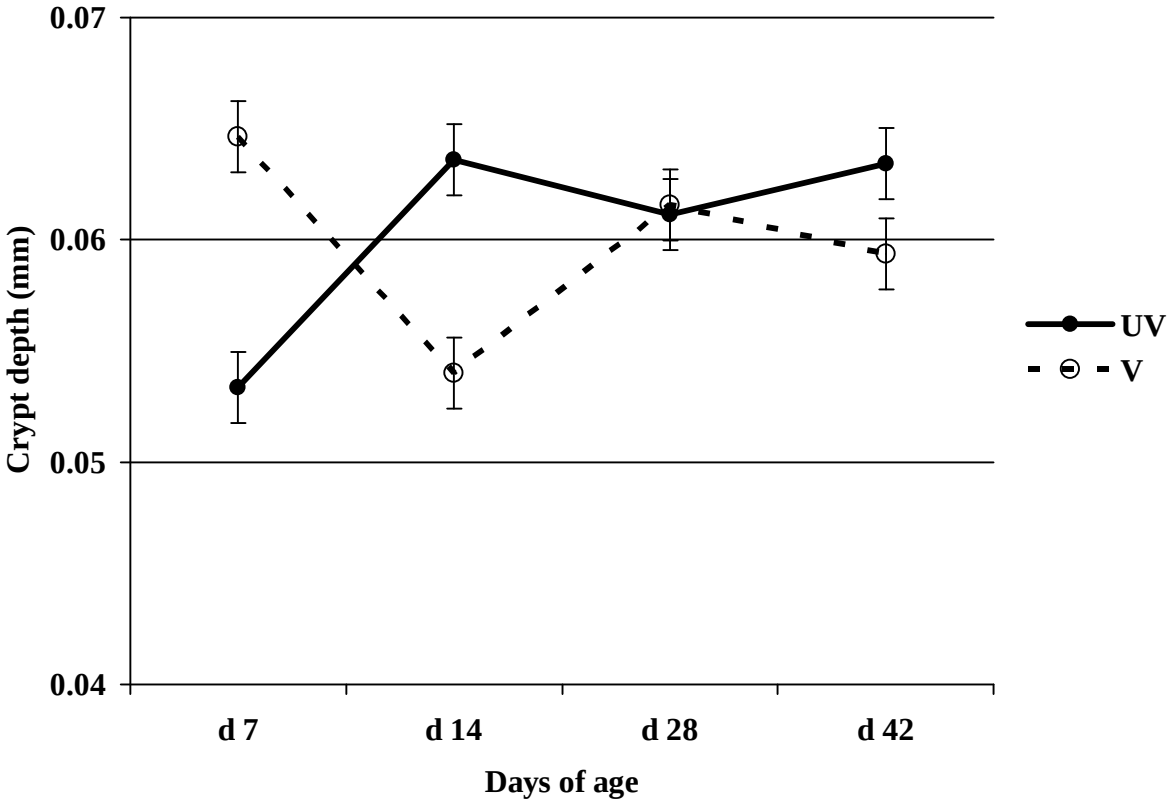

Figure 4.8. Interaction of vaccination and age on jejunum crypt depth. UV=non-vaccinated; V=vaccinated with Coccivac® -T. Interaction ($P<0.0001$) of vaccination and age was significant. Error bars indicate SEM.

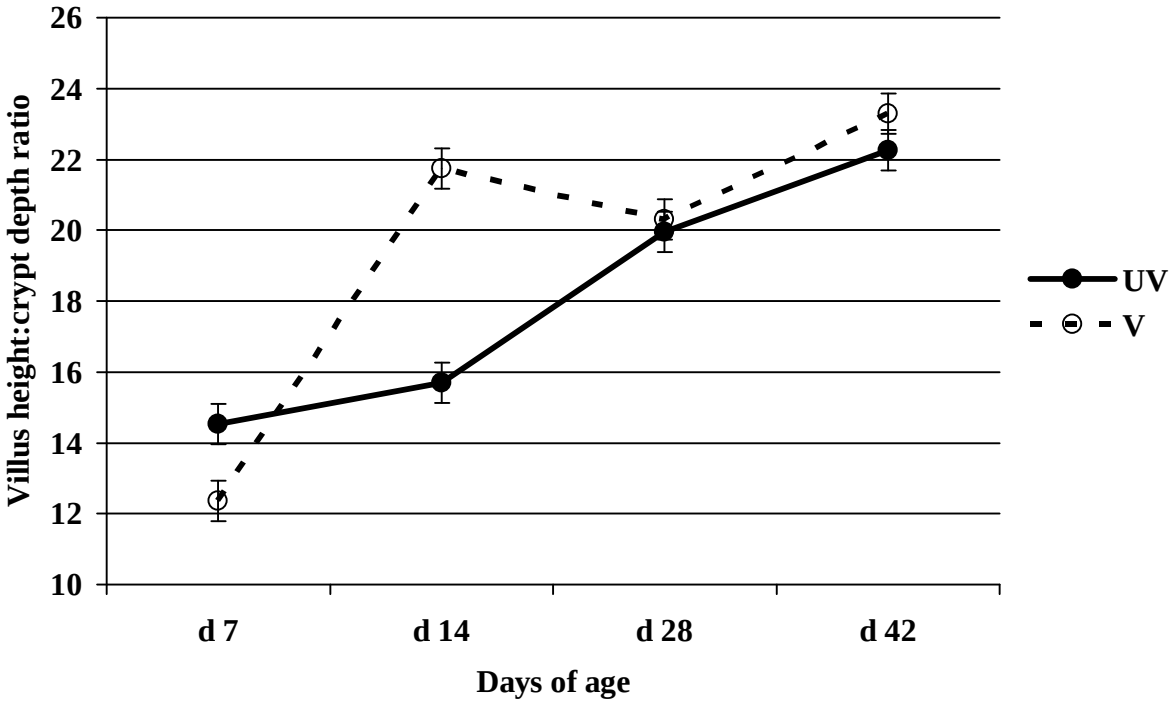

Figure 4.9. Interaction of vaccination and age on jejunum villus height to crypt depth ratio.
UV=non-vaccinated; V=vaccinated with Coccivac®-T. Interaction ($P<0.0001$) of vaccination and age was significant. Error bars indicate SEM.

Table 4.10. Effect of level and source of Zn combined with Cu, Mn, and Se supplementations on mucin producing goblet cells (number/mm^2) of commercial turkeys

	Duodenum	Jejunum
Diet[1]		
SI	933.01	1757.07
RI	954.97	1804.82
O1	970.33	1766.80
O2	960.74	1760.53
SEM[2]	16.85	32.41
P	0.4204	0.7139
Vaccine[3]		
UV	936.90	1751.61
V	972.63	1793.00
SEM[4]	11.92	22.91
P	0.0351	0.2029
Age		
d 7	958.08	1410.9
d 14	916.01	1564.10
d 28	959.01	1866.94
d 42	985.96	2247.28
SEM[5]	16.85	32.40
P	0.0298	<0.0001
Interaction		
Diet*Vaccine	0.1479	0.4284
Diet*Age	0.0358	0.0027
Vaccine*Age	0.0035	0.0117
Diet*Vaccine*Age	0.0291	0.0005

[1] SI=standard inorganic, inorganic Zn (150 ppm) with inorganic sources of Mn (165 ppm), Cu (10 ppm), and Se (0.2 ppm); RI=reduced inorganic, inorganic Zn, Mn, Cu at 10% of SI, and inorganic Se (0.2ppm); O1= organic 1, organic Zn (15 ppm) with organic sources of Mn (16.5 ppm), Cu (1 ppm), and Se (0.2 ppm); O2= organic 2, organic Zn (30 ppm) with the same level and source of other organic trace minerals as O1.
[2] Means are an average of 18 pens per treatment.
[3] UV=non-vaccinated; V=vaccinated with Coccivac®-T.
[4] Means are an average of 36 pens per treatment.
[5] Means are an average of 72 pens per treatment.

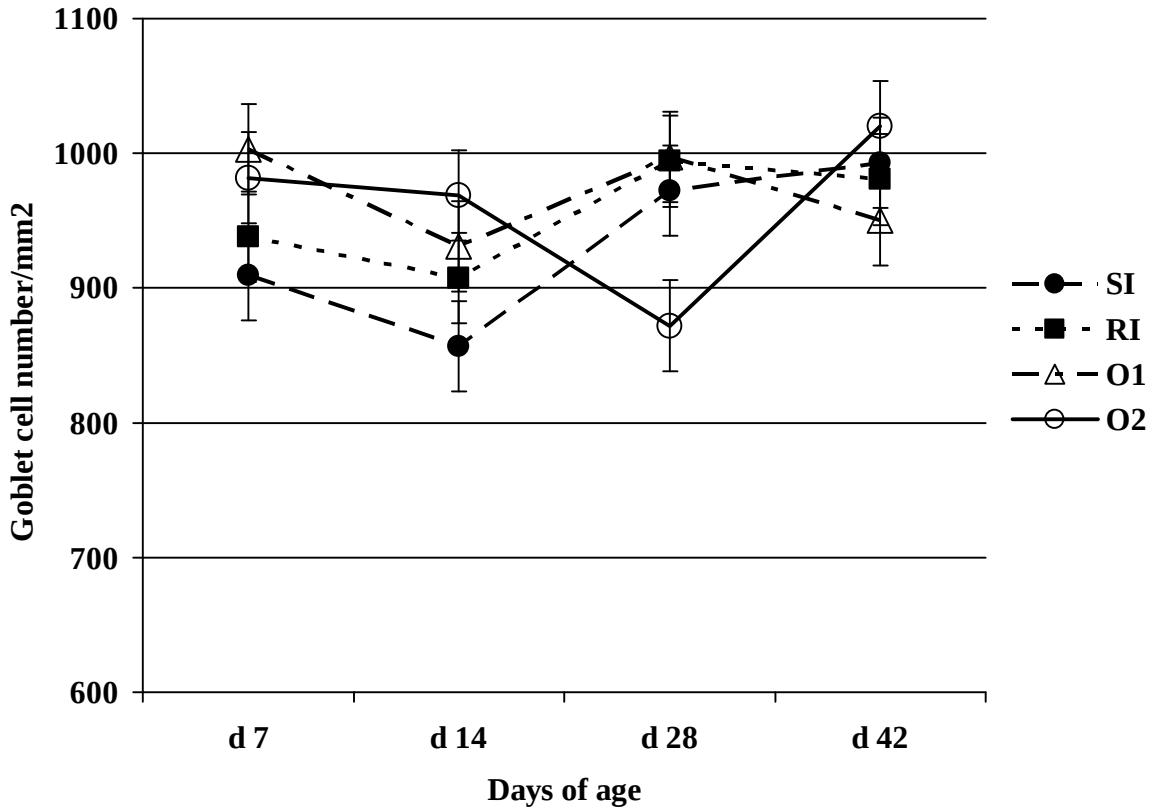

Figure 4.10. Interaction of diet and age on number of goblet cells in the duodenum. SI=standard inorganic, inorganic Zn (150 ppm) with inorganic sources of Mn (165 ppm), Cu (10 ppm), and Se (0.2 ppm); RI=reduced inorganic, inorganic Zn, Mn, Cu at 10% of SI, and inorganic Se (0.2ppm); O1= organic 1, organic Zn (15 ppm) with organic sources of Mn (16.5 ppm), Cu (1 ppm), and Se (0.2 ppm); O2= organic 2, organic Zn (30 ppm) with the same level and source of other organic trace minerals as O1. Interaction (P =0.0358) of diet and age was significant. Error bars indicate SEM.

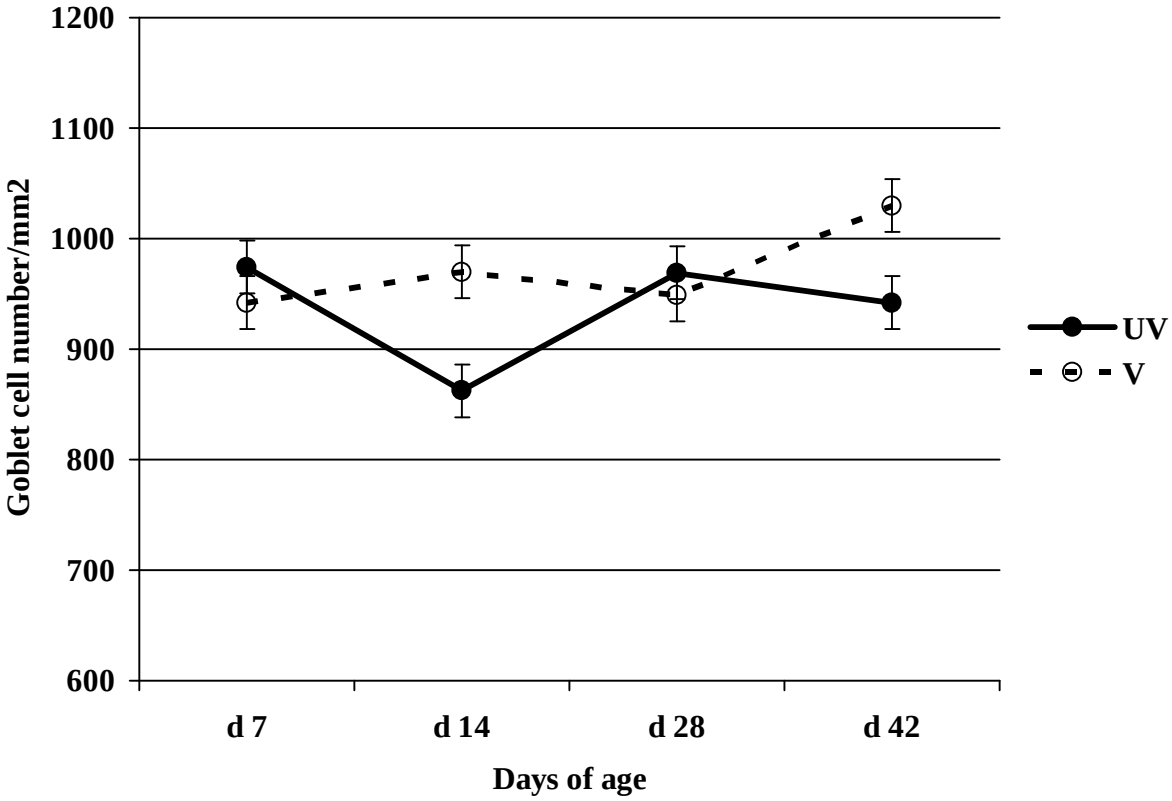

Figure 4.11. Interaction of vaccination and age on number of goblet cells in the duodenum.
UV=non-vaccinated; V=vaccinated with Coccivac®-T. Interaction (P =0.0035) of vaccination and age was significant. Error bars indicate SEM.

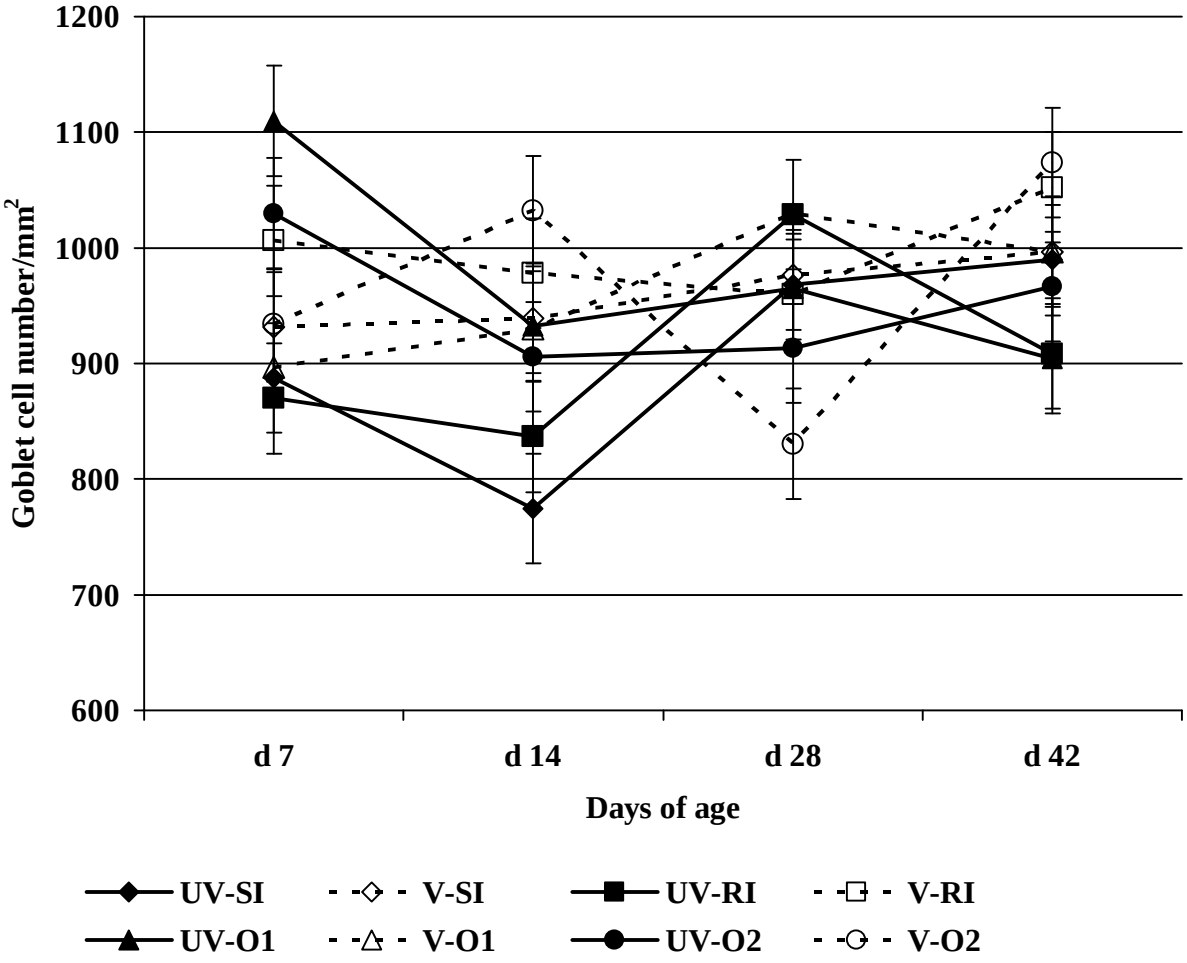

Figure 4.12. Interaction of diet, vaccination and age on number of goblet cells in the duodenum.
SI=standard inorganic, inorganic Zn (150 ppm) with inorganic sources of Mn (165 ppm), Cu (10 ppm), and Se (0.2 ppm); RI=reduced inorganic, inorganic Zn, Mn, Cu at 10% of SI, and inorganic Se (0.2ppm); O1= organic 1, organic Zn (15 ppm) with organic sources of Mn (16.5 ppm), Cu (1 ppm), and Se (0.2 ppm); O2= organic 2, organic Zn (30 ppm) with the same level and source of other organic trace minerals as O1. UV=non-vaccinated; V=vaccinated with Coccivac®-T. Interaction (P =0.0291) of diet, vaccination, and age was significant. Error bars indicate SEM.

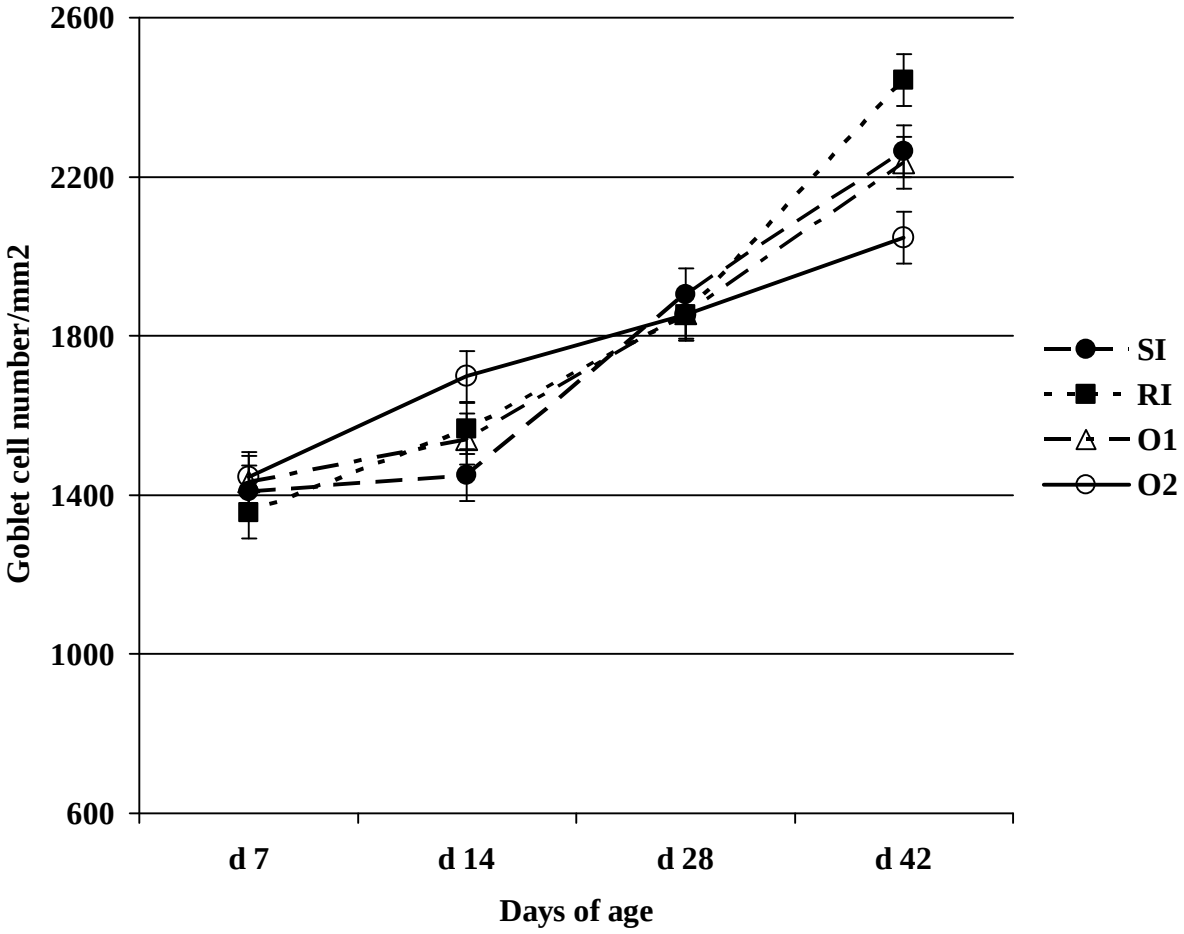

Figure 4.13. Interaction of diet and age on number of goblet cells in the jejunum. SI=standard inorganic, inorganic Zn (150 ppm) with inorganic sources of Mn (165 ppm), Cu (10 ppm), and Se (0.2 ppm); RI=reduced inorganic, inorganic Zn, Mn, Cu at 10% of SI, and inorganic Se (0.2ppm); O1= organic 1, organic Zn (15 ppm) with organic sources of Mn (16.5 ppm), Cu (1 ppm), and Se (0.2 ppm); O2= organic 2, organic Zn (30 ppm) with the same level and source of other organic trace minerals as O1. Interaction (P =0.0027) of diet and age was significant. Error bars indicate SEM.

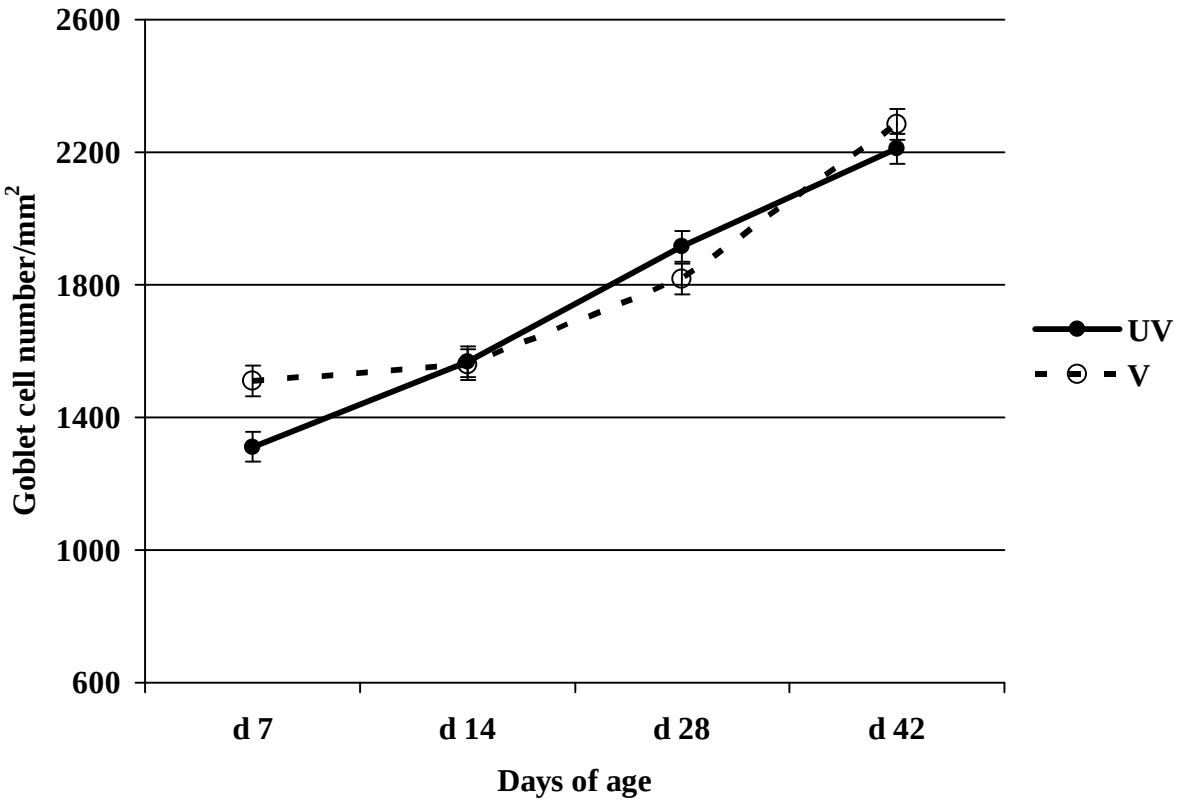

Figure 4.14. Interaction of vaccination and age on number of goblet cells in the jejunum. UV=non-vaccinated; V=vaccinated with Coccivac®-T. Interaction (P =0.0117) of vaccination and age was significant. Error bars indicate SEM.

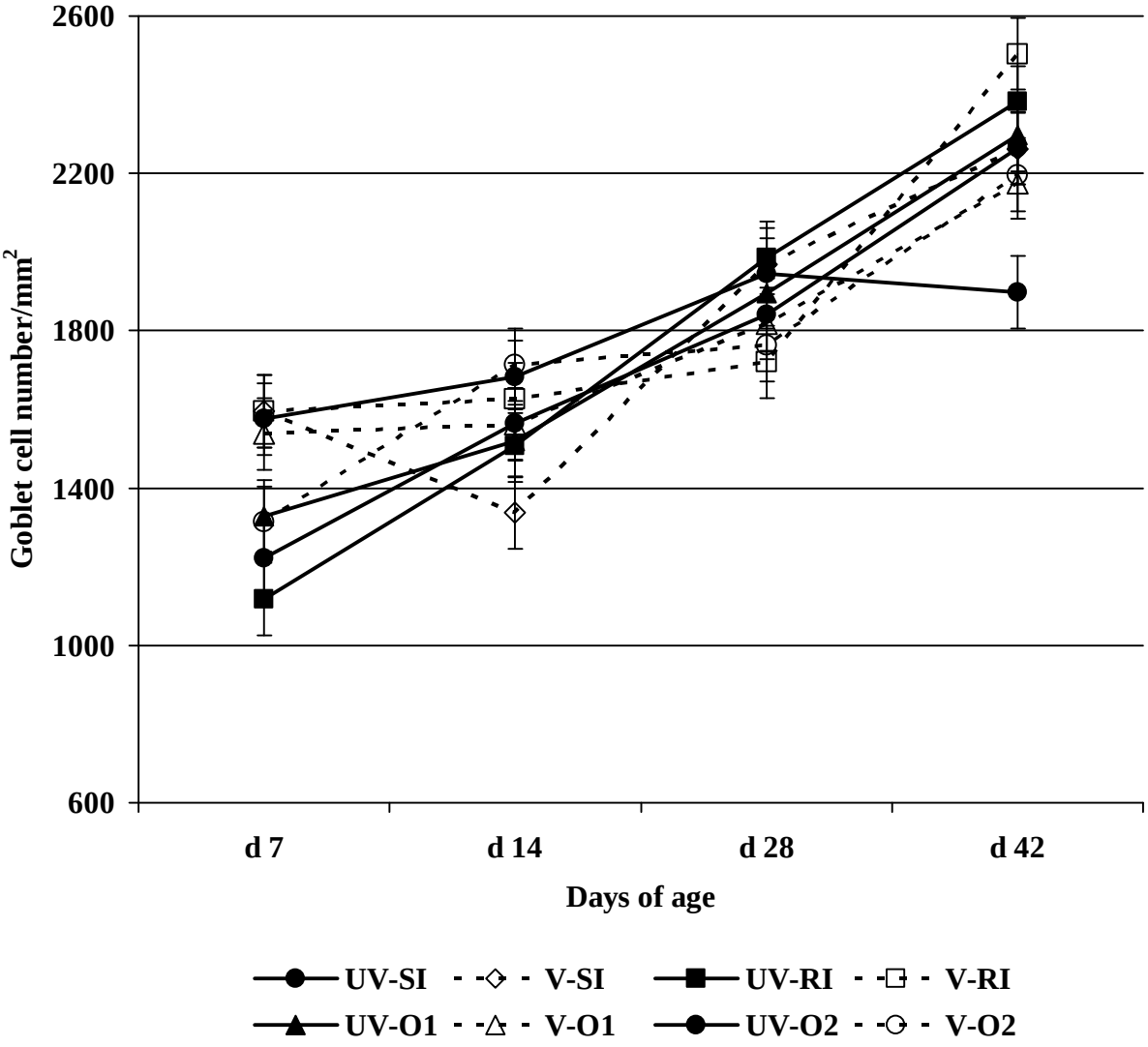

Figure 4.15. Interaction of diet, vaccination and age on number of goblet cells in the jejunum.
SI=standard inorganic, inorganic Zn (150 ppm) with inorganic sources of Mn (165 ppm), Cu (10 ppm), and Se (0.2 ppm); RI=reduced inorganic, inorganic Zn, Mn, Cu at 10% of SI, and inorganic Se (0.2ppm); O1= organic 1, organic Zn (15 ppm) with organic sources of Mn (16.5 ppm), Cu (1 ppm), and Se (0.2 ppm); O2= organic 2, organic Zn (30 ppm) with the same level and source of other organic trace minerals as O1. UV=non-vaccinated; V=vaccinated with Coccivac®-T. Interaction (P =0.0005) of diet, vaccination, and age was significant. Error bars indicate SEM.

Chapter V

Epilogue

Modern poultry strains have been selected for improved growth rate or increased egg output over the last 40 years. Therefore, the recommended requirements for trace minerals in the NRC (1994) most likely do not represent the needs of modern strains of commercial poultry. As a result, commonly used excessive levels of dietary minerals provides a safety margin for poultry performance, while it exacerbates the hazard of environment contamination by unabsorbed minerals. Data of the present study indicated that the mineral content (Mn, Zn) in excreta and litter were significantly reduced by decreasing the diet trace mineral levels, changing diet trace minerals source, balancing diet trace minerals complex, and phase feeding during 19 wk feeding in turkeys. Reducing the dietary trace mineral supplement may be an effective strategy to reduce excess mineral burden and reduce the risk of soil phytotoxicity. When minerals are supplemented in excess of the animal's requirement, minerals tend to be excreted in the feces because of decreased utilization efficiency. Therefore, using a smaller safety margin when formulating poultry diets may be a potential way of reducing the risk of trace mineral accumulation in the soil, especially in areas of intensive farming.

Feed ingredient analysis is a pivotal step to reduce the appearance of soil contamination, since it could ensure a safety margin of trace minerals in the diet and avoid the occurence of trace mineral deficiency. Feedstuffs grown in certain geographic areas may be plentiful, marginal, or even deficient in specific elements because of varied uptake of minerals caused by variation of trace minerals in the soil. An excessive concentration of one mineral may result in a deficiency of absorption in other minerals

due to mineral interactions. Thus, analysis of feed ingredients is an absolutely necessary approach to confirm the appropriate amount of trace minerals that the birds intake.

The requirements for Mn and Zn in the NRC (1994) were 60 ppm and 70 ppm for turkeys from wk 0 to wk 8, respectively. In this study, the concentration of Mn and Zn were 88 ppm and 90 ppm in the RI and O1 diets (the treatments with the lowest supplemental level of trace minerals) by feed ingredient analysis, respectively, which is still higher than the published requirement for trace minerals in NRC (1994). The performance of turkeys with the RI or O1 diets was similar to or improved compared with the poults with higher supplemental levels of trace minerals, even with the challenge of a coccidial vaccine. The results suggested that the supplementation level of trace minerals may be decreased, even below the NRC requirement. Although there was no significant difference in performance, immune response, or intestinal morphology between RI and O1 treatments during the experiments, the effect of trace mineral source may also need to be considered. It is reported that organic minerals usually show better bioavailability than inorganic forms. Since organic minerals are usually chelated with protein/amino acids, one of the most acceptable absorption mechanisms is absorption intact into the blood system by peptide or amino acid uptake pathways rather than the normal metal ion uptake mechanism. However, no one really knows the exact absorption mechanism of organic minerals. Therefore, when we reduce the level of trace minerals in further investigations, we should also consider the effect of mineral source.

The conclusions relating to immune response with the different diets in the second study were only based on *Eimeria* infection. In commercial poultry flocks, the birds have to face multiple diseases and stress. Beyond *Eimeria*, there are many other infectious

agents such as viruses, bacteria, parasites and fungi involved in turkey diseases. Infectious agents can be introduced and spread through turkey farms by horizontal and vertical routes, which cause large economic losses due to decreased weight gain, increased mortality rates, medication costs, and feed conversion rates. In the modern turkey industry, enteric disorders, respiratory diseases, leg weakness, cannibalism and breast blisters are common in meat turkeys. Usually the development of any disease in poultry flocks is influenced by three systems, which include the management, the host, and the infectious agent (Hafez, 2000). The severity of clinical signs, duration of the disease and mortality are influenced by virulence of the agent, nutritional and immune status of the birds, and management. Poor nutrition and feed quality can either increase a bird's susceptibility to enteric disorders or directly cause them. Because of importance in protein, fat and carbohydrate metabolism, epithelial tissue integrity, bone development, immune response, and cell protection, trace minerals are necessary to maintain animal health and resist diseases. However, the requirement of trace minerals in the diet may change, since the causes and mechanisms of diseases are different. Therefore, we need to investigate the effect of source and low levels of trace minerals in the diet on other turkey diseases or stressful conditions before we conclude that the decreased supplemental levels of trace minerals are adequate for all situations. The objective of further research could be to determine the optimum levels of trace minerals in the diet to guarantee maximum performance and immune response while decreasing environmental contamination.

