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Trace Mineral Nutrition in Poultry and Swine*

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ABSTRACT : Trace minerals such as zinc, copper, and manganese are essential cofactors for hundreds of cellular enzymes and transcription factors in all animal species, and thus participate in a wide variety of biochemical processes. Immune development and response, tissue and bone development and integrity, protection against oxidative stress, and cellular growth and division are just a few examples. Deficiencies in trace minerals can lead to deficits in any of these processes, as well as reductions in growth performance. As such, most animal diets are supplemented with inorganic and/or organic forms of trace minerals. Inorganic trace minerals (ITM) such as sulfates and oxides form the bulk of trace mineral supplementation, but these forms of minerals are well known to be prone to dietary antagonisms. Feeding high-quality chelated trace minerals or other classes of organic trace minerals (OTM) can provide the animal with more bioavailable forms of the minerals. Interestingly, many, if not most, published experiments show little or no difference in the bioavailability of OTMs versus ITMs. In some cases, it appears that there truly is no difference. However, real differences in bioavailability can be masked if source comparisons are not made on the linear portion of the dose-response curve. When highly bioavailable chelated minerals are fed, they will better supply the biochemical systems of the cells of the animal, leading to a wide variety of benefits in both poultry and swine. Indeed, the use of certain chelated trace minerals has been shown to enhance mineral uptake, and improve the immune response, oxidative stress management, and tissue and bone development and strength. Furthermore, the higher bioavailability of these trace minerals allows the producer to achieve similar or improved performance, at reduced levels of trace mineral inclusion. (**Key Words :** Chelate, Trace Mineral, Poultry, Swine, Immune Response, MINTREX)

INTRODUCTION

Trace minerals such as zinc, copper, and manganese are crucial for a wide variety of physiological processes in all animals. The biological importance of zinc is underscored by the understanding that zinc is a required co-factor for several hundred enzymes, representing all six enzyme classes, and a wide variety of transcription factors (Vallee and Falchuk, 1993; Underwood and Suttle, 1999). Indeed, proteins with zinc-binding domains are estimated to represent approximately 10% of the human proteome, and the same would be expected to be true in production animals as well (Andreini et al., 2006). As such, zinc plays essential roles in a wide array of processes including cell proliferation and animal growth, immune development and response, reproduction, gene regulation, and defense against oxidative stress and damage (Shankar and Prasad, 1998; Underwood and Suttle, 1999; Fraker et al., 2000; Blanchard et al., 2001; Ibs and Rink, 2003; Song et al., 2009). Zinc's role in gene regulation is based on its incorporation into the structure of various transcription factors, including the zinc-finger transcription factors and hormone receptor proteins (Coleman, 1992; Blanchard et al., 2001; Dreosti, 2001; Cousins et al., 2003). Likely reflecting its role in gene regulation, zinc is required for the synthesis of a variety of enzymes and other proteins. Two key structural proteins, collagen and keratin, both require zinc for their synthesis (Underwood and Suttle, 1999). Keratin is the major structural protein of the hoof horn, feathers, skin, beaks and claws, while collagen is the major structural protein of the extracellular matrix and connective tissues in internal tissues, including cartilage and bone. Decreases in collagen and keratin synthesis rates in zinc-deficiency can lead to a variety of defects including bone abnormalities, poor feathering, decreased tissue strength and dermatitis (Underwood and Suttle, 1999; Leeson and Summers, 2001). Furthermore, collagen turnover rates are also decreased in zinc-deficiency, presumably because the collagenases/ matrix metalloproteinases (MMPs) are zinc-dependent enzymes (Starcher et al., 1980; Pardo and Selman, 2005). Decreases in collagen synthesis and turnover rates would be predicted to cause decreases in tissue strength. This is important both for animal health and for post mortem

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processing. Decreased intestinal strength, for example, would be predicted to result in higher rates of intestinal breaks and condemnations during processing. Finally, zinc plays important roles in the development and proper functioning of the immune system (Shankar and Prasad, 1998; Fraker et al., 2000; Ibs and Rink, 2003). Deficiencies in zinc can lead to decreased immune function, as demonstrated by reduced T cell function, lower antibody titers and other deficits.

