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Does Inclusion at Low Levels of Organically Complexed Minerals Versus Inorganic Forms Create a Weakness in Performance or Antioxidant Defense System in Broiler Diets?

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Abstract: Inorganic trace mineral participates, like sulfate or oxide salts, are a critical component in commercial poultry feeds. However, inorganic trace minerals can suffer from high rates of loss due to dietary antagonism which cause a significantly reduce in their bioavailability. As a result, more inorganic trace minerals are supplied than are actually needed. An excess of supplemental inorganically complexed minerals leads to waste and environmental. The use of lower levels of organically complexed minerals in poultry diets has become a common method to solve this problem in recent years. Organically complexed minerals are biotechnological products which inorganic mineral integrated to organic structures such as protein or polysaccharide. Research regarding this kind of supplementation is still at a nascent stage, though and not enough data exists to determine optimal levels of organically complexed minerals and to quantify differences in excretion rates between inorganic and organic sources. In addition, it is unclear that using at lower levels of organically complexed minerals in diet whether creates a weakness on systems, in which the mineral plays an active role.

Key words: Broiler, organic mineral, performance, antioxidant defense system

INTRODUCTION

Trace minerals role in immune function and related physiological roles have been studied in recent years. In livestock diets, trace mineral supplementation is a quite complex issue due to their resources, functions and interactions. In commercial poultry diets, majority of trace minerals are commonly supplemented in the form of inorganic salts, such as sulfates, oxides and carbonates, to provide levels of minerals that prevent clinical deficiencies and allow the birds to reach their genetic growth potential (Bao *et al*., 2007). As traditionally, trace mineral supplementation is much higher than those recommended by National Research Council (NRC, 1994; Inal *et al*., 2001). Excessive mineral supplementation leads to wasteful and the environmental contamination. Due to increasing concerns about potential mineral pollution, there has been considerable interest in discussion on how to reduce mineral excretion without any negative effect on production performance. The use of organically complexed or chelated minerals at a much lower concentration for livestock diets have been suggested as an answer to this discussion, based on the hypothesis that such mineral complexes have a higher bioavailability than inorganic salt analogues (Aksu *et al*., 2011; Saripinar Aksu *et al*., 2010b; Bao *et al*., 2007; Nollet *et al*., 2008). In recent years, use of organically complexed minerals at lower levels in livestock rations has become widespread especially due to their ecological and physiological contributions. Organically complexed minerals are biotechnological products which inorganic mineral integrated to organic structures such as protein or polysaccharide. Therefore, new organically complexed minerals have been patented and marketed providing a more available form of trace minerals for poultry. The intent of this presentation is to do such a review of established structures and functions of organically complexed minerals, as well as a possible weakness that may occur in reducing trace minerals in the poultry diets.

Description of the problem: Minerals and other nutrients in feed help prevent clinical and pathological problems in domestic animals. Trace minerals are necessary for encouraging adequate and healthy growth and development, maintaining many metabolic processes, building strong immunity and increasing appetite (Underwood and Suttle, 1999). Mineral deficiency often

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occurs as a symptom of typical metabolic disorders, though and reduces overall production and causes loss of appetite and failure of the reproductive and immune system (Vanderklis and Keme, 2002). Signs of trace mineral deficiency change depending on the severity of impairment. Poultry initially exhibit problems related to immune and enzyme functions and fertility. Body weight gain rates then reduce, stop, or even reverse. At this stage, more acute clinical symptoms begin to appear in the form of various disorders and a decrease in animal production performance. If the origin of deficiency is not determined in the first stages, though, or the solution is defined with the wrong approach, then important economic losses follow (Wikse, 1992). Traditionally, these key trace minerals are supplemented in the form of inorganic salts, such as sulfates, oxides and carbonates, to provide levels of minerals that prevent clinical deficiencies and allow the bird to reach its genetic growth potential (Bao *et al.*, 2007). Farmers cannot be sure that poultry are receiving proper levels of these key elements in all conditions without knowing the bioavailability of trace minerals.