Using diet composition to manipulate the immune system is probably one of the most cost-effective and practical control measures to prevent infection. However, the immune response is very complicated during infection. It has been shown that the

chicken intestinal immune response involves multiple cytokines, chemokines, and T cell subsets following *Eimeria* infection. In the present study, CD4+, CD8+, and ratios of CD4+ and CD8+ T cells were measured in peripheral blood. Since the anti-CD3+ does not cross react well with the turkey CD3+ antigen, there was no way to evaluate its effect. Therefore, we could not evaluate the immune response induced by CD3+ lymphocytes and related cytokines regulated by CD3+. Further investigations need to be executed to evaluate lymphocyte subpopulations in the intestine, which may indicate the exact immune response caused by *Eimeria* infection, since dynamic changes in intestinal T-lymphocytes are not adequately represented in peripheral lymph nodes or blood. Our understanding is limited with respect to whether immune phenotypic and functional alterations in intestinal immune cells are adequately reflected by circulating immune cells in peripheral blood and the relationship between systemic immune response and local tissue T-lymphocyte response during *Eimeria* infection. To achieve these aims, we also need to observe the altered cytokine responses in peripheral blood and intestinal mucosa during the infection. The studies on the effect of other trace minerals (Mn, Cu, and Se) on CD4+ and CD8+ in *Eimeria* infection is recommended. Research on the relationship between blood levels of trace minerals and immune response should also be executed during *Eimeria* infection.

Eimeria infection affects the expression levels of gene transcripts encoding pro-inflammatory, Th1 and Th2 cytokines, such as, IFN-γ, TNF-α, IL-1β, and IL-6, in intestinal intraepithelial lymphocytes. In this study, we did not measure the expression of these cytokines in intestinal tissue since there was no significant effect on lymphocyte subpopulations and ratios in peripheral blood between dietary treatments during the

whole experiment. Most of the immune response happens in the intestine during acute infection phase. However, the measurement of lymphocyte subpopulation started from d 21, which may have neglected the immune response in acute infection. Therefore, further study should be focused on the effect of trace minerals on expression of the related cytokines in intestinal tissue during the acute phase of infection as well as the recovery phases.

In summary, the present study suggested that low levels of trace minerals in the diet resulted in similar or improved performance, immune response, and intestinal morphology, with decreased environmental pollution when compared with NRC recommended levels in the diet. Future studies should focus on determination of the optimal safety margin of trace mineral supplementation, either organic or inorganic, and underlying mechanisms of action, based on performance and immune response under different challenge situations.

Literature Cited

AAFCO. 2005. Official publication. Pages 307-308. Association of American Feed Control Officials, Atlanta, GA.

Allen, P. C., and R. H. Fetterer. 2002. Recent advances in biology and immunobiology of Eimeria species and in diagnosis and control of infection with these coccidian parasites of poultry. Clin. Microbiol. Rev. 15:58-65.

Ao, T., J. L. Pierce, R. Power, K. A. Dawson, A. J. Pescatore, A. H. Cantor, and M. J. Ford. 2006. Evaluation of bioplex Zn as an organic zinc source for chicks. International Journal of Poultry Sciences 5: 808-811.

AOAC, 1995. Official Methods of Analysis. Association of Official Analytical Chemists, Washington, DC.

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

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

Arredondo, M., and M. T. Nunez. 2005. Iron and copper metabolism. Mol Aspects Med 26:313-327.

Aschner, J. L., and M. Aschner. 2005. Nutritional aspects of manganese homeostasis. Mol Aspects Med 26:353-362.

Atuma, C., V. Strugala, A. Allen, and L. Holm. 2001. The adherent gastrointestinal mucus gel layer: thickness and physical state in vivo. Am J Physiol Gastrointest Liver Physiol 280:G922-929.

Bafundo, K. W., D. H. Baker, and P. R. Fitzgerald. 1984. Zinc utilization in the chick as influenced by dietary concentrations of calcium and phytate and by Eimeria acervulina infection. Poult Sci 63:2430-2437.

Baker, D. H., and C. B. Ammerman. 1995. Zinc bioavailability. Pages 367-398 in Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins. C. B. Ammerman, D. H. Baker, A. J. Lewis, ed. Academic Press, San Diego.

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.

Bala, S., M. L. Failla, and J. K. Lunney. 1991. Alterations in splenic lymphoid cell subsets and activation antigens in copper-deficient rats. J Nutr 121: 745-753.

Balevi, T., and B. Coskun. 2004. Effects of dietary copper on production and egg cholesterol content in laying hens. Br Poult Sci 45:530-534.

Banks, K. M., K. L. Thompson, J. K. Rush, and T. J. Applegate. 2004. Effects of copper source on phosphorus retention in broiler chicks and laying hens. Poult Sci 83: 990-996.

Bao, B., A. S. Prasad, F. W. Beck, and M. Godmere. 2003. Zinc modulates mRNA levels of cytokines. Am J Physiol Endocrinol Metab 285:E1095-1102.

Bar-Shira, E., D. Sklan, and A. Friedman. 2003. Establishment of immune competence in the avian GALT during the immediate post-hatch period. Dev Comp Immunol 27:147-157.

Bartlett, J. R., and M. O. Smith. 2003. Effects of different levels of zinc on the performance and immunocompetence of broilers under heat stress. Poult Sci 82:1580-1588.

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, and R. M. Leach. 1978. Copper deficiency in the laying hen. J Nutr 108:804-811.

Beach, J. R., and J. C. Corl. 1925. Studies in the control of avian coccidiosis. Poult Sci 4:83-93.

Beach, R. S., M. E. Gershwin, and L. S. Hurley. 1982. Reversibility of developmental retardation following murine fetal zinc deprivation. J Nutr 112:1169-1181.

Beck, F. W., A. S. Prasad, J. Kaplan, J. T. Fitzgerald, and G. J. Brewer. 1997. Changes in cytokine production and T cell subpopulations in experimentally induced zinc-deficient humans. Am J Physiol 272:E1002-1007.

Befus, A. D., N. Johnston, G. A. Leslie, and J. Bienenstock. 1980. Gut-associated lymphoid tissue in the chicken. I. Morphology, ontogeny, and some functional characteristics of Peyer's patches. J Immunol 125:2626-2632.

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.

Bourlioux, P., B. Koletzko, F. Guarner, and V. Braesco. 2003. The intestine and its microflora are partners for the protection of the host: report on the Danone Symposium "The Intelligent Intestine," held in Paris, June 14, 2002. Am J Clin Nutr 78:675-683.

Bozkaya, L. A., R. Oztürk-Urek, T. Aydemir, and L. Tarhan. 2001. Effects of Se, Cu and Se + vitamin E deficiency on the activities of CuZnSOD, GSH-Px, CAT and LPO levels in chicken erythrocytes. Cell Biochem Funct 19:153-157.

Broom, L. J., H. M. Miller, K. G. Kerr, and J. S. Knapp. 2006. Effects of zinc oxide and Enterococcus faecium SF68 dietary supplementation on the performance, intestinal microbiota and immune status of weaned piglets. Res Vet Sci 80:45-54.

Brown, D. R., and L. L. Southern. 1985. Effect of Eimeria acervulina infection in chicks fed excess dietary cobalt and/or manganese. J Nutr 115:347-351.

Brown, P. J., B. G. Miller, C. R. Stokes, N. B. Blazquez, and F. J. Bourne. 1988. Histochemistry of mucins of pig intestinal secretory epithelial cells before and after weaning. J Comp Pathol 98:313-323.

Burrell, A. L., W. A. Dozier, A. J. Davis, M. M. Compton, M. M. Freeman, P. F. Vendrell, and T. L. Ward. 2004. Responses of broilers to dietary zinc concentrations and sources in relation to environmental implications. Br. Poult. Sci. 45: 255-263.

Byrnes, S., R. Eaton, and M. Kogut. 1993. In vitro interleukin-1 and tumor necrosis factor-alpha production by macrophages from chickens infected with either Eimeria maxima or Eimeria tenella. Int J Parasitol 23:639-645.

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.

Cario, E., S. Jung, J. Harder D'Heureuse, C. Schulte, A. Sturm, B. Wiedenmann, H. Goebell, and A. U. Dignass. 2000. Effects of exogenous zinc supplementation on intestinal epithelial repair in vitro. Eur J Clin Invest 30:419-428.

Carlson, M. S., S. L. Hoover, G. M. Hill, J. E. Link, and J. R. Turk. 1998. Effect of pharmacological zinc on intestinal metallothionein concentration and morphology in the nursery pig. J Anim Sci 76 (Suppl. 1):57 (abstr.).

Carlton, W. W., and W. Henderson. 1964. Skeletal lesions in experimental copper-deficiency in chickens. Avian Dis 8:48-55.

Case, C. L., and M. S. Carlson. 2002. Effect of feeding organic and inorganic sources of additional zinc on growth performance and zinc balance in nursery pigs. J Anim Sci 80:1917-1924.

Cebra, J. J. 1999. Influences of microbiota on intestinal immune system development. Am J Clin Nutr 69:1046S-1051S.

Chapman, H. D. 1997. Biochemical, genetic and applied aspects of drug resistance in Eimeria parasites of the fowl. Avian Pathol 26:221-244.

Chapman, H. D., B. Roberts, M. W. Shirley, and R. B. Williams. 2005. Guidelines for evaluating the efficacy and safety of live anticoccidial vaccines, and obtaining approval for their use in chickens and turkeys. Avian Pathol 34:279-290.

Cheng, T. K., T. M. Fakler, C. J. Rapp, and T. L. Ward. 2005. Organic versus inorganic trace minerals in poultry: the zinc experience. Pages 101-106 in 32nd Annual Carolina Poultry Nutrition Conference, Sheraton Imperial Hotel, RTP, NC.

Chesters, J. K. 1992. Trace element-gene interactions. Nutr Rev 50:217-223.

Choct, M., A. J. Naylor, and N. Reinke. 2004. Selenium supplementation affects broiler growth performance, meat yield and feather coverage. Br. Poult. Sci. 45: 677-683.

Coleman, J. E. 1992. Zinc proteins: enzymes, storage proteins, transcription factors, and replication proteins. Annu Rev Biochem 61:897-946.

Collins, N. E., and E. T. Moran. 1999. Influence of supplemental manganese and zinc on live performance and carcass quality of diverse broiler strains J Appl Poult Res 8:228-235.

Colnago, G. L., L. S. Jensen, and P. L. Long. 1984. Effect of selenium and vitamin E on the development of immunity to coccidiosis in chickens. Poult Sci 63:1136-1143.

Cook-Mills, J. M., and P. J. Fraker. 1993. The role of metals in the production of toxic oxygen metabolites by mononuclear phagocytes. Pages 127-140 in Nutrient Modulation of the Immune Response. C. Cunningham-Rundles, ed. Marcel Dekker, New York.

Coppen, D. E., and N. T. Davies. 1987. Studies on the effects of dietary zinc dose on 65Zn absorption in vivo and on the effects of Zn status on 65Zn absorption and body loss in young rats. Br J Nutr 57:35-44.

Cousins, R. J. 1985. Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiol. Rev 65:238-309.

Cousins, R. J. 1998. A role of zinc in the regulation of gene expression. Proc Nutr Soc 57:307-311.

Cragg, R. A., S. R. Phillips, J. M. Piper, J. S. Varma, F. C. Campbell, J. C. Mathers, and D. Ford. 2005. Homeostatic regulation of zinc transporters in the human small intestine by dietary zinc supplementation. Gut 54:469-478.

Creech, B. L., J. W. Spears, W. L. Flowers, G. M. Hill, K. E. Lloyd, T. A. Armstrong, and T. E. Engle. 2004. Effect of dietary trace mineral concentration and source (inorganic vs. Chelated) on performance, mineral status, and fecal mineral excretion in pigs from weaning through finishing. J. Anim. Sci. 82: 2140-2147.

Cromwell, G. L., and C. Kirk-Baer. 2005. Setting the NRC standards for minerals-were

we right? Nottingham University Press, Nottingham.

Dalloul, R. A., and H. S. Lillehoj. 2005. Recent advances in immunomodulation and vaccination strategies against coccidiosis. Avian Dis 49:1-8.

Dalloul, R. A., and H. S. Lillehoj. 2006. Poultry coccidiosis: recent advancements in control measures and vaccine development. Expert Rev Vaccines 5:143-163.

Dardenne, M., and J. M. Bach. 1993. Rationale for the mechanism of zinc interaction in the immune system. Pages 501-509 in Nutrient Modulation of the Immune Response. C. Cunningham-Rundles, ed. Marcel Dekker Inc., New York.

Davis, C. D., L. Zech, and J. L. Greger. 1993. Manganese metabolism in rats: an improved methodology for assessing gut endogenous losses. Proc Soc Exp Biol Med 202:103-108.

Degen, W. G., N. Daal, L. Rothwell, P. Kaiser, and V. E. Schijns. 2005. Th1/Th2 polarization by viral and helminth infection in birds. Vet Microbiol 105:163-167.

DePasquale-Jardieu, P., and P. J. Fraker. 1984. Interference in the development of a secondary immune response in mice by zinc deprivation: persistence of effects. J Nutr 114:1762-1769.

Deplancke, B., and H. R. Gaskins. 2001. Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. Am J Clin Nutr 73:1131S-1141S.

Dewar, W. A., P. A. Wight, R. A. Pearson, and M. J. Gentle. 1983. Toxic effects of high concentrations of zinc oxide in the diet of the chick and laying hen. Br Poult Sci 24: 397-404.

Dibner, J. J., J. D. Richards, M. L. Kitchell, and M. A. Quiroz. 2007. Metabolic challenges and early bone development. J Appl Poult Res 16:126-137.

Dowd, P. S., J. Kelleher, and P. J. Guillou. 1986. T-lymphocyte subsets and interleukin-2 production in zinc-deficient rats. Br J Nutr 55:59-69.

Dozier, W. A., 3rd, A. J. Davis, M. E. Freeman, and T. L. Ward. 2003. Early growth and environmental implications of dietary zinc and copper concentrations and sources of broiler chicks. Br Poult Sci 44:726-731.

Driessen, C., K. Hirv, H. Kirchner, and L. Rink. 1995. Zinc regulates cytokines induction by superantigens and lipopolysaccharide. Immunology 84:272-277.

Driscoll, D. M., and P. R. Copeland. 2003. Mechanism and regulation of selenoprotein synthesis. Annu Rev Nutr 23:17-40.

Dunsford, B. R., W. E. Haensly, and D. A. Knabe. 1991. Effects of diet on acidic and neutral goblet cell populations in the small intestine of early weaned pigs. Am J Vet

Res 52:1743-1746.

Edwards, H. M., 3rd, and D. H. Baker. 2000. Zinc bioavailability in soybean meal. J Anim Sci 78:1017-1021.

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

Falchuk, K. H. 1998. The molecular basis for the role of zinc in developmental biology. Mol Cell Biochem 188:41-48.

Falchuk, K. H., and M. Montorzi. 2001. Zinc physiology and biochemistry in oocytes and embryos. Biometals 14:385-395.

Ferket, P. R., and M. A. Qureshi. 1992. Effect of level of inorganic and organic zinc and manganese on the immune function of turkey toms. Poult Sci 71 (Suppl. 1):60 (abstr.).

Finley, J. W., and C. D. Davis. 1999. Manganese deficiency and toxicity: are high or low dietary amounts of manganese cause for concern? Biofactors 10:15-24.

Fletcher, M. P., M. E. Gershwin, C. L. Keen, and L. Hurley. 1988. Trace element deficiencies and immune responsiveness in humans and animal models. Pages 215-239 in Nutrition and Immunology. R. K. Chandra, ed. Alan R. Liss Inc, New York.

Flinchum, J. D., C. F. Nockels, and R. E. Moreng. 1989. Aged hens fed zinc-methionine had chicks with improved performance. Poult Sci 68 (Suppl. 1):55.

Fontaine, N., J. C. Meslin, S. Lory, and C. Andrieux. 1996. Intestinal mucin distribution in the germ-free rat and in the heteroxenic rat harbouring a human bacterial flora: effect of inulin in the diet. Br J Nutr 75:881-892.

Forstner, J. F., and G. G. Forstner. 1994. Gastrointestinal mucus. Pages 1255-1284 in Physiology of the Gastrointestinal Tract. L. R. Johnson, ed. 3rd ed. Raven, New York.

Fox, M. C., D. R. Brown, and L. L. Southern. 1987. Effect of dietary buffer additions on gain, efficiency, duodenal pH, and copper concentration in liver of Eimeria acervulina-infected chicks. Poult Sci 66:500-504.

Fraker, P., and L. King. 1998. Changes in regulation of lymphopoiesis and myelopoiesis in the zinc-deficient mouse. Nutr Rev 56:S65-69.

Fraker, P. J. 2005. Roles for cell death in zinc deficiency. J Nutr 135:359-362.

Fraker, P. J., R. Caruso, and F. Kierszenbaum. 1982. Alteration of the immune and

nutritional status of mice by synergy between zinc deficiency and infection with Trypanosoma cruzi. J Nutr 112:1224-1229.

Fraker, P. J., S. M. Haas, and R. W. Luecke. 1977. Effect of zinc deficiency on the immune response of the young adult A/J mouse. J Nutr 107:1889-1895.

Gaither, L. A., and D. J. Eide. 2001. Eukaryotic zinc transporters and their regulation. Biometals 14:251-270.

Garrett-Sinha, L. A., H. Eberspaecher, M. F. Seldin, and B. de Crombrugghe. 1996. A gene for a novel zinc-finger protein expressed in differentiated epithelial cells and transiently in certain mesenchymal cells. J Biol Chem 271: 31384-31390.

Gaskins, H. R. 1997. Immunological aspects of host/microbal interactions at the intestinal epithelium. Pages 537-587 in Gastrointestinal Microbiology. R. I. Mackie, B. A. White, R. E. Isaacson, ed. Chapman & Hall, New York.

Giraldo, C., and L. L. Southern. 1988. Role of compensatory gain in Eimeria acervulina-induced liver copper accumulation in chicks. J Nutr 118:871-876.

Gogal, R. M., S. A. Ahmed, and C. T. Larsen. 1997. Analysis of avian lymphocyte proliferation by a new, simple, nonradioactive assay (Lympho-pro). Avian Dis 41:714-725.

Gross, W. B., and P. B. Siegel. 1997. Why some get sick. J. Appl. Poultry Res. 6: 453-460.

Guemouri, L., Y. Artur, B. Herbeth, C. Jeandel, G. Cuny, and G. Siest. 1991. Biological variability of superoxide dismutase, glutathione peroxidase, and catalase in blood. Clin Chem 37:1932-1937.

Guerinot, M. L. 2000. The ZIP family of metal transporters. Biochim Biophys Acta 465:190-198.

Gunshin, H., B. Mackenzie, U. V. Berger, Y. Gunshin, M. F. Romero, W. F. Boron, S. Nussberger, J. L. Gollan, and M. A. Hediger. 1997. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature 388:482-488.

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.

Hafez, H. M., Factors influencing turkey diseases, 2000, World Poult (Spec.) 6-8.

Hahn, J. D., and D. H. Baker. 1993. Growth and plasma zinc responses of young pigs fed pharmacologic levels of zinc. J Anim Sci 71:3020-3024.

Harris, E. D. 2002. Cellular transporters for zinc. Nutr Rev 60:121-124.

Heresi, G., Castillo-Duran, C., Munoz, C., Arevalo, M., and L. Schlesinger. 1985. Phagocytosis and immunoglobulin levels in hypocupremic infants. Nutr Res 5:1327–34.

Higuchi, S., A. Higashi, T. Nakamura, Y. Yanabe, and I. Matsuda. 1991. Anti-neutrophil antibodies in patients with nutritional copper deficiency. Eur J Pediatr 150: 327-330.

Hill, D. A., E. R. Peo, Jr., A. J. Lewis, and J. D. Crenshaw. 1986. Zinc-amino acid complexes for swine. J Anim Sci 63:121-130.

Ho, E. 2004. Zinc deficiency, DNA damage and cancer risk. J Nutr Biochem 15:572-578.

Holben, D. H., and A. M. Smith. 1999. The diverse role of selenium within selenoproteins: a review. J Am Diet Assoc 99:836-843.

Hong, Y. H., H. S. Lillehoj, E. P. Lillehoj, and S. H. Lee. 2006. Changes in immune-related gene expression and intestinal lymphocyte subpopulations following Eimeria maxima infection of chickens. Vet Immunol Immunopathol 114:259-272.

Hopkins, R. G., and M. L. Failla. 1997. Copper deficiency reduces interleukin-2 (il-2) production and il-2 mrna in human t-lymphocytes. J Nutr 127: 257-262.

Hopkins, R. G., and M. L. Failla. 1999. Transcriptional regulation of interleukin-2 gene expression is impaired by copper deficiency in jurkat human t lymphocytes. J Nutr 129: 596-601.

Iji, P. A., A. Saki, and D. R. Tivey. 2001. Body and intestinal growth of broiler chicks on a commercial starter diet. 1. Intestinal weight and mucosal development. Br Poult Sci 42:505-513.

Isolauri, E., Y. Sutas, P. Kankaanpaa, H. Arvilommi, and S. Salminen. 2001. Probiotics: effects on immunity. Am J Clin Nutr 73:444S-450S.

Jeffers, T. K. 1975. Attenuation of Eimeria tenella through selection for precociousness. J Parasitol 61:1083-1090.

Jeurissen, S. H., E. M. Janse, A. N. Vermeulen, and L. Vervelde. 1996. Eimeria tenella infections in chickens: aspects of host-parasite: interaction. Vet Immunol Immunopathol 54:231-238.

Ji, F., X. G. Luo, L. Lu, B. Liu, and S. X. Yu. 2006a. Effect of manganese source on manganese absorption by the intestine of broilers. Poult Sci 85:1947-1952.

Ji, F., X. G. Luo, L. Lu, B. Liu, and S. X. Yu. 2006b. Effects of manganese source and calcium on manganese uptake by in vitro everted gut sacs of broilers' intestinal segments. Poult Sci 85:1217-1225.

Kambe, T., Y. Yamaguchi-Iwai, R. Sasaki, and M. Nagao. 2004. Overview of mammalian

zinc transporters. Cell Mol Life Sci 61:49-68.

Kandori, H., K. Hirayama, M. Takeda, and K. Doi. 1996. Histochemical, lectin-histochemical and morphometrical characteristics of intestinal goblet cells of germfree and conventional mice. Exp Anim 45:155-160.

Katz, J. P., N. Perreault, B. G. Goldstein, C. S. Lee, P. A. Labosky, V. W. Yang, and K. H. Kaestner. 2002. The zinc-finger transcription factor klf4 is required for terminal differentiation of goblet cells in the colon. Development 129: 2619-2628.