Like zinc, copper is essential for a wide variety of health and performance-related functions in all animal species. Functions performed by zinc are often enhanced by copper-dependent enzymes. For example, lysyl oxidase, the enzyme that crosslinks collagen subunits into mature protein forms to increase their strength, is copper-dependent (Rucker et al., 1998). Lysyl oxidase also crosslinks the structural protein elastin, which is found in connective tissue, primarily in the cardiovascular system, intestines, and other tissues that change size as a consequence of fill. Because of its role in collagen crosslinking, copper promotes skin, bone, tendon and intestinal strength. Experiments in poultry have demonstrated that bone breaking strength correlates strongly with the extent of collagen crosslinking (Rath et al., 1999). In copper-deficient animals, therefore, the elastin and collagen may be unable to withstand the mechanical stresses typical of the cardiovascular or skeletal systems, respectively (O'Dell et al., 1961; Guenthner et al., 1978). Indeed, severe copper deficiencies have been reported to cause aortic rupture in multiple species, and bones may be fragile and easily broken (Guenthner et al., 1978; Opsahl et al., 1982; Underwood and Suttle, 1999). Like collagen, keratin synthesis requires zinc, and keratin crosslinking is copper-dependent.

Manganese is essential for growth and fertility of animals (Gallup and Norris, 1939; Underwood and Suttle, 1999). Furthermore, it plays a very important role in bone development, both in the embryo and after birth or hatch. The ground substance of developing bone, particularly the proteoglycan matrix in which collagen and elastin are embedded, requires Mn for glycosylation of its protein core molecule (Fawcett, 1994). Proper development of this matrix is required for later stages of bone development. In a Mn-deficient animal, therefore, there can be a failure of endochondral ossification, resulting in chondrodystrophy and perosis (Underwood and Suttle, 1999). Evidence that Mn-deficiency causes these defects is shown by the fact that these two conditions can be corrected by manganese supplementation.

All three of these trace minerals play key roles in managing oxidative stress. Reactive oxygen species (ROS) generation is a normal byproduct of cellular energy production, and a primary weapon of the innate immune response (Mayne, 2003; Iqbal et al., 2004). Unfortunately, these ROS are damaging to cellular lipids, proteins and DNA and if left unchecked can induce a variety of undesirable consequences. The superoxide dismutase (SOD) enzymes form a first-line defense that converts oxygen radicals to hydrogen peroxide, which is a less toxic molecule (Hydrogen peroxide is then converted to water through the action of glutathione peroxidase, a selenium-containing enzyme). There are two forms of SOD in animal cells, the copper and zinc dependent form, in the cytoplasm, and the manganese-dependent form in the mitochondria (Underwood and Suttle, 1999). Decreases in SOD activity, for example in a mineral deficiency, can lead to increased amounts of lipid, protein and nucleic acid damage, which can induce cellular death (Rothstein et al., 1994; Troy and Shelanski, 1994; Kokoszka et al., 2001). The actions of SOD should not be underemphasized. Both the Cu/Zn- and Mn-dependent isoforms of SOD are two of only a handful of enzymes whose activity correlates with lifespan in simple organisms (Orr and Sohal, 1994; Parkes et al., 1998; Honda and Honda, 1999). Zinc has been proposed to directly or indirectly manage oxidative stress in other ways as well, including through the induction of metallothionein, and as a required cofactor in the p53 transcription factor, which mediates the repair of DNA damaged by oxidative stress or other means (Ho and Ames, 2002; Formigari et al., 2007). Zinc deficiency has been shown in a variety of publications to increase oxidative stress *in vitro* and *in vivo*, as shown by increases in the prevalence of markers of oxidized lipids and damaged DNA, and the production of free radicals (Ho et al., 2003; Song et al., 2009). In zinc-deficiency, p53 loses its ability to bind DNA and promote repair, which can result in increased rates of apoptosis (Ho and Ames, 2002; Fraker, 2005). Indeed, zinc deficiency has been shown to increase cellular turnover rates in the small intestine (Cui et al., 1999; Richards et al., 2005).

Inorganic and organic forms of trace minerals

Historically, zinc, copper and manganese have been supplemented in animal diets using inorganic salts such as oxides and sulfates. However, trace mineral salts tend to dissociate in the low pH environment of the upper gastrointestinal tract, leaving the minerals susceptible to various nutrient and ingredient antagonisms that impair absorption (and thus reduce bioavailability) (Underwood and Suttle, 1999). Antagonisms can occur between minerals. For example, high levels of zinc reduce the availability of copper, and the opposite is also true (O'Dell, 1989; Leeson and Summers, 2001; Zhao et al., 2008). In addition, phytic acid is able to form complexes with trace minerals that are very stable and highly insoluble, rendering the minerals unavailable for absorption (Oberleas et al., 1966; Leeson and Summers, 2001). The phytic acid-mediated antagonism

is amplified in the presence of calcium (Oberleas et al., 1966).

A potential advantage of chelated trace minerals is that the binding of the organic ligand(s) to the mineral should provide stability of the complex in the upper gastrointestinal system, thereby minimizing mineral losses to antagonists and allowing the complex to be delivered to the absorptive epithelium of the small intestine for mineral uptake (Leeson and Summers, 2001). It should be noted that different organic trace minerals are not equally stable at low pH, and therefore will not necessarily increase the bioavailability of a given mineral to the same extent (Brown and Zeringue, 1994; Cao et al., 2000; Guo et al., 2001).

Measuring trace mineral bioavailability

Measuring the deposition or storage of minerals into selected tissues (e.g. tibia or plasma zinc, liver copper, tibia manganese, etc.) is the most common output in trace mineral relative bioavailability (RBV) experiments (Underwood and Suttle, 1999). More recently, the use of mineral-responsive biomarkers, such as changes in gene or protein expression, or the activity of a mineral-dependent enzyme, has become more common (Payne and Southern, 2005; Richards et al., 2007; Huang et al., 2009; Richards et al., 2010). Regardless of the response variables measured, it is surprising how many papers report little or no difference in OTM vs. ITM bioavailability. An extensive review of the zinc bioavailability literature in poultry, swine and other species was published in 1995 (Baker and Ammerman, 1995). In this review, the average bioavailabilities of a variety of inorganic (zinc oxide, zinc sulfate, zinc chloride, zinc carbonate, elemental zinc) and organic zinc sources (zinc lysine, zinc methionine, and zinc proteinate) were compared. In poultry, relative to the sulfate, acetate or chloride forms of zinc (defined as 100% bioavailable in individual experiments), the average RBV of zinc methionine and a zinc proteinate was 125% and 100%, respectively (Baker and Ammerman, 1995; and references therein). In swine, there were no differences between organic zinc (zinc methionine or zinc lysine) and inorganic zinc (zinc sulfate or zinc chloride) (Baker and Ammerman, 1995; and references therein). (Interestingly, zinc oxide ranged from 55% to 100% as available as zinc sulfate.) While this review is 15 years old, subsequent papers in the literature often report similar results. It seems likely that some OTMs truly will not be more bioavailable than ITMs, due to their inability to stay chelated or complexed in the low pH environment of the upper GI tract (Brown and Zeringue, 1994; Cao et al., 2000; Guo et al., 2001). On other occasions, however, true differences in bioavailability could be masked by experimental design. Using tibia zinc content as the measure of bioavailability, Wedekind and colleagues have indicated that the bioavailability of zinc methionine relative to zinc sulfate ranges from 117% to 206% in broiler chicks, depending on the diet matrix (Wedekind et al., 1992). The differences in RBV were reduced in crystalline or semi-purified diets that contain low levels of antagonists such as fiber or phytic acid, and increased in corn-soy diets. Furthermore, these authors emphasized the importance of measuring RBV on the linear portion of the dose-response curve, rather than on the plateau. Regardless of the response variables utilized, measuring the response on the plateau of the curve will minimize true differences in RBV. Indeed, in this same paper, Wedekind et al. reported no difference in zinc methionine vs. zinc sulfate RBV when supplementing at zinc levels above the tibia zinc breakpoint (Wedekind et al., 1992). Recent experiments with other chelated minerals support this finding. A study of zinc chelated by the methionine hydroxy analogue (HMTBa-chelated zinc, or MINTREX® Zn) performed on the linear portions of the dose response curves indicated that the zinc from this source was approximately 160% or 250% as available as the zinc from zinc sulfate, depending on the response variable measured (tibia zinc; or the small intestinal expression of the zinc responsive biomarker, metallothionein; respectively) (Richards et al., 2010).