Imbalances in these rationed mineral levels may create an antagonistic effect and be the root of reduced bioavailability because absorption and metabolism of the minerals are insufficient. In classical practice, therefore, mineral premix included much higher levels of these minerals than was actually required. Moreover, much of the information, even in recent National Research Council (NRC, 1994) documents, is actually based on research from the 1960s and 1970s, when the birds and their management were substantially different (Nollet *et al.*, 2008). Fecal excretion levels of inorganic mineral resources are higher due to the use of high concentrations of minerals in diets and the inability of birds to digest, absorb and metabolize inorganic trace minerals. Obviously, this problem is not only wasteful but also harmful to the environment (Leeson, 2003). Modern and high-scale livestock enterprises constitute a major threat in this sense, especially in regions with intensive livestock production. For example, poultry manure applied on an N basis contains Zn and Cu that is 660% and 560%, respectively, in excess of crop requirements (Dozier *et al.*, 2003). In addition, due to concerns of build-up of heavy metals when applying poultry litter to cropland, environmental protection agencies around the world have pressed for lower levels of excess mineral applied to land (Bao *et al.,* 2007). Therefore, nutritionists have started to discuss how to reduce mineral excretion without any negative effect on production performance due to increasing concerns about potential mineral pollution. The use of complex organic or chelated minerals at a much lower concentration for livestock diets has been suggested as an answer to this discussion, based on the hypothesis that such mineral complexes have higher bioavailability than inorganic salt analogues (Saripinar Aksu *et al.*, 2011; Bao and Choct, 2009).

Interaction between minerals: Uncertainties related to the absorption and utilization of inorganically complexed minerals and low-cost of inorganic salts lead to the excessive use of trace mineral in these diets. Weak mineral absorption causes negative interactions between the minerals and a mineral deficiency from reduced bioavailability may result, even with high level of minerals in the diet. The use of high amounts of trace minerals in inorganic forms such as sulfates, oxides and carbonates may reduce nutrient absorption and bioavailability (Underwood and Suttle, 1999).

There are many factors that could affect an animal's response to trace mineral supplementation such as the duration and concentration of trace mineral supplementation, physiological status of an animal (i.e., pregnant vs. non pregnant), the absence or presence of dietary antagonists, environmental factors and the influence of stress on trace mineral metabolism (Baker *et al.*, 2003).

Using organically complexed minerals in livestock feed could reduce the loss of trace minerals in the digestive system by recovering the mineral from antagonistic factors and creating complex compounds that move trace minerals into the absorptive epithelium of the intestine. Thus, bioavailability of trace minerals can be increased and environmental pollution can be decreased. Organically complexed minerals are a bound form and are not affected from the intensity of the other minerals in ambient. Metal complexes or chelates are stable in the digestive system and preserve the complex structure forming minerals. Therefore, these type of minerals are more readily absorbed (Spears, 1996; Puchala *et al.*, 1999; Uchida *et al.*, 2001) and almost all of the absorbed amounts can be evaluated in metabolism, thus the amount of mineral excretion would significantly reduce (Aksu *et al.*, 2011; Nollet *et al.*, 2008).

What is an organic trace mineral?: Organically complexed minerals are metal ions formed by a complex structure of amino acids, peptides, proteins and polysaccharides. Commercially available organic trace mineral supplements vary in regard to the type of ligand or ligands used to form the metal complex or chelate. Most of the organic minerals marketed are classified as complexes, chelates, or proteinates. Chelation refers to a special type of complex formed between a ligand and a metal ion. To be classified as a chelate, a ligand or chelating agent must contain a minimum of 2 functional groups (oxygen, nitrogen, amino, hydroxyl), each capable of donating a pair of electrons to combine (via coordinate covalent bonding) with a metal and must form a heterocyclic ring structure with the metal (Kratzer and Vohra, 1986).

Organically complexed minerals can be produced when metal salts react with organic compounds in the suitable conditions or by adding soluble metal salts in yeast cultures in proper media, including, for example, biological media biological (AAFCO, 1997; Hale and Olson, 2002). In livestock diets, organic forms of trace mineral such as chelates, proteinates, polysaccharides and organic acids can be used instead of inorganic forms of those minerals.

Minerals with an organic structure are defined by The American Association of Feed Control (AAFCO) as follows [AAFCO, 1997): A *Metal Amino Acid Chelate* (57.142) is the product resulting from the reaction of a soluble metal salt with amino acids with a mole ratio of one mole of metal to one to three (preferably two) moles of amino acids to form coordinate covalent bonds. The average weight of the hydrolyzed amino acids must be approximately 150 and the resulting molecular weight of the chelate must not exceed 800.

A *Metal Amino Acid Complex* (57.150) is the product resulting from complexing of a soluble metal salt with an amino acid(s).

A *Metal (Specific amino acid) Complex* (57.151) is the product resulting from complexing of a soluble metal salt with a specific amino acid.

A *Metal Proteinate* (57.23) is the product resulting from the chelation of a soluble salt with amino acids and/or partially hydrolyzed protein.

A *Metal Polysaccharide Complex* (57.29) is the product resulting from complexing of a soluble salt with a polysaccharide solution declared as an ingredient as the specific metal complex.

The organic forms of many trace minerals such as zinc, copper and manganese are commonly used in livestock feed. All of these products do not have equal efficiency even though all are used to increase bioavailability of trace minerals. The primary chelated minerals used in animal feed are the trace elements iron, manganese, cobalt, copper and zinc. These “Transitional Elements” on the Periodic Table have chemical characteristics intermediate between metal and non-metal elements. Transitional elements prefer to form coordinate covalent bonds, a hybrid form of linkage that gives them their unique ability to form stable complexes--coordination complexes or chelates (Hale and Olson, 2002).

Solubility, absorbability and bioavailability in organically complexed minerals: The value of feeds (or contributions) used as a mineral source depends not only on mineral contents but also the mineral amounts that can be used by animals. This value is defined as “bioavailability.” The degree of bioavailability is determined by four main factors (Hazell, 1985; Ammerman *et al.*,1995):

Accessibility: The amount of minerals absorbed by the mucosa

Absorbability: The potential transfer of the mineral that can be absorbed in the mucosa to the system to be used

Continuity: The potential retention of the moved mineral

Functionality: The potential cooperation of the detained mineral with the functional forms of other minerals