Keen, C. L., J. L. Ensunsa, M. H. Watson, D. L. Baly, S. M. Donovan, M. H. Monaco, and M. S. Clegg. 1999. Nutritional aspects of manganese from experimental studies. Neurotoxicology 20:213-223.

Kelly, M. P., and R. F. Power. 1995. Fractionation and identification of the major selenium containing compounds in selenized yease. J Dairy Sci 78 (Suppl 1):237.

Kelvay, L. M., L. Inman, L. K. Johnson, M. Lawler, J. R. Mahalko, D. B. Miline, H. C. Lukaski, M. Bolonchuk, and H. H. Sandstead. 1984. Increased cholesterol in plasma in a young man during experimental copper depletion. Metabolism 33:1112-1118.

Kidd, M. T., N. B. Anthony, and S. R. Lee. 1992. Progeny performance when dams and chicks are fed supplemental zinc. Poult Sci 71:1201-1206.

Kidd, M. T., N. B. Anthony, L. A. Newberry, and S. R. Lee. 1993. Effect of supplemental zinc in either a corn-soybean or a milo and corn-soybean meal diet on the performance of young broiler breeders and their progeny. Poult Sci 72:1492-1499.

Kidd, M. T., P. R. Ferket, and M. A. Qureshi. 1996. Zinc metabolism with special reference to its role in immunity. Worlds Poult Sci J 52:309-324.

Kidd, M. T., M. A. Qureshi, P. R. Ferket, and L. N. Thomas. 1994a. Blood clearance of Escherichia coli and evaluation of mononuclear-phagocytic system as influenced by supplemental dietary zinc methionine in young turkeys. Poult Sci 73:1381-1389.

Kidd, M. T., M. A. Qureshi, P. R. Ferket, and L. N. Thomas. 1994b. Dietary zinc-methionine enhances mononuclear-phagocytic function in young turkeys. Biological Trace Element Research 1994: 217-229.

Kidd, M. T., M. A. Qureshi, P. R. Ferket, and L. N. Thomas. 2000. Turkey hen zinc source affects progeny immunity and disease resistance J Appl Poult Res 9:414-423.

Kim, W. K., and P. H. Patterson. 2004. Effects of dietary zinc supplementation on broiler performance and nitrogen loss from manure. Poult Sci 83:34-38.

Kincaid, R. L., B. P. Chew, and J. D. Cronrath. 1997. Zinc oxide and amino acids as sources of dietary zinc for calves: effects on uptake and immunity. J Dairy Sci 80:1381-1388.

Kindon, H., C. Pothoulakis, L. Thim, K. Lynch-Devaney, and D. K. Podolsky. 1995. Trefoil peptide protection of intestinal epithelial barrier function: cooperative interaction with mucin glycoprotein. Gastroenterology 109:516-523.

King, L. E., F. Osati-Ashtiani, and P. J. Fraker. 2002. Apoptosis plays a distinct role in the loss of precursor lymphocytes during zinc deficiency in mice. J Nutr 132:974-979.

Kingery, W. L., C. W. Wood, D. P. Delaney, J. C. Williams, G. L. Mullins, and E. van Santen. 1993. Implications of long-term land application of poultry litter on Tall Fescue pastures. J Prod Agric 6:390-395.

Klotz, L. O., K. D. Kroncke, D. P. Buchczyk, and H. Sies. 2003. Role of copper, zinc, selenium and tellurium in the cellular defense against oxidative and nitrosative stress. J Nutr 133:1448S-1451S.

Kohlmeier, M. 2003. Nutrient Metabolism. Academic Press, Boston.

Koller, L. D., Mulhern, S. A., Frankel, N.C., Steven, M. G., and J. R. Williams. 1987. Immune dysfunction in rats fed a diet deficient in copper. Am J Clin Nutr 45:997–1006.

Konjufca, V. H., G. M. Pesti, and R. I. Bakalli. 1997. Modulation of cholesterol levels in broiler meat by dietary garlic and copper. Poult Sci 76:1264-1271.

Krebs, N. F. 2000. Overview of zinc absorption and excretion in the human gastrointestinal tract. J Nutr 130:1374S-1377S.

Krebs, N. F., J. E. Westcott, J. W. Huffer, and L. V. Miller. 1998. Absorption of exogenous zinc and secretion of endogenous zinc in the human small intestine. FASEB J 12:A345.

Kumar, N., A. K. Garg, V. Mudgal, R. S. Dass, V. K. Chaturvedi, and V. P. Varshney. 2008. Effect of different levels of selenium supplementation on growth rate, nutrient utilization, blood metabolic profile, and immune response in lambs. Biol Trace Elem Res 126 Suppl 1: S44-56.

Lamm, M. E. 1976. Cellular aspects of immunoglobulin A. Adv Immunol 22:223-290.

Larsen, C. T., F. W. Pierson, and W. B. Gross. 1997. Effect of dietary selenium on the response of stressed and unstressed chickens to Escherichia coli challenge and antigen. Biol Trace Elem Res 58:169-176.

Laurent, F., R. Mancassola, S. Lacroix, R. Menezes, and M. Naciri. 2001. Analysis of chicken mucosal immune response to Eimeria tenella and Eimeria maxima infection by quantitative reverse transcription-PCR. Infect Immun 69:2527-2534.

Leach, R. M. 1976. Metabolism and function of manganese. Pages 235-247 in Trace

Elements in Human Health and Disease. A. S. Prasad, ed. Academic Press, New York.

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.

Lee, H. H., A. S. Prasad, G. J. Brewer, and C. Owyang. 1989. Zinc absorption in human small intestine. Am J Physiol 256:G87-91.

Lee, J., J. R. Prohaska, S. L. Dagenais, T. W. Glover, and D. J. Thiele. 2000. Isolation of a murine copper transporter gene, tissue specific expression and functional complementation of a yeast copper transport mutant. Gene 254:87-96.

Leeson, S. 2005. Trace mineral requirements of poultry- validity of the NRC recommendations. Pages 107-118 in Re-defining Mineral Nutrition. JA Taylor-Pickard, LA Tucker, ed. Nottingham University Press, Nottingham.

Lesson, S., and J. D. Summers. 2005. Feeding programs for turkeys. Pages 345-369 in Commercial Poultry Nutrition. University Books, Guelph.

Leeson, S., H. Namkung, L. Caston, S. Durosoy, and P. Schlegel. 2008. Comparison of selenium levels and sources and dietary fat quality in diets for broiler breeders and layer hens. Poult Sci 87: 2605-2612.

Lillehoj, H. S. 1989. Intestinal intraepithelial and splenic natural killer cell response to eimerian infections in inbred chickens. Infect Immun 57:1879-1884.

Lillehoj, H. S. 1998. Role of T lymphocytes and cytokines in coccidiosis. Int J Parasitol 28:1071-1081.

Lillehoj, H. S., and K. D. Choi. 1998. Recombinant chicken interferon-gamma-mediated inhibition of Eimeria tenella development in vitro and reduction of oocyst production and body weight loss following Eimeria acervulina challenge infection. Avian Dis 42:307-314.

Lillehoj, H. S., and E. P. Lillehoj. 2000. Avian coccidiosis. A review of acquired intestinal immunity and vaccination strategies. Avian Dis 44:408-425.

Lillehoj, H. S., W. Min, K. D. Choi, U. S. Babu, J. Burnside, T. Miyamoto, B. M. Rosenthal, and E. P. Lillehoj. 2001. Molecular, cellular, and functional characterization of chicken cytokines homologous to mammalian IL-15 and IL-12. Vet Immunol Immunopathol 82:229-244.

Lillehoj, H. S., W. Min, and R. A. Dalloul. 2004. Recent progress on the cytokine regulation of intestinal immune responses to Eimeria. Poult Sci 83:611-623.

Lillehoj, H. S., and J. M. Trout. 1996. Avian gut-associated lymphoid tissues and intestinal immune responses to Eimeria parasites. Clin Microbiol Rev 9:349-360.

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

Linh, B. K., T. Hayashi, and Y. Horii. 2008. Eimeria vermiformis infection reduces goblet cells by multiplication in the crypt cells of the small intestine of C57BL/6 mice. Parasitol Res.

Liu, Z., M. M. Bryant, and D. A. Roland. 2005. Layer performance and phytase retention as influenced by copper ssulfate pentahydrate and tribasic copper chloride. J Appl Poult Res 14:499-505.

Liuzzi, J. P., R. K. Blanchard, and R. J. Cousins. 2001. Differential regulation of zinc transporter 1, 2, and 4 mRNA expression by dietary zinc in rats. J Nutr 131:46-52.

Lu, Z., F. Jehan, C. Zierold, and H. F. DeLuca. 2000. Isolation and characterization of the chicken vitamin d receptor gene and its promoter. J Cell Biochem 77: 92-102.

Lu, L., X. G. Luo, C. Ji, B. Liu, and S. X. Yu. 2007. Effect of manganese supplementation and source on carcass traits, meat quality, and lipid oxidation in broilers. J Anim Sci 85:812-822.

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.

Malecki, E. A., G. M. Radzanowski, T. J. Radzanowski, D. D. Gallaher, and J. L. Greger. 1996. Biliary manganese excretion in conscious rats is affected by acute and chronic manganese intake but not by dietary fat. J Nutr 126:489-498.

Mateo, R. D., J. E. Spallholz, R. Elder, I. Yoon, and S. W. Kim. 2007. Efficacy of dietary selenium sources on growth and carcass characteristics of growing-finishing pigs fed diets containing high endogenous selenium. J. Anim. Sci. 85: 1177-1183.

Matsler, P. L., and H. D. Chapman. 2006. Characterization of a strain of Eimeria meleagridis from the turkey. Avian Dis 50:599-604.

Matsler, P. L., and H. D. Chapman. 2007. Selection for early (precocious) development of Eimeria meleagridis in the turkey. Avian Dis 51:122-124.

McClung, J. P., and D. J. Bobilya. 1999. The influence of zinc status on the kinetics of zinc uptake into cultured endothelial cells. J Nutr Biochem 10:484-489.

McDougald, L. R., and W. M. Reid. 1997. Coccidiosis. Pages 865-883 in Diseases of Poultry. Tenth ed. B. W. Calnek, ed. Iowa State University Press, Iowa.

McDougald, L. R. 1998. Intestinal protozoa important to poultry. Poult Sci 77:1156-1158.

McMahon, R. J., and R. J. Cousins. 1998a. Mammalian zinc transporters. J Nutr 128:667-670.

McMahon, R. J., and R. J. Cousins. 1998b. Regulation of the zinc transporter ZnT-1 by dietary zinc. Proc Natl Acad Sci U S A 95:4841-4846.

Meslin, J. C., N. Fontaine, and C. Andrieux. 1999. Variation of mucin distribution in the rat intestine, caecum and colon: Effect of the bacterial flora. Comp Biochem Physiol A Mol Integr Physiol 123: 235-239.

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

Miller, R. S., and L. C. Hoskins. 1981. Mucin degradation in human colon ecosystems. Fecal population densities of mucin-degrading bacteria estimated by a "Most probable number" Method. Gastroenterology 81: 759-765.

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.

Molina, H., and M. Garcia. 1997. Enzymatic defenses of the rat heart against lipid peroxidation. Mech Ageing Dev 97:1-7.

Moore, P. A. 1998. Best management practices for poultry manure utilization that enhance agricultural productivity and reduce pollution. Pages 89-124 in Animal Waste Utilization: Effective Use of Manure as a Soil Resource. J.L. Hatfield and B.A. Stewart ed. Lewis Publishers.

Moreno-Reyes, R., D. Egrise, J. Neve, J. L. Pasteels, and A. Schoutens. 2001. Selenium deficiency-induced growth retardation is associated with an impaired bone metabolism and osteopenia. J Bone Miner Res 16: 1556-1563.

