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## Chelated Minerals for Poultry

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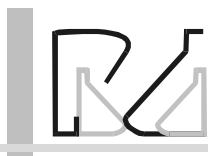
### ABSTRACT

Organic minerals have been subject of an increasing number of investigations recently. These compounds can be considered the most significant event regarding commercial forms of minerals targeting animal supplementation in the last decades. Minerals, especially metals, are usually supplemented in poultry feeds using cheap saline sources and have never required a lot of attention in terms of quality. On the other hand, definitions of organic minerals are very broad and frequently lead to confusion when decision-making becomes necessary. Organic minerals include any mineral bound to organic compounds, regardless of the type of existing bond between mineral and organic molecules. Proteins and carbohydrates are the most frequent candidates in organic mineral combinations. Organic fraction size and bond type are not limitations in organic mineral definition; however, essential metals (Cu, Fe, Zn, and Mn) can form coordinated bonds, which are stable in intestinal lumen. Metals bound to organic ligands by coordinated bonds can dissociate within animal metabolism whereas real covalent bonds cannot. Chelated minerals are molecules that have a metal bound to an organic ligand through coordinated bonds; but many organic minerals are not chelates or are not even bound through coordinated bonds. Utilization of organic minerals is largely dependent on the ligand; therefore, amino acids and other small molecules with facilitated access to the enterocyte are supposed to be better utilized by animals. Organic minerals with ligands presenting long chains may require digestion prior to absorption. After absorption, organic minerals may present physiological effects, which improve specific metabolic responses, such as the immune response. Many studies have demonstrated the benefits of metal-amino acid chelates on animal metabolism, but the detection positive effects on live performance is less consistent.

### INTRODUCTION

Inorganic and organic mineral forms are found in animal tissues, both in variable concentrations. Inorganic elements are found in the ash left after combustion of live tissues mostly as oxides, carbonates, and sulfates (Underwood e Suttle, 1999). Out of the 109 known elements, 26 are considered essential for animals (Underwood, 1977). From these, 11 are macro elements (carbon, hydrogen, oxygen, nitrogen, sulfur, calcium, phosphorus, potassium, sodium, chlorine, and magnesium) and 15 are microelements (iron, zinc, copper, manganese, nickel, cobalt, molybdenum, selenium, chromium, iodine, fluorine, tin, silicon, vanadium, and arsenic).

The presence of a mineral in animal tissues does not imply its essentiality. Minerals can be natural contaminants and, in this case, they follow a similar distribution as that found in the surrounding



environment. Essential elements, however, exist in a normal and expected symmetry, and their deficiency results in impairment or loss of organic functions (Uderwood, 1977). The presence of essential elements in animal tissues follows cell function and, therefore, they have typical concentrations for each organ. As occurs with any other nutrients, minerals can cause toxicity if ingested in high levels or for long periods.

Minerals are nutrients involved in a high number of metabolic pathways, and it is largely accepted that many of their functions are still not well understood. Macroelements are expressed as a percentage of feeds or tissues, and are mostly involved in structural functions. Microelements, or trace minerals, are more difficult to be assessed through analysis due to their very low concentrations in animal tissues and to the limitations in equipment and techniques. In addition to being present in very low concentrations, microelements share very few common functions. However, it is possible to generalize their primary function as catalysts of enzymatic cell systems with a very broad range of functions. In these systems, minerals are frequently associated with proteins in a fixed proportion as metalloenzymes in which interactions between minerals and proteins improve catalytic activities but also reduce protein turnover.

Mineral utilization by animals primarily depends of their absorption from the ingested feed. In the feed, minerals are found in a wide range of chemical forms. They are found as organic molecules or as part of salts of varying solubility. Supplementary sources of organic minerals in the market are still recent; however, they brought attention to a group of nutrients that have been set aside for many years as less important. The interest in organic forms include differences in availability, but also it is also related to possible improvements of their specific actions at cell level.

Organic microelements have been primarily defined by AAFCO (2000). Some definitions are: Specific Metal Amino Acid Complex, resulting from complexing a soluble metal salt with a specific amino acid; Metal Amino Acid Complex, resulting from complexing a soluble metal salt with amino acids; Metal Amino Acid Chelate, resulting from the reaction of a metal ion from a soluble salt with amino acids in a molar ratio of 1 mol of the metal to 1 – 3 (preferably 2) moles of amino acids, which form coordinated bonds (molecular weight of the hydrolyzed chelate should exceed 800 Daltons); Proteinate Metal, resulting from the chelation of a soluble salt with amino acids and/or partially solubilized protein; Polysaccharide Metal Complex, resulting from

complexing a soluble salt with a solution of polysaccharides declared as integral part of an specific complex.

The word chelate derives from the Greek “chele”, which means tweezers or claw. The reasons for this definition become clear when the structure of chelates is examined. They are the result of electron sharing between a metal and a ligand. A ligand is usually an anion or a molecule, which has an atom or a pair of electrons with available valences. Common ligands contain oxygen, nitrogen, sulfur, halogens, or a combination of these due to their electronic structure. Chelated minerals have non-metallic ligands, and are therefore organic. Atoms, which are able to donate their electrons, are called donor atoms. Ligands with only one donor atom are called monodentate, whereas those with two or more are called polydentate. Only polydentate are able to form chelates, since they can bind a metal within their electronic dents or claws.

Di- or trivalent metals are well-adapted to form coordinated bonds, which are characteristic of chelates. A coordinated bond, also known as complex bond, consists of a metal and a ligand aligned in such a way that the available electrons from the donor atoms are very close to those from the metal. Mineral chelation is very important in the biological systems. Most enzymes require a chelated metal in their structures to become effective. Vitamins, such as vitamin B12 (cyanocobalamin), have a metal (in this case Co) complexed to a tetradent porphyrin group, nitrogen, and a pseudonucleotide. Porphