

# **EARLY NUTRITION OF ZINC AND COPPER IN CHICKS AND POULTS: IMPACT ON GROWTH AND IMMUNE FUNCTION**

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## **Summary**

One area of early nutrition that needs more research attention is mineral nutrition. Mineral requirements for breeding hens have been established with regard to fertile egg development and hatchability but not for progeny quality. Among the systems most responsive to mineral status is the immune system. Weights of the primary immune organs bursa and thymus in progeny respond to hen mineral nutrition and appear to benefit from organic forms of zinc (Zn). Progeny from hens fed organic trace minerals (OTM) appear to have an advantage in cellular immunity after hatch compared to those fed inorganic trace minerals (ITM). This has been shown for Zn with few other minerals being studied.

At the time of hatch, there is residual mineral available to the bird in its liver, residual yolk and yolk sac membrane. The amount of this mineral is not sufficient, however, to support even the first week of growth and development. In the case of ducklings, feeding a deficient diet results in a significantly reduced bursa weight at 7 days (Cui *et al.*, 2003). Benefits of feeding OTM during the immune response to a vaccination can be seen in specific antibody production and later, in resistance to a challenge. In the case of coccidiosis, a disease reported to be more detrimental in mineral deficient birds, the feeding of OTM gives better performance and Zn status following challenge.

## **Introduction**

The requirements for minerals in the incubation and early performance of poultry are areas of early nutrition that need more research attention. First, the mineral requirements of the breeder hen for optimum egg production, hatchability, and chick quality are not well defined (Council, 1994). It has been clearly shown that relatively severe deficiencies of minerals, including calcium, phosphorous, magnesium, manganese, potassium, sodium, and zinc lead to poor egg production and hatchability (Blamburg *et al.*, 1960; Burns *et al.*, 1952, 1953; Hajj and Sell, 1969; Kienholz *et al.*, 1961; Mehring, 1965; Nott and Combs, 1969; Rogler *et al.*, 1959; Sell *et al.*, 1967). Deficiency studies, however, do not lead to a broader understanding of the requirement during these periods, nor do they address the effect of marginal deficiency or excess. Similarly, effects of Mn and/or Zn deficiency on chick quality have been described as resulting primarily in bone deformities (Caskey *et al.*, 1939; Kienholz *et al.*, 1961; Lyons and Insko Jr, 1937). However, it is not nearly as clear what mineral levels in breeder diets can do to optimize chick quality or subsequent performance. Thus, the starting point for hatchling mineral status is often not well defined in terms of amount or variability for required minerals.

The effects of mineral deficiencies on chick performance are well studied. Deficiencies of all macro and trace minerals have been evaluated in terms of chick performance, and effects on particular metabolic processes have been described (Butler, 1983; Leeson and Summers, 2001). Such studies typically emphasize mineral deficiencies over the three week starter period. The effects on early nutrition (the first week of life) are not separately evaluated nor are the requirements for minerals in the chick or poult during the immediate post-hatch period. This report will cover the developmental functions of Zn and copper (Cu) and some data from the literature on the effect of these minerals in breeder hen diets on

progeny performance. It will also cover some data from Novus research on the effect of early mineral nutrition on chick gut and immune development. Much of the work that has been done on these subjects compares inorganic sources of trace minerals with organic sources and these comparisons will be presented. Organic sources to be described in this report include Mintrex™ zn, Organic Trace Mineral Supplement, a source of protected Zn in which the organic ligand used is 2-hydroxy-4-(methylthio)butanoic acid (HMTBA). This unique mineral-ligand combination provides both increased mineral availability and a source of methionine activity in one product.

## **Zinc**

Zn is essential for development. In the form of Zn finger proteins, it is involved in the regulation of DNA transcription (Luscombe *et al.*, 2000) which controls the differentiation of many cell types, including T-lymphocytes (Staal *et al.*, 2001) and myeloid precursor cells (Shivdasani, 2001). In fact, an engineered Zn finger protein has been used to cause the differentiation of embryonic stem cells (Bartsevich *et al.*, 2003). Thus, Zn is literally essential for embryonic development of all species, including poultry.