Morris, B. C., H. D. Danforth, D. J. Caldwell, F. W. Pierson, and A. P. McElroy. 2004. Intestinal mucosal mast cell immune response and pathogenesis of two Eimeria acervulina isolates in broiler chickens. Poult Sci 83:1667-1674.

Mosmann, T. R., H. Cherwinski, M. W. Bond, M. A. Giedlin, and R. L. Coffman. 1986. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 136:2348-2357.

Mosmann, T. R., and R. L. Coffman. 1989. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 7:145-173.

Muir, W. I. 1998. Avian intestinal immunity: basic mechanisms and vaccine. Poult Avian

Bio Rev 9:87-106.

Mullan, B., and D' Souza, D. 2005. The role of organic minerals in modern pig production. Pages 89-106 in Re-defining Mineral Nutrition. JA Taylor-Pickard, LA Tucker, ed. Nottingham University Press, Nottingham.

Munoz, C., E. Rios, J. Olivos, O. Brunser, and M. Olivares. 2007. Iron, copper and immunocompetence. Br J Nutr 98 Suppl 1: S24-28.

Naveh, Y., L. Bentur, and E. Diamond. 1988. Site of zinc absorption in dog small intestine. J Nutr 118:61-64.

Neutra, M. R., N. J. Mantis, and J. P. Kraehenbuhl. 2001. Collaboration of epithelial cells with organized mucosal lymphoid tissues. Nat Immunol 2:1004-1009.

Nys, Y., J. Gautron, M. D. McKee, J. M. Garcia-Ruiz, and M. T. Hincke. 2001. Biochemical and functional characterisation of eggshell matrix proteins in hens. Worlds Poult Sci J 57:401-413.

Opsahl, W., H. Zeronian, M. Ellison, D. Lewis, R. B. Rucker, and R. S. Riggins. 1982. Role of copper in collagen cross-linking and its influence on selected mechanical properties of chick bone and tendon. J Nutr 112: 708-716.

Osati-Ashtiani, F., L. E. King, and P. J. Fraker. 1998. Variance in the resistance of murine early bone marrow B cells to a deficiency in zinc. Immunology 94:94-100.

Overcash, M. R., F. J. Humenik, and J. R. Miner. 1983. Livestock waste management, volume 1. CRC Press, Boca Raton, Florida.

Oviedo, E. O., and P. R. Ferket. 2005. Nutritional factors that affect leg problems in meat poultry: a review. Pages 58-88 in 32nd Annual Carolina Poultry Nutrition Conference, Sheraton Imperial Hotel, RTP, NC.

Ovington, K. S., L. M. Alleva, and E. A. Kerr. 1995. Cytokines and immunological control of Eimeria spp. Int J Parasitol 25:1331-1351.

Ozkan, S., H. B. Malayoglu, S. Yalcin, F. Karadas, S. Kocturk, M. Cabuk, G. Oktay, S. Ozdemir, E. Ozdemir, and M. Ergul. 2007. Dietary vitamin E (alpha-tocopherol acetate) and selenium supplementation from different sources: performance, ascites-related variables and antioxidant status in broilers reared at low and optimum temperatures. Br Poult Sci 48:580-593.

Palacios, C. 2006. The role of nutrients in bone health, from A to Z. Crit. Rev. Food Sci. Nutr. 46: 621-628.

Pan, C., K. Huang, Y. Zhao, S. Qin, F. Chen, and Q. Hu. 2007. Effect of selenium source and level in hen's diet on tissue selenium deposition and egg selenium concentrations. J Agric Food Chem 55:1027-1032.

Panda, S. K., and A. T. Rao. 1994. Effect of a vitamin E-selenium combination on chickens infected with infectious bursal disease virus. Vet Rec 134:242-243.

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

Papavasiliou, P. S., S. T. Miller, and G. C. Cotzias. 1966. Role of liver in regulating distribution and excretion of manganese. Am J Physiol 211:211-216.

Papp, L. V., J. Lu, A. Holmgren, and K. K. Khanna. 2007. From selenium to selenoproteins: synthesis, identity, and their role in human health. Antioxid Redox Signal 9:775-806.

Payne, R. L., T. K. Lavergne, and L. L. Southern. 2005. Effect of inorganic versus organic selenium on hen production and egg selenium concentration. Poult Sci 84:232-237.

Payne, R. L., and L. L. Southern. 2005. Comparison of inorganic and organic selenium sources for broilers. Poult. Sci. 84: 898-902.

Percival, S. S. 1998. Copper and immunity. Am J Clin Nutr 67: 1064S-1068S.

Rath, N. C., G. R. Huff, W. E. Huff, and J. M. Balog. 2000. Factors regulating bone maturity and strength in poultry. Poult. Sci. 79: 1024-1032.

Rebel, J. M., J. T. van Dam, B. Zekarias, F. R. Balk, J. Post, A. Flores Minambres, and A. A. ter Huurne. 2004. Vitamin and trace mineral content in feed of breeders and their progeny: Effects of growth, feed conversion and severity of malabsorption syndrome of broilers. Br. Poult. Sci. 45: 201-209.

Richards, M. P., P. C. Augustine, and S. E. Darcey. 1985. Serum and liver zinc, copper and iron in chicks infected with Eimeria acervulina or Eimeria tenella. Poult Sci 64 (Suppl. 1):169 (abstr).

Rink, L., and P. Gabriel. 2000. Zinc and the immune system. Proc Nutr Soc 59:541-552.

Rink, L., and H. Kirchner. 2000. Zinc-altered immune function and cytokine production. J Nutr 130:1407S-1411S.

Rivera, M. T., A. P. De Souza, T. C. Araujo-Jorge, S. L. De Castro, and J. Vanderpas. 2003. Trace elements, innate immune response and parasites. Clin Chem Lab Med 41: 1020-1025.

Roch, G., M. Boulianne, and L. De Roth. 2000. Effects of dietary vitamin E and selenium source on incidence of ascites, growth performance and blood parameters cold-stressed broilers. Poult Sci 79 (Suppl. 1):41.

Romagnani, S. 1997. The Th1/Th2 paradigm. Immunol Today 18:263-266.

Rose, M. E. 1987. Immunity to Eimeria infections. Vet Immunol Immunopathol 17:333-343.

Rose, M. E., P. Hesketh, and D. Wakelin. 1992. Immune control of murine coccidiosis: CD4+ and CD8+ T lymphocytes contribute differentially in resistance to primary and secondary infections. Parasitology 105 (Pt 3):349-354.

Rose, M. E., H. S. Joysey, P. Hesketh, R. K. Grencis, and D. Wakelin. 1988. Mediation of immunity to Eimeria vermiformis in mice by L3T4+ T cells. Infect Immun 56:1760-1765.

Roth, J. A. 2006. Homeostatic and toxic mechanisms regulating manganese uptake, retention, and elimination. Biol Res 39:45-57.

Rucker, R. B., T. Kosonen, M. S. Clegg, A. E. Mitchell, B. R. Rucker, J. Y. Uriu-Hare, and C. L. Keen. 1998. Copper, lysyl oxidase, and extracellular matrix protein cross-linking. Am J Clin Nutr 67:996S-1002S.

Ruiz, J. A., A. M. Perez-Vendrell, and E. Esteve-Garcia. 2000. Effect of dietary iron and copper on performance and oxidative stability in broiler leg meat. Br Poult Sci 41:163-167.

Sakaguchi, S., Y. Iizuka, S. Furusawa, Y. Tanaka, M. Takayanagi, and Y. Takayanagi. 2000. Roles of selenium in endotoxin-induced lipid peroxidation in the rats liver and in nitric oxide production in J774A.1 cells. Toxicol Lett 118:69-77.

Salgueiro, M. J., M. Zubillaga, A. Lysionek, G. Cremaschi, C. G. Goldman, R. Caro, T. De Paoli, A. Hager, R. Weill, and J. Boccio. 2000. Zinc status and immune system relationship: a review. Biol Trace Elem Res 76:193-205.

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.

Sandoval, M., P. R. Henry, X. G. Luo, R. C. Littell, R. D. Miles, and C. B. Ammerman. 1998. Performance and tissue zinc and metallothionein accumulation in chicks fed a high dietary level of zinc. Poult Sci 77:1354-1363.

Scheuhammer, A. M., and M. G. Cherian. 1985. Binding of manganese in human and rat plasma. Biochim Biophys Acta 840:163-169.

Schiavon, S., L. Bailoni, M. Ramanzin, R. Vincenzi, A. Simonetto, and G. Bittante. 2000. Effect of proteinate or sulfate mineral sources on traceelements in blood and liver of piglets. Anim. Sci. 71: 131-139.

Schat, K. A., and T. J. Myers. 1991. Avian intestinal immunity. Crit Rev Poult

Biol:19-34.

Schroeder, H. A., J. J. Balassa, and I. H. Tipton. 1996. Essential trace metals in man: manganese, a study in homeostasis. J Chron Dis 19:545-571.

Scott, M. L., M. C. Nesheim, and R. J. Young. 1976. Nutrition of the Chicken. M. L. Scott of Ithica, New York.

Shankar, A. H., and A. S. Prasad. 1998. Zinc and immune function: the biological basis of altered resistance to infection. Am J Clin Nutr 68:447S-463S.

Sharma, R., F. Fernandez, M. Hinton, and U. Schumacher. 1997. The influence of diet on the mucin carbohydrates in the chick intestinal tract. Cell Mol Life Sci 53: 935-942.

Sharp, P. 2003. Ctr1 and its role in body copper homeostasis. Int J Biochem Cell Biol 35:288-291.

Sharp, P. 2004. The molecular basis of copper and iron interactions. Proc Nutr Soc 63:563-569.

Shelton, J. L., and L. L. Southern. 2006. Effects of phytase addition with or without a trace mineral premix on growth performance, bone response variables, and tissue mineral concentrations in commercial broilers. J. Appl. Poultry Res. 15: 94-102.

Shirley, M. W., and B. J. Millard. 1986. Studies on the immunogenicity of seven attenuated lines of Eimeria given as a mixture to chickens. Avian Pathol 15:629-638.

Shrimali, R. K., R. D. Irons, B. A. Carlson, Y. Sano, V. N. Gladyshev, J. M. Park, and D. L. Hatfield. 2008. Selenoproteins mediate t cell immunity through an antioxidant mechanism. J Biol Chem 283: 20181-20185.

Singh, H., S. Sodhi, and R. Kaur. 2006. Effects of dietary supplements of selenium, vitamin E or combinations of the two on antibody responses of broilers. Br Poult Sci 47:714-719.

Skrivan, M., M. Marounek, G. Dlouha, and S. Sevcikova. 2008. Dietary selenium increases vitamin e contents of egg yolk and chicken meat. Br Poult Sci 49: 482-486.

Skrivan, M., V. Skrivanova, and M. Marounek. 2005. Effects of dietary zinc, iron, and copper in layer feed on distribution of these elements in eggs, liver, excreta, soil, and herbage. Poult Sci 84:1570-1575.

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

Skrivan, M., V. Skrivanova, and M. Marounek. 2006. Effect of various copper supplements to feed of laying hens on cu content in eggs, liver, excreta, soil, and herbage. Arch. Environ. Contam. Toxicol. 50: 280-283.

Smit-McBride, Z., J. J. Mattapallil, M. McChesney, D. Ferrick, and S. Dandekar. 1998. Gastrointestinal t lymphocytes retain high potential for cytokine responses but have severe cd4(+) t-cell depletion at all stages of simian immunodeficiency virus infection compared to peripheral lymphocytes. J Virol 72: 6646-6656.

Smirnov, A., R. Perez, E. Amit-Romach, D. Sklan, and Z. Uni. 2005. Mucin dynamics and microbial populations in chicken small intestine are changed by dietary probiotic and antibiotic growth promoter supplementation. J Nutr 135:187-192.