Impact of trace mineral supplementation on tissue development and strength

As described above, zinc and copper play key roles in the synthesis and proper assembly of collagen. An experiment was conducted to test the effects of copper source and level on intestinal breaking strength (IBS) in broilers. Broilers were fed diets that were adequate for zinc, but were unsupplemented for copper (9 ppm Cu from ingredients), or supplemented with 25 ppm copper from copper sulfate, a copper proteinate, copper lysine, or HMTBa-chelated Cu (MINTREX® Cu). There was a significant improvement in IBS in all supplemented diets, but birds that were supplemented with HMTBa-chelated Cu had greater IBS than all other treatments (Figure 1). One likely explanation to understand these results is that the extent of collagen and elastin crosslinking in the control birds may have been low due to suboptimal copper status in the control diets. Addition of the various copper sources, but especially HMTBa-chelated copper, may have promoted collagen and elastin crosslinking beyond what occurred in the low-copper treatment.

As described above, zinc and copper play important roles in bone development via their actions on collagen, while manganese-dependent enzymes promote formation of the proteoglycan matrix in the cartilage model for developing bone. Tibial dyschondroplasia (TD) is a common developmental defect in fast growing birds. In this condition, the cartilage model at the epiphysial plate fails to

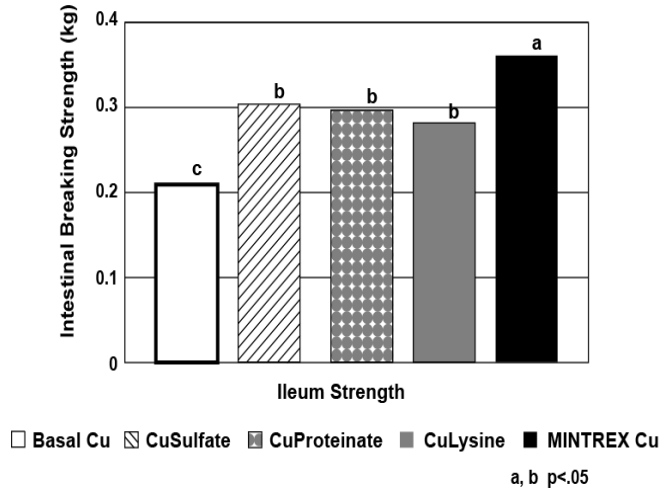

Figure 1. Increase in intestinal breaking strength in broilers fed diets either unsupplemented for copper (Basal Cu), or supplemented with 25 ppm copper from the indicated sources. Bars lacking a common superscript differ significantly ($p<0.05$).

ossify, resulting in plugs of cartilage in place of true bone. Bones are therefore weak, and can result in bone breaks when the bird grows heavy. Rath and colleagues have demonstrated that tibias exhibiting TD have normal collagen content, but reduced amounts of sulfated glycosaminoglycans and MMP activity when compared to normal tibias (Rath et al., 1997). These results suggest supplementation with manganese and zinc may alleviate TD. In a separate paper, this group also showed that the extent of collagen crosslinking in tibia correlated with bone breaking strength (Rath et al., 1999). These results imply an important role for copper. Given the high incidence of TD and bone weakness in fast growing meat birds, one would expect the incidence of these problems to be reduced with improved trace mineral nutrition. Indeed, recent commercial and university experiments in turkey flocks have demonstrated reduced TD, reduced lameness and increased bone breaking strength when the diets were supplemented with HMTBa-chelated trace minerals (Dibner et al., 2007; Ferket et al., 2009). It is interesting to note that in the control treatments, ITM levels were formulated at commercial levels, far exceeding published requirements. Thus, one might have predicted that these animals would not be deficient in trace minerals. Yet the physiological responses of these animals to chelated mineral supplementation indicate that the controls were deficient, at least with respect to supplying the minerals needed for optimal structural development.