After hatch, Zn finger proteins continue to bind to DNA and regulate its transcription in all tissues. In addition, Zn containing proteins are the receptors for steroid and thyroid hormones (Luscombe *et al.*, 2000). After binding the appropriate ligand, these receptors move to the nucleus, where they regulate the DNA transcription of hormone response elements (Freedman and Luisi, 1993).

Another Zn function of fundamental importance to embryonic and post-hatch development is its role in the regulation of cell turnover. A Zn containing protein, p53, regulates the fate of cells with DNA damage: death or repair (Bray *et al.*, 1998). Abnormalities of the p53 gene are the single most common molecular abnormality seen in cancer, itself a failure of cell turnover regulation (Hainaut *et al.*, 1997). The importance of p53 is that it can trigger apoptosis or can halt the progression of the cell cycle, allowing the repair of DNA damage (Clarke *et al.*, 1993; Hall *et al.*, 1993; Ho and Ames, 2002; Kastan *et al.*, 1991; Kastan *et al.*, 1992; Lowe *et al.*, 1993; Yonish-Rouach *et al.*, 1991). This effect on cell turnover may be responsible for the cell cycle phase changes in immune organs of chicks and ducklings fed a Zn deficient diet (Cui *et al.*, 2003, 2004).

In addition to these functions, Zn is a constituent or activator in hundreds of enzymes. It is the most common mineral constituent in metalloenzymes (Underwood and Suttle, 2001). These range in function from protection against oxidation by decomposition of superoxide radicals (Bannister *et al.*, 1971; Cook-Mills and Fraker, 1993) to facilitation of cellular migration by digestion of basement membranes (Goodsell, 2000).

Zn is also required in the synthesis of two key functional proteins: collagen and keratin (Underwood and Suttle, 2001). The importance of these two proteins in the developing animal are obvious: keratin is the structural protein of feathers, skin, beaks and claws while collagen is the major structural protein of the internal tissues, including cartilage and bone. Some of the first and most common symptoms of Zn deficiency are related to these two structural proteins, from bone and beak malformation during embryonic development to shortening of bone, perosis, skin scaling and very poor feathering (Leeson and Summers, 2001). Another key function of Zn in relation to chick development is its effect on appetite. Deficiency and excess of Zn are both associated with anorexia (Leesen and Summers, 2001).

## **Copper**

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Cu is essential for reproduction and development of embryonic birds, including poultry. Hens on a severely deficient diet display defective shell formation (Baumgartner *et al.*, 1978), reduced egg production and hatchability (Savage, 1968). Embryos from these hens showed hemorrhage, anemia, bone abnormalities, and retarded development. The effect on anemia appears to be mediated by an effect of Cu on iron transport and mobilization (Leeson and Summers, 2001). In contrast to Zn, many of the effects of Cu deficiency can be traced to its role as a constituent of various metalloenzymes rather than as a regulator of gene expression or cell turnover. For example, Cu affects many biological systems by virtue of its role in the proper crosslinking of collagen and elastin. The enzyme lysyl oxidase is a Cu metalloenzyme that is reduced in Cu deficient animals. The result is that the elastin and collagen from such animals are unable to withstand the mechanical stresses typical of the cardiovascular or skeletal systems (Guenther *et al.*, 1978; O'Dell *et al.*, 1961). Bones may be fragile and easily broken (Opsahl *et al.*, 1982).

Cu deficiency is associated with changes in the central nervous system that may affect the developing embryo (Leeson and Summers, 2001). These may be related to reduced activity of cytochrome c oxidase, a Cu containing enzyme in the electron transport chain (Chao *et al.*, 1994). Cu is also required for the development of strength and elasticity of the skin. Cu is involved in pigmentation because the enzyme polyphenoloxidase which catalyzes the production of melanin, is a Cu containing enzyme. Melanin is an essential component of the system for protecting the skin from ultraviolet radiation damage. Finally, Cu is required in the oxidation reaction between cysteine molecules that yields disulfide bonds (Leeson and Summers, 2001). These bonds are essential in developing and stabilizing the tertiary structure of proteins.

### ***Effects of Mineral Nutrition of Breeder Hens on Egg Minerals***

Effects of breeder hen diet on egg content of trace minerals are not widely reported. In the case of calcium, the hen will deplete her own mineral stores to provide for the developing egg and shell (Underwood and Suttle, 2001), although prolonged calcium deficiency will ultimately halt egg production (Buckner *et al.*, 1930). There are not similar data for other minerals such as Zn or Cu.

There is more Zn in yolk than in albumen, with a total of more than 90% of the embryo's supply found in yolk soluble and granule fractions (Richards, 1997). The transfer of Zn to the yolk is mediated by vitellogenin, an estrogen-dependent protein formed in the liver that binds Zn and Cu and transfers them via the bloodstream to the ovary (Richards, 1997). The developing oocyte accumulates circulating vitellogenin-bound minerals by endocytosis (Lambson, 1970; Mobbs and McMillian, 1979; Shen *et al.*, 1993). Once there, the vitellogenin is converted to lipovitellin and phosvitin via proteolysis (Shen, *et al.*, 1993). These proteins are both found in the granule fraction of the yolk (Bellairs *et al.*, 1972). For Cu, however, only about 45% is found in the yolk, with the rest to be found in the albumen, shell membrane and shell (Richards, 1997). The shell membrane is particularly rich in Cu, although its nutritional value in the development of the embryo is not established (Richards, 1997). During incubation, mineral use is not constant but shows peaks for Cu at 6-8 days before hatch and for Zn at 1-2 days post hatch in turkey poults (Richards, 1997). The significance of these differences is not yet clear.

### ***Inorganic vs Organic forms of Trace Minerals***

Historically, trace minerals have been supplemented in poultry diets using the inorganic salts such as Zn and Cu oxide (ZnO, CuO) or Zn and Cu sulfate (ZnSO<sub>4</sub>, CuSO<sub>4</sub>). Inorganic minerals have also been used in the development of mineral requirements for poultry diets (NRC,1994). However, use of inorganic salts can result in poor bioavailability of the mineral, primarily because of the numerous nutrient and ingredient antagonisms that impair absorption (Underwood and Suttle, 2001).

Perhaps the most important antagonism in the mineral nutrition of poultry is that between the divalent minerals, including Zn and Cu, and phytate. Phytate is able to form chelates of these minerals that are very stable and highly insoluble (Leeson and Summers, 2001). The antagonism is mutual in that the binding of calcium, Zn and other minerals also reduces the availability of phytin phosphorous, even in the presence of exogenous phytase (Tamim and Angel, 2003). Antagonisms also occur between one mineral and another. For example, high levels of Zn reduce the availability of Cu (Evans *et al.*, 1975). Availability of inorganic sources of minerals can also be reduced by other nutrients; for example, Cu availability is reduced by ascorbic acid (Carlton and Henderson, 1965).

The common denominator in these interactions is the dissociation of the inorganic salt in the relatively low pH of the upper gastrointestinal system. When the mineral reaches the higher pH of later gut segments it ionizes and can bind to a number of minerals, nutrients and non-nutritive components of the digesta that render it insoluble. Insoluble forms of minerals are excreted.

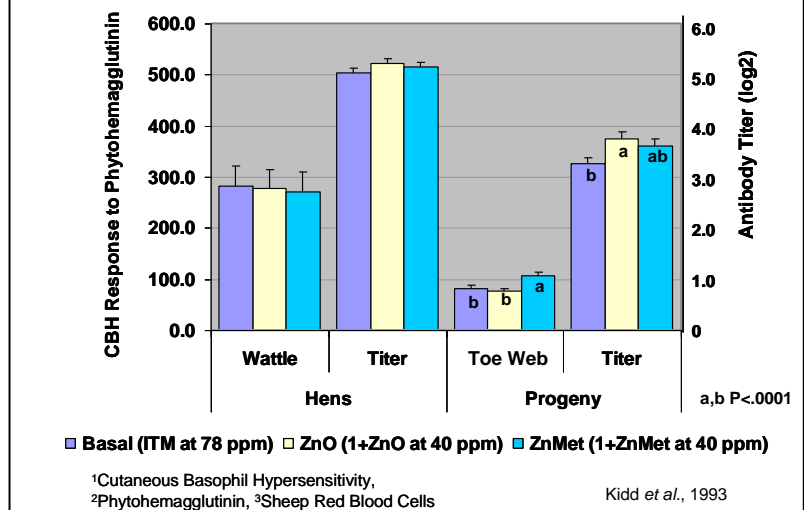
The advantage of organic trace minerals is that the binding of the mineral to the organic ligand provides stability of the complex in the upper gastrointestinal system. Organic trace minerals resist dissociation in the crop, proventriculus and gizzard, thus allowing the intact complex to be delivered to the absorptive epithelium of the small intestine (Leeson and Summers, 2001). The organic forms of many minerals, including Zn, Cu and manganese (Mn) are widely used in animal agriculture. The increased availability of organic Zn and Cu compared to inorganic forms has been demonstrated (Cao *et al.*, 2002; Paik *et al.*, 1999; Wedekind *et al.*, 1992). It should be noted that levels of other dietary minerals will affect the relative bioavailability (Scheideler, 1991) and also that different organic ligands will not necessarily increase the bioavailability of a given mineral to the same extent (Cao *et al.*, 2002). The next section of this report will include results using organic sources of Zn, made with either HMTBA (Mintrex zn) or dl-methionine (ZnMet) as the organic ligand.