Accessibility is directly affected by the interaction of minerals in the feed with current minerals or antagonists in the intestine. For that reason, the most basic determinant of the availability and its effect on fulfilling an animal’s nutritional requirement is accessibility. Absorbability is determined by the mineral-taking capacity of mucosa. Another element can decrease the mineral-taking or its absorbability if the taking-mineral mechanism of mucosa is not specific, such as the interaction between iron and manganese. Continuity is the ability of minerals to escape through the stomach and the intestinal excretion of mineral. Functionality of minerals may be affected by the location in which minerals are absorbed and kept. For example, absorbed iron is not more absorbed from mucosa (Ammerman *et al.*,1995; Boland, 2003). If inorganic salts may be converted into their free ions in the digestive system, they can be metabolized through intestinal absorption. However, the absorption of ionized minerals may be partially or completely blocked by creating complex compounds with other elements or with some antagonistic nutrients in the intestinal environment. As a result, it is impossible to determine with precision how much of the inorganic salts will be digested and metabolized. The availability of inorganic resources depends on the animal's ability to convert these resources into the organic compounds biologically. It is again unclear whether the absorbed mineral as free ions combines, turns into a useful form and metabolizes. Organically complexed minerals, except for large-molecule proteinates, are absorbed and metabolized without any change or are stored in various tissues. Large-molecule organically complexed minerals bond to amino acids or peptides before they become free ions. Therefore, the absorption and bioavailability of these forms and the absorption of some inorganic forms of trace minerals are very high, including sodium selenite and copper sulfate (Spears, 1996). In several studies (Aspila, 1991; Koenig *et al.*,1997) related to selenium, no differences were observed in the absorption rates of seleno-methionin and sodium selenite. However, selenite or sulphate minerals are excreted by urine more quickly after being metabolized. Bioavailability studies address some of these contradictory results. A study (Cao *et al*., 2000) investigated the bioavailability of four commercial organic zinc compounds (with methionin, polysaccharide, lysine-chelated and proteinate) in lambs and chickens. Of these compounds, only Zn-proteinate had higher bioavailability than Zn-sulphate. Bioavailabity of trace minerals is generally determined by determining the stored and accumulated amounts of minerals in the liver and bone (specifically, the tibia). High mineral density in tissues may protect the animals against short-term mineral inefficiencies and various stresses and shows that the bioavailability of trace minerals is higher. However, the most important failure of this assessment is to measure only a portion of the mineral. The amount of mineral in tissues shows storage of mineral pools but does not completely calculate the amount of all absorbed minerals. In recent years, a different and new technological approach was tested for measurement of the genes that are responsible for mineral absorption in the intestine. The function of most genes is related to minerals. One of these special genes is Metallothionein (MT) and is responsible for the regulation of zinc absorption (Lu *et al*., 1990; Cao *et al*., 2002; Martinez *et al*., 2004). The MT gene is used in determining the amount of zinc present or measuring the bioavailability of different zinc resources. An unwarranted technique to measure the value of MT with real-polymerase chain reaction analysis has important consequences on this issue (Cengiz, 2008).

What is the advantage of organically complexed form?: It is possible to reduce the excretion of Cu by 75%, Zn by 50% and Fe by 14% by using organic trace minerals (Bao and Choct, 2009). In recent years, there has been a considerable reduction in the recommended concentrations of organically complex minerals in broiler chicken diets without any negative effects on broiler performance (Bao *et al*., 2007; Nollet *et al*., 2008; Petrovic *et al*., 2009; Aksu *et al*., 2011a), antioxidant defense systems (Saripinar Aksu *et al*., 2010a), hematological and biochemical parameters (Saripinar Aksu *et al*., 2010b) and meat quality parameters of broilers (Aksu *et al*., 2011b). The contribution of organically complexed minerals into feed also supports the immunity, protecting chickens from intestinal pathogens (Abdallah *et al*., 2009). Ao *et al*. (2011) observed that chicks fed 12mg organic Zn per kg of diet had the same growth rate but lower tibia Zn content compared with those fed either 40mg of Zn as sulphate or 24mg of Zn as organic. They reported that lower than suggested NRC or commercial levels of Zn provided by organic sources can be used in broiler diets and may better support optimum growth of broiler chicks. In another novel study (Aksu *et al*., 2011a) with 200 Ross 308 broiler chickens, the effect of reducing organic Cu, Zn and Mn by one-third instead of using inorganic forms of these minerals were measured by looking at the mineral concentrations in the tibia and mineral excretions. The results showed that organically complex trace minerals can be used at a much lower concentration than the current recommended levels without a negative impact on performance and with decreased excess mineral excretion. Researchers stated that further studies are needed to determine the proper level of organic trace minerals by taking tibia mineral levels into consideration. In a previous study (Ao *et al*., 2011) where organic forms of zinc, copper, manganese and iron (peptide chelate at the rate of 50% or 100% of the total requirements of the elements recommended for Ross 308 broiler chicks) were used in broiler diets, replacing inorganically complexed minerals with organic sources improved bird's performance and enhanced immune response of chicks. In another experiment with 2,040 Ross 308 broiler chickens (Nollet *et al*., 2008), the effect of substituting inorganic with organically complexed minerals in broiler feed on performance showed significantly lower ($P<0.05$) excretion rates for all minerals in fecal samples while no significant differences were observed in any of the productive performance parameters measured during the trial. Fecal levels of Mn, Zn, Fe and Cu were also found to be as 46, 63, 73 and 55%, respectively in this study.