Feeding chelated minerals can improve structural integrity of tissues even when fed at reduced inclusion rates. In a recent trial with 120,000 broilers, birds fed reduced levels of supplemental zinc, copper and manganese (32 ppm, 8 ppm and 32 ppm, respectively) as HMTBa chelates had significantly improved footpads relative to broilers fed much higher levels of zinc, copper, and manganese (100 ppm, 125 ppm and 90 ppm, respectively) as sulfates (Manangi et al., 2010). It is interesting to note that growth performance was not different between these two treatments, while trace mineral excretion was reduced in the birds fed chelates. These data demonstrate that the HMTBa-chelated trace minerals met the requirement of these birds, even though they were supplemented at low levels.

Impact of supplemental trace minerals on the immune response

As described above, trace minerals, especially zinc, are required for proper immune development and function (Shankar and Prasad, 1998; Fraker et al., 2000; Ibs and Rink, 2003). Deficiencies in zinc can cause decreased antibody responses to vaccination. Previous results with multiple inorganic (sulfate and oxide) and organic zinc sources (zinc methionine and HMTBa-chelated zinc) in poultry have demonstrated source differences in both immune development and response to antigenic challenge (Dibner, 2005; Moghaddam and Jahanian, 2009). In each of these papers, supplementation with the organic zinc source enhanced cellular or antibody responses to vaccination. We wished to test the effect of feeding chelated trace minerals on the immune response to vaccination in pigs. Replacement gilts (50 per treatment) were fed diets supplemented with 165 ppm zinc, 16.5 ppm Cu and 38.6 ppm manganese, either as ITMs or an equal mixture of ITMs and HMTBa-chelated minerals. The pigs were vaccinated with a commercial vaccine for *Mycoplasma hyopneumoniae* on weeks 0 and 2 postweaning, and bled for antibody titers on weeks 0, 2, 4, 8 and 12. Titers were measured by a commercially-available ELISA. Log titers below 2.8 are considered to be negative titers according to the kit instructions. While both groups of pigs achieved a similar titer by 12 weeks, the gilts supplemented with the

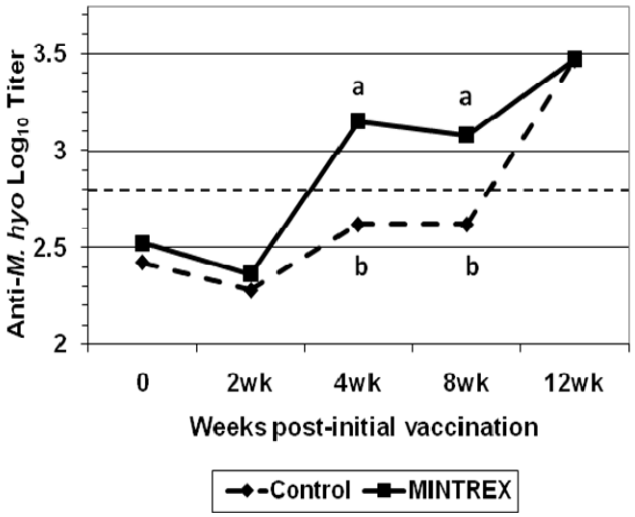

Figure 2. Vaccine-induced anti-*M. hyopneumoniae* antibody titers of gilts supplemented with 165 ppm zinc, 16.5 ppm Cu and 38.6 ppm manganese, either as ITMs or an equal mixture of ITMs and HMTBa-chelated minerals (MINTREX® Zn, Cu, and Mn). Titers were measured using a commercially-available ELISA kit (IDEXX Laboratories). Titers that fall below the horizontal dotted line are considered negative by the kit instructions. Data points lacking a common superscript within a timepoint differ significantly ($p<0.05$).

HMTBa chelates reached a positive titer 8 weeks prior to the gilts fed the control diet (Figure 2). These data suggest that for those eight weeks, the replacement gilts fed ITMs were not as protected against *M. hyopneumoniae* as the gilts fed the HMTBa-chelated minerals.