## Research Trials

### *Effect of Breeder Hen Mineral Source on Progeny Performance*

Comparisons of progeny from broiler breeder hens supplemented with inorganic or organic forms of Zn have only rarely been reported to improve growth or performance. Several groups have reported no effect of breeder hen OTM supplementation on progeny performance (Hudson *et al.*, 2004a; Kidd *et al.*, 1992; Rebel *et al.*, 2004; Virden *et al.*, 2003). In contrast, benefits have been observed for progeny livability (Virden *et al.*, 2003), tibia weight (Kidd *et al.*, 1992), and various immune parameters, including thymus weight (Kidd *et al.*, 1992), cellular immune response (Kidd *et al.*, 1993; Virden *et al.*, 2002), or humoral immune response (Kidd *et al.*, 1993). The cellular immune parameter that was most consistently

**Figure 1. Effect of Turkey Hen Mineral Source and Level on Progeny CBH<sup>1</sup> to PHA<sup>2</sup> and Antibodies to SRBC<sup>3</sup>**



improved with OTM Zn was cutaneous basophil hypersensitivity (CBH) elicited by phytohemagglutinin (Kidd *et al.*, 1994, 2000), although negative results have also been reported (Hudson *et al.*, 2004a) with this test.

Figure 1 shows the effect of breeder hen mineral source on breeder hen and progeny immune function (Kidd *et al.*, 1993). Breeder hens were fed a basal diet that met the NRC (1994) requirement for Zn (78 ppm) and included either ZnO or ZnMet at 40 ppm above the basal Zn. Progeny poults were fed a common basal diet meeting the NRC (1994) requirement for Zn. Hens (at 37 wk) and their progeny (at 10 days) were tested for cellular immune response using a cutaneous basophil hypersensitivity (CBH) test (wattle or toe web injection of phytohemagglutinin) and for a humoral antibody response to sheep red blood cells (SRBC). The supplemental Zn in the breeder hen diet did not affect cellular immunity of the hens but did affect cellular response in the progeny. The organic Zn source significantly enhanced the CBH response in toe web. Humoral response for ZnMet, however, was not significantly better than the control.

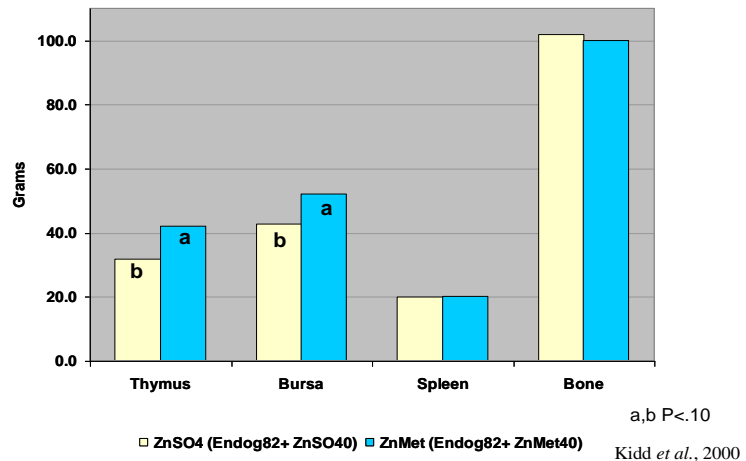
The supplemental Zn in the breeder hen diet did not affect cellular immunity of the hens but did affect cellular response in the progeny. The organic Zn source significantly enhanced the CBH response in toe web. Humoral response for ZnMet, however, was not significantly better than the control.