Smirnov, A., D. Sklan, and Z. Uni. 2004. Mucin dynamics in the chick small intestine are altered by starvation. J Nutr 134:736-742.

Sone, T., K. Yamaoka, Y. Minami, and H. Tsunoo. 1987. Induction of metallothionein synthesis in Menkes' and normal lymphoblastoid cells is controlled by the level of intracellular copper. J Biol Chem 262:5878-5885.

Southern, L. L., and D. H. Baker. 1982a. Eimeria acervulina infection in chicks fed excess copper in the presence or absence of excess dietary methionine. J Anim Sci 54:989-997.

Southern, L. L., and D. H. Baker. 1982b. Iron status of the chick as affected by Eimeria acervulina infection and by variable iron ingestion. J Nutr 112:2353-2362.

Southern, L. L., and D. H. Baker. 1983a. Eimeria acervulina infection and the zinc-copper interrelationship in the chick. Poult Sci 62:401-404.

Southern, L. L., and D. H. Baker. 1983b. Zinc toxicity, zinc deficiency and zinc-copper interrelationship in Eimeria acervulina-infected chicks. J Nutr 113:688-696.

Spallholz, J. E., L. M. Boylan, and H. S. Larsen. 1990. Advances in understanding selenium's role in the immune system. Ann N Y Acad Sci 587: 123-139.

Spears, J. W. 1989. Zinc methionine for ruminants: relative bioavailability of zinc in lambs and effects of growth and performance of growing heifers. J Anim Sci 67:835-843.

Spears, J. W., and E. B. Kegley. 2002. Effect of zinc source (zinc oxide vs zinc proteinate) and level on performance, carcass characteristics, and immune response of growing and finishing steers. J Anim Sci 80:2747-2752.

Specian, R. D., and M. G. Oliver. 1991. Functional biology of intestinal goblet cells. Am J Physiol 260:C183-193.

Stahl, J. L., J. L. Greger, and M. E. Cook. 1989. Zinc, copper and iron utilisation by

chicks fed various concentrations of zinc. Br Poult Sci 30:123-134.

Stefanidou, M., C. Maravelias, A. Dona, and C. Spiliopoulou. 2006. Zinc: a multipurpose trace element. Arch Toxicol 80:1-9.

Stevenson, M. H., S. W. Gibson, and N. Jackson. 1987. Withdrawal of zinc oxide-containing diets from mature, female domestic fowl: effects on selected tissue mineral contents. Br Poult Sci 28:449-459.

Storey, M. L., and J. L. Greger. 1987. Iron, zinc and copper interactions: chronic versus acute responses of rats. J Nutr 117:1434-1442.

Strous, G. J., and J. Dekker. 1992. Mucin-type glycoproteins. Crit Rev Biochem Mol Biol 27:57-92.

Surai, P. F. 2000. Effect of selenium and vitamin e content of the maternal diet on the antioxidant system of the yolk and the developing chick. Br. Poult. Sci. 41: 235-243.

Surai, P. F. 2002. Selenium in poultry nutrition. 1. Antioxidant properties, deficiency and toxicity. Worlds Poult Sci J 58:333-347.

Swain, B. K., T. S. Johri, and S. Majumdar. 2000. Effect of supplementation of vitamin E, selenium and their different combinations on the performance and immune response of broilers. Br Poult Sci 41:287-292.

Swanson, C. A. 1987. Comparative utilization of selenite, selenomethionine, and selenized yeast by the laying hen. Nutr Res 7:529-537.

Swinkels, J. W., E. T. Kornegay, W. Zhou, M. D. Lindemann, K. E. Webb, Jr., and M. W. Verstegen. 1996. Effectiveness of a zinc amino acid chelate and zinc sulfate in restoring serum and soft tissue zinc concentrations when fed to zinc-depleted pigs. J Anim Sci 74:2420-2430.

Tacnet, F., F. Lauthier, and P. Ripoche. 1993. Mechanisms of zinc transport into pig small intestine brush-border membrane vesicles. J Physiol 465:57-72.

Tako, E., P. R. Ferket, and Z. Uni. 2005. Changes in chicken intestinal zinc exporter mRNA expression and small intestinal functionality following intra-amniotic zinc-methionine administration. J Nutr Biochem 16:339-346.

Taylor, G. A., and P. J. Blackshear. 1995. Zinc inhibits turnover of labile mRNAs in intact cells. J Cell Physiol 162:378-387.

Taylor-Pickard, J. A. 2005. Feeding the modern genotype-trace elements. Pig J. 56: 179-191.

Thomson, C. D., and R. D. Stewart. 1973. Metabolic studies of (75Se) selenomethionine and (75Se) selenite in the rat. Br J Nutr 30:139-147.

Tierney, J. B., E. Matthews, S. D. Carrington, and G. Mulcahy. 2007. Interaction of eimeria tenella with intestinal mucin in vitro. J Parasitol 93: 634-638.

Todd, W. R., C. A. Elvehjem, and E. B. Hart. 1933. Zinc in the nutrition of the rat. Am J Physiol 107:146-156.

Trout, J. M., and H. S. Lillehoj. 1993. Evidence of a role for intestinal CD8+ lymphocytes and macrophages in transport of Eimeria acervulina sporozoites. J Parasitol 79:790-792.

Trout, J. M., and H. S. Lillehoj. 1995. Eimeria acervulina infection: evidence for the involvement of CD8+ T lymphocytes in sporozoite transport and host protection. Poult Sci 74:1117-1125.

Trout, J. M., and H. S. Lillehoj. 1996. T lymphocyte roles during Eimeria acervulina and Eimeria tenella infections. Vet Immunol Immunopathol 53:163-172.

Turk, D. E. 1974. Intestinal parasitism and nutrient absorption. Fed Proc 33:106-111.

Turk, D. E. 1981. Coccidial infections and iron absorption. Poult Sci 60:323-326.

Turk, D. E., and J. F. Stephens. 1966. Effect of intestinal damage produced by Eimeria necatrix infection in chicks upon absorption of orally administered zinc-65. J Nutr 88:261-266.

Turk, D. E., and J. F. Stephens. 1967. Upper intestinal tract infection produced by E. acervulina and absorption of 65Zn and 131-I-labeled oleic acid. J Nutr 93:161-165.

Turnlund, J. R., W. R. Keyes, H. L. Anderson, and L. L. Acord. 1989. Copper absorption and retention in young men at three levels of dietary copper by use of the stable isotope 65Cu. Am J Clin Nutr 49:870-878.

Turnlund, J. R., W. R. Keyes, G. L. Peiffer, and K. C. Scott. 1998. Copper absorption, excretion, and retention by young men consuming low dietary copper determined by using the stable isotope 65Cu. Am J Clin Nutr 67:1219-1225.

Ueno, H., G. Hasegawa, R. Ido, T. Okuno, and K. Nakamuro. 2008. Effects of selenium status and supplementary seleno-chemical sources on mouse t-cell mitogenesis. J Trace Elem Med Biol 22: 9-16.

Uni, Z., A. Geyra, H. Ben-Hur, and D. Sklan. 2000. Small intestinal development in the young chick: crypt formation and enterocyte proliferation and migration. Br Poult Sci 41:544-551.

Uni, Z., Y. Noy, and D. Sklan. 1995. Posthatch changes in morphology and function of the small intestines in heavy- and light-strain chicks. Poult Sci 74:1622-1629.

Uni, Z., A. Smirnov, and D. Sklan. 2003. Pre- and posthatch development of goblet cells

in the broiler small intestine: effect of delayed access to feed. Poult Sci 82:320-327.

van Heugten, E., J. W. Spears, E. B. Kegley, J. D. Ward, and M. A. Qureshi. 2003. Effects of organic forms of zinc on growth performance, tissue zinc distribution, and immune response of weanling pigs. J Anim Sci 81:2063-2071.

Vasto, S., E. Mocchegiani, M. Malavolta, I. Cuppari, F. Listi, D. Nuzzo, V. Ditta, G. Candore, and C. Caruso. 2007. Zinc and inflammatory/immune response in aging. Ann N Y Acad Sci 1100:111-122.

Vendeland, S. C., J. T. Deagen, J. A. Butler, and P. D. Whanger. 1994. Uptake of selenite, selenomethionine and selenate by brush border membrane vesicles isolated from rat small intestine. Biometals 7:305-312.

Virden, W. S., J. B. Yeatman, S. J. Barber, K. O. Willeford, T. L. Ward, T. M. Fakler, R. F. Wideman, Jr., and M. T. Kidd. 2004. Immune system and cardiac functions of progeny chicks from dams fed diets differing in zinc and manganese level and source. Poult Sci 83:344-351.

Virden, W. S., J. B. Yeatman, S. J. Barber, C. D. Zumwalt, T. L. Ward, A. B. Johnson, and M. T. Kidd. 2003. Hen mineral nutrition impacts progeny livability. J Appl Poult Res 12:411-416.

Wang, K., B. Zhou, Y. Kuo, J. Zemansky, and J. Gitschier. 2002. A novel member of a Zinc transporter family is defective in acrodermatitis enteropathica. Am J Hum Genet. 71:66-73.

Wapnir, R. A. 1990. Copper absorption and body distribution: Role of protein and other nutrients. Pages 181-209 in Protein Nutrition and Mineral Absorption. CRC Press, Boca Raton.

Ward, J. D., and J. W. Spears. 1993. Comparison of copper lysine and copper sulfate as copper sources for ruminants using in vitro methods. J Dairy Sci 76:2994-2998.

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.

Wideman, R. F., Y. Kochera Kirby, T. L. Barton, and D. Clark. 1996. Excess dietary copper triggers enlargement of the proventriculus in broilers. J Appl Poult Res 5:219-230.

Williams, R. B. 1998. Epidemiological aspects of the use of live anticoccidial vaccines for chickens. Int J Parasitol 28:1089-1098.

Williams, R. B. 1999. A compartmentalised model for the estimation of the cost of coccidiosis to the world's chicken production industry. Int J Parasitol 29:1209-1229.

Williams, R. C., and R. J. Gibbons. 1972. Inhibition of bacterial adherence by secretory immunoglobulin A: a mechanism of antigen disposal. Science 177:697-699.

Williams, C. M., J. C. Barker, and J. T. Sims. 1999. Management and utilization of poultry wastes. Rev. Environ. Contam. Toxicol. 162: 105-157.

Williams, R. B., W. W. H. Carlyle, D. R. Bond, and I. A. G. Brown. 1999. The efficacy and economic benefits aof Paracox, a live attenuated anticoccidial vaccine, in commercial trials with standard broiler chickens in the United Kingdom. Int J Parasitol 29:341-355.

Wintergerst, E. S., S. Maggini, and D. H. Hornig. 2007. Contribution of selected vitamins and trace elements to immune function. Ann Nutr Metab 51:301-323.

Wolffram, S., F. Ardüser, and E. Scharrer. 1985. In vivo intestinal absorption of selenate and selenite by rats. J Nutr 115:454-459.

Yoon, I., T. M. Werner, and J. M. Butler. 2007. Effect of source and concentration of selenium on growth performance and selenium retention in broiler chickens. Poult Sci 86:727-730.

Yun, C. H., H. S. Lillehoj, and E. P. Lillehoj. 2000. Intestinal immune response to coccidiosis. Dev Comp Immunol 24:303-324.

Zanetti, M. A., P. R. Henry, C. B. Ammerman, and R. D. Miles. 1991. Estimation of the relative bioavailability of copper sources in chicks fed on conventional dietary amounts. Br Poult Sci 32:583-588.

Zimmermann, M. B., and J. Kohrle. 2002. The impact of iron and selenium deficiencies on iodine and thyroid metabolism: Biochemistry and relevance to public health. Thyroid 12: 867-878.