Effect of trace mineral supplementation on oxidative stress

Oxidative stress results when the production of reactive oxygen species (ROS) exceeds the body's ability to detoxify the reactive species, or to repair the damage caused by them. Inappropriately high or chronic levels of oxidative stress can damage lipids, proteins and DNA, leading to high rates of cell death and turnover (Dibner et al., 1996; Girotti, 1998; Mayne, 2003). Poor oxidative stress management in production animals can result in decreased performance, compromised immune function, increased morbidity and poor meat quality (Sheehy et al., 1994; Buckley et al., 1995; Iqbal et al., 2004; Spears and Weiss, 2008). Although the roles that natural and synthetic antioxidants play in managing oxidative stress are well recognized, it is important to understand that trace minerals also participate in these processes (Underwood and Suttle, 1999; Ho and Ames, 2002; Formigari et al., 2007; Song et al., 2009). One method to estimate oxidative stress in a group of animals is to measure specific oxidized forms of lipids, proteins and nucleic acids from blood or tissue samples (Mayne, 2003). A battery study was conducted in broilers to investigate whether different inorganic and organic trace mineral forms could reduce oxidative stress. All treatments except the negative control were supplemented with 30 ppm zinc, 20 ppm manganese and 5 ppm copper. As an indicator of

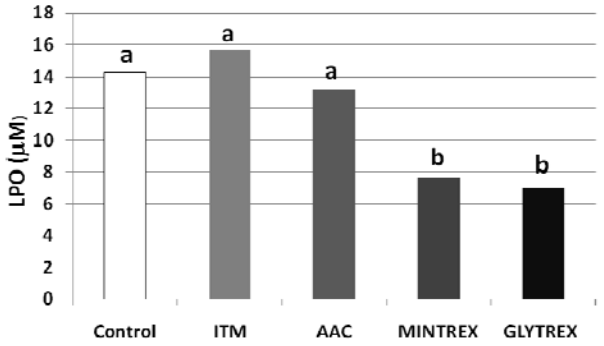

Figure 3. Plasma lipid hydroperoxide (LPO) concentrations in broilers fed diets unsupplemented for zinc, copper and manganese, or supplemented with 30 ppm zinc, 20 ppm manganese and 5 ppm copper from ITMs, amino acid complexes (AAC), HMTBa-chelated trace minerals (MINTREX), or glycine-chelated trace minerals (GLYTREX™). LPO levels were measured on day 29 post-hatch using a commercially-available kit (Cayman Chemical). Bars lacking a common superscript differ significantly ($p\leq0.01$).

oxidative stress, the concentration of lipid hydroperoxides (LPO) was measured in plasma. As shown in Figure 3, birds fed either the glycine-chelated trace minerals (GLYTREX™ chelated minerals) or the HMTBa-chelated trace minerals (MINTREX®) exhibited significantly ($p<0.01$) lower levels of lipid hydroperoxides in their blood versus the control, indicating lower oxidative stress in these birds. Inclusion of ITM or amino acid complexes did not reduce plasma LPO relative to the control ($p>0.45$) or each other ($p>0.2$).

CONCLUSIONS

Essential trace minerals such as zinc, copper and manganese play a wide variety of biological and physiological roles in animal development and health. These minerals take part in the antioxidant defense and DNA repair, bone and tissue development, and immune function. The importance of these minerals in animal agriculture is widely recognized, and virtually all diets are supplemented with these minerals. However, growing evidence supports the conclusion that the trace mineral requirements of production animals are not easily or consistently met by feeding inorganic forms of these minerals. Well-designed experiments that investigate the relative bioavailability of trace mineral sources demonstrate that certain organic trace minerals can more effectively satisfy the trace mineral requirements of production animals. By providing a more bioavailable source of minerals, OTM supplementation has been shown to exert a variety of positive effects, including improved immune responses, enhanced bone and tissue development and strength, and reduced oxidative stress. Finally, due to their higher bioavailability, the requirement can be met at lower levels of inclusion in the diet, resulting in reduced excretion into the environment.

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