Figure 2 shows the effect of breeder hen mineral source on progeny organ weight. The only differences noted were in the two primary immune organs, bursa and thymus. Note that the basal diet was itself adequate in Zn. The 40 ppm Zn from the two treatments was added on top of 50 ppm Zn sulfate.

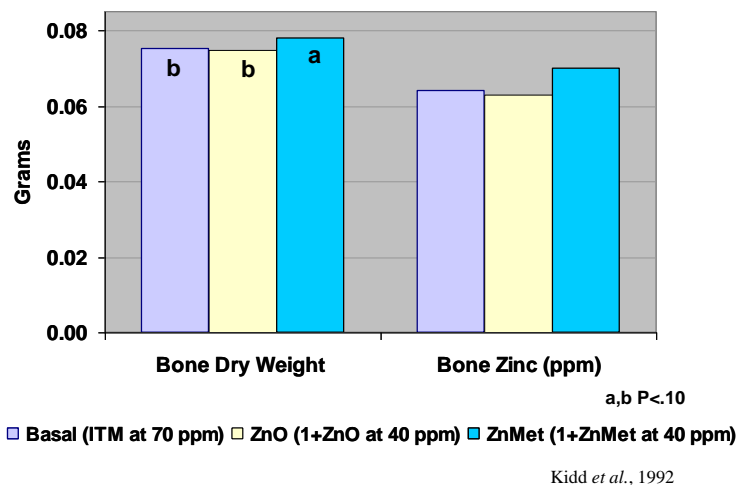
Figure 3 indicates that, in contrast to turkeys, feeding broiler breeders an organic mineral source was associated with greater tibia weight and a numerical increase in bone Zn in the progeny. Weights of other organs were not reported (Kidd *et al.*, 1992). Once again, the treatments – ZnO and ZnMet – were added to a common basal containing greater than NRC (1994) levels of inorganic Zn. Thus, the effect of organic Zn

supplementation on the performance of progeny appears to be primarily in the development of the immune system. This has been reported to result in improved resistance to infection (Virden *et al.*, 2003).