Indeed, a variety of trials have demonstrated greater bioavailability of organically complex trace minerals which in turn would allow for lower inclusion rates and reduced excretion.

Yet there has been little research into the effects of these minerals in lower levels on hematological and biochemical parameters, lipid peroxidation and antioxidant defense systems. Inadequate mineral intake may increase the susceptibility of tissues to oxidative stress by causing weakness in protective antioxidant systems. Reactive Oxygen Species (ROS) are essential for proper cell functioning and are widely produced during normal cell metabolism. Low levels of ROS are necessary for many cell-signaling procedures. Under normal physiological conditions, a balance exists between the levels of ROS produced during cellular metabolism and the levels of endogenous antioxidants which serve to protect tissues from oxidative damage. Imbalance or loss of cellular redox homeostasis results in oxidative stress, causing severe damage to cellular components (Sies, 1991). An excessive level of ROS leads to a variety of pathological conditions, including lipid peroxidation, apoptosis and tissue damage. Lipid peroxidation can compromise the integrity of cell membranes and increase cell membrane fluidity which adversely affects immune responses and lipid peroxidation inactivate membrane bound receptors and enzymes (Bendich, 1993). Research has shown that maintenance of the redox balance is important to the health of broiler chicks due to their high body lipid content which is the cause of lipid peroxidation (Zhang *et al.*, 2008). Against to lipid peroxidation and oxidative damage, all living organisms have evolved an interdependent antioxidant system that includes enzymatic and non-enzymatic components in the liver (Ohtsuka *et al.*,1998) and erythrocytes (Orzechhoswki *et al.*, 2000). The major antioxidant enzymes are Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx). Reduced Glutathione (GSH), melatonin, Ceruloplasmin (Cp) and albumin are non-enzymatic antioxidants (Halliwell and Gutteridge, 1986). Copper, zinc and manganese are essential trace minerals that are important co-factors of SOD and CAT and participate in the structure of ceruloplasmin (Haddad *et al.*, 2008).

Studies related to dietary supplementation with lower levels of organically complex minerals on hematological and biochemical parameters and antioxidant defense systems in poultry are quite inadequate. Previous studies of dietary supplementation of individual organically complexed minerals have found that a zinc and copper combination is involved in the formation of Cu/Zn-SOD and the level of this enzyme is elevated when these minerals added to the diet (Jia-Perng *et al.*, 2001; Sahin *et al.*, 2005). A study conducted to compare the effects of dietary supplementation of organically complex Zn-proteinate (10 mg/kg), Cu-proteinate (50 mg/kg) and Mn-proteinate (5 mg/kg) with oxide forms of Zn (50 mg/kg), Cu (200 mg/kg) and Mn (30 mg/kg) on plazma Malondialdehyde (MDA), nitric oxide (NO), glutation (GSH) and antioxidant activity in laying hens reported that both organic and inorganic sources of Zn and Mn decreased the oxidative stress in laying hens, whereas especially organic copper increased this effect (Bulbul *et al.*, 2008). In a recent study with 200 Ross 308 broiler chickens, the effect of using of organically complex minerals at 1/3 (L1), 2/3 (L2) and 3/3 (L3) proportions of Cu, Zn and Mn on antioxidant defense systems showed that plasma Zn levels significantly increased when plasma Cu levels significantly decreased ($p<0.05$) in chickens fed at the 2/3 and 3/3 levels (Saripinar *et al.*, 2010a). The plasma malondialdehyde (MDA) level of experimental chickens was decreased in groups receiving levels of organic Cu, Zn and Mn in comparison to those fed inorganic forms ($p<0.01$). Erythrocyte Superoxide Dismutase (SOD) activity was higher in all groups receiving organic mineral supplements in comparison to those fed inorganic forms ($p<0.01$). No differences were observed on either the erythrocyte catalase (CAT) activity, the plasma ceruloplasmin (Cp) levels, the liver MDA levels, or the liver CAT and SOD activities in any of the groups that received the organic supplements of Cu, Zn and Mn. It was concluded that supplementation of lower levels of organically complex copper, zinc and manganese instead of their inorganic forms in diets had no negative effects on the antioxidant defense systems in broiler chickens. The same researchers also speculated that supplementation levels of organic copper, zinc and manganese in broiler feeds can be reduced at 1/3 levels without any negative impact effect on hematological and biochemical blood parameters. Results of this study indicated that the plasma Zn levels significantly increased as the serum Cu levels significantly decreased ($P<0.05$) in chickens fed at 2/3 and 3/3 levels of organically complexed minerals. Hemoglobi concentrations and packed cell volume were significantly higher in chickens fed at 1/3 level of organically complexed minerals. Total leukocyte count and peripheral blood leukocyte type were in the normal range in both the control and organic mineral supplemented groups. As the High Density Lipoprotein (HDL) cholesterol level increased, Low Density Lipoprotein (LDL) cholesterol and total cholesterol levels decreased in chickens fed organically complex minerals (Saripinar *et al.*, 2010b). On the other hand, it has been widely established that a deficient, as well as an excessive, intake of trace elements could cause oxidative stress (e.g., lipid peroxidation) in poultry (Surai, 2002) but the negative consequences of Lipid Peroxidation (LPO) can be overcome by adequate dietary supplementation of antioxidants. Cu, Zn, Fe, Mn and Se are considered antioxidant nutrients-Fe as part of catalase; Cu, Zn and Mn as part of superoxide dismutase; and Se as part of glutathione peroxidase and all play interdependent roles in the antioxidant protection of organisms through their termination of LPO reactions. By contrast, the same elements, as transitional metal ions, have the ability to generate highly reactive hydroxyl radicals through their interactions with reactive oxygen species (Aruoma *et al.*, 1991). Poultry meat quality and colour are easily influenced by several dietary and environmental variables. As colour references vary from country to country, the colour of poultry meat that is attractive to consumers and how to develop said colours are obviously important factors to be considered by the poultry industry. Future research will thus focus on the effects of substitute and reduced mineral diets on meat quality properties.

As far as we are aware, there has been no directly relevant study published on the effects of organically complexed minerals on the colour characteristics of broiler meat. In a study conducted directly related to the effects of organically-complexed minerals on meat quality of chickens (Aksu *et al.*, 2011b), it was reported that concentration of organically complexed mineral in the diet had no statistically significant effect on pH values of breast fillets. Treatments significantly affected the Thiobarbituric Acid Reactive Substances (TBARS) values. Lightness (L* value) of the fillet from broilers fed the diet containing inorganic minerals was significantly lower than that for broilers fed on the diets containing organically complexed minerals. The redness (a* value) and Chroma (C* value) significantly decreased while Hues (H* value) were higher in the fillet from broilers fed organically complexed minerals compared with those fed inorganic minerals. The yellowness (b* value) was not changed by dietary treatment.

Conclusion: Use of organic feed ingredients and dietary organically complex minerals in animal diets is becoming more important with the growing interest in natural animal products. Therefore, organically complex mineral will have an important place in the future to improve animal performance, protect animal health and environment and fruitful animal production. Consequently, organically complexed minerals will continue to be an important issue for current and intensive research for animal nutritionists. Future research on adding these minerals to different basal diets will be helpful in understanding both the mechanisms and impacts of organically complexed trace minerals on meat quality

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