**Figure 2. Effect of Turkey Breeder Hen Mineral Source on Progeny Organ Weight**



**Figure 3. Effect of Hen Mineral Source and Level on Progeny Tibia Weight and Zinc Content**

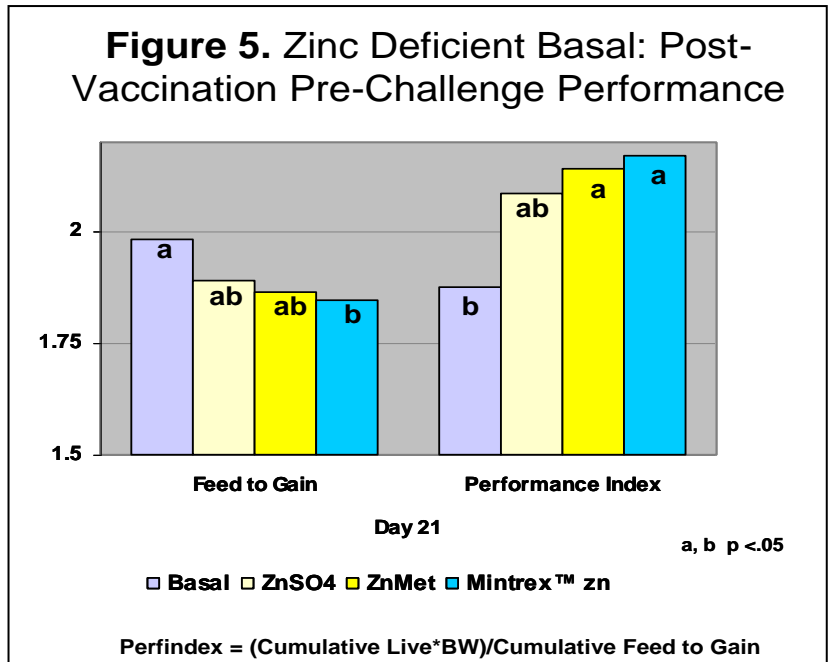
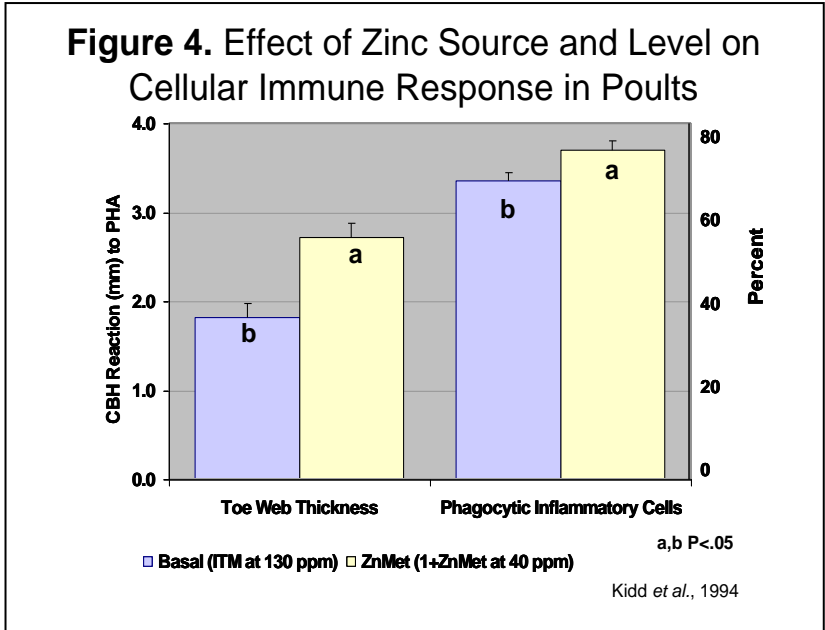


**Effect of Mineral Source on Early Nutrition of Zn and Copper**

Under normal circumstances, the hatchling is provided with highly available minerals in the liver, residual yolk, and yolk sac membrane at the time of hatch (Richards, 1997). Despite this, the early feeding of supplemental organic Zn can improve cellular immunity in 20 day old chicks. Figure 4 shows an improved CHB response and proportion of phagocytic inflammatory cells in poult fed organic Zn. Thus, the immune system of hatchlings that are fed supplemental organic Zn show increases in activity similar to those seen in progeny of hens fed organic Zn.

Coccidiosis is caused by an intracellular protozoan parasite. The host response to coccidiosis is a cellular immune response that has been shown to be responsive to supplemental Zn (Bafundo *et al.*, 1984; Southern and Baker, 1983). A coccidial challenge has been shown to result in significant reductions in plasma Zn in conjunction with an increase in hepatic metallothionein (Richards and Augustine, 1988). One of the most common species, *Eimeria acervulina*, infects the duodenum and has been shown to disrupt mineral absorption during the acute phase of the infection (Turk and Stephens, 1966, 1970). Coccidiosis vaccination provides a controlled dose of parasite and vaccinated chicks may be considered infected with a sub-clinical dose of parasite.

A study in broilers was done to test whether Zn source could affect the response to coccidiosis vaccination and challenge (Figure 5). Birds were vaccinated on day 0 while being fed a Zn-deficient basal, ZnSO<sub>4</sub>, ZnMet or Mintrex zn, all at 40 ppm Zn. Figure 5 shows that Zn supplementation did improve performance of vaccinated birds, which was significant in a single degree of freedom contrast between the basal and the other three treatments. Performance index, a measure of total performance that includes livability, body weight and feed efficiency indicated that the organic sources of Zn were significantly better than the basal. Once again, the contrast between the basal and the supplemented birds was significant.



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Figure 6 shows the effect of Zn source on the immune response to a coccidial vaccination. Total immunoglobulin levels did not differ between treatments (data not shown) but antibodies to a specific coccidial antigen, a microneme protein involved in parasite invasion, were significantly higher in birds fed Mintrex. This should be an indicator of overall immune response to the vaccine.

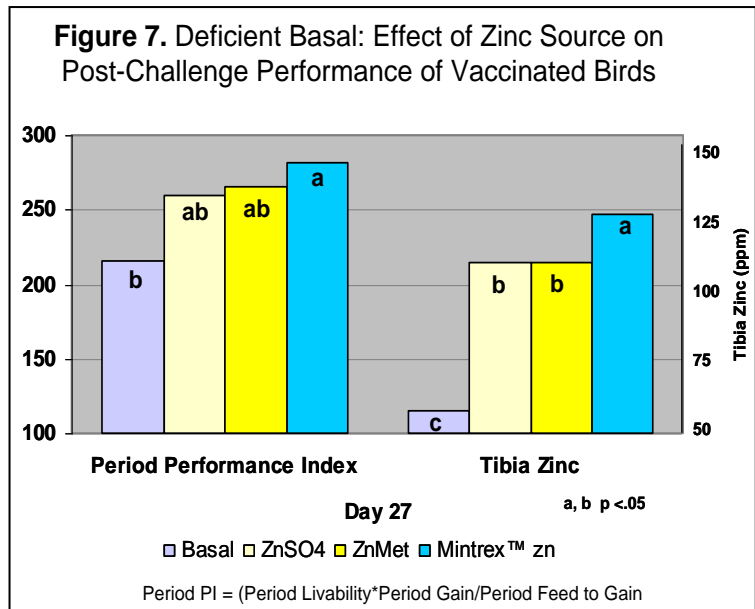
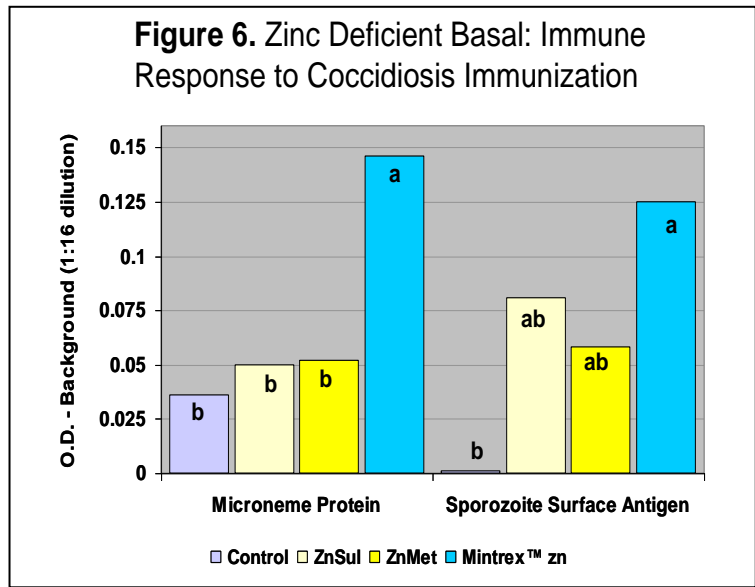
Results in Figure 7 show that these vaccinated birds were better able to withstand a mixed species coccidial challenge administered on day 23. The Mintrex fed birds showed performance significantly better than the basal and the challenge resulted in less depletion of tibia Zn than all other treatments. In this study, the benefit of early Zn supplementation with an organic source is seen in an improved vaccine response that resulted in better performance and bone Zn retention after a coccidiosis challenge.

**Conclusions**

Mineral status of breeder hens can affect the production and hatchability of fertile eggs. Progeny from hens fed organic sources of Zn appear to have a better-developed or more active cellular immune response after hatch (Kidd *et al.*, 1993). During incubation, mineral use is not constant but shows peaks for Cu at 6-8 days before hatch and for Zn at 1-2 days post hatch in turkey poults (Richards, 1997). Some mineral is left at the end of incubation and is found in the hatchling liver, yolk sac membrane and residual yolk. This highly available mineral is consumed very rapidly and a dietary Zn deficiency can be detected as early as 7 days post hatch by a decrease in bursa weight (Cui *et al.*, 2003).

Zn is required for development and differentiation of all tissues due to its regulatory role in the cell cycle, programmed cell death and gene transcription. Deficiency can affect development of the immune system, particularly the cellular response. The immune system also appears to be responsive to mineral form, with organic sources of Zn being associated with early cellular immune development, response to vaccination, and improved livability, performance, and bone mineral retention after a disease challenge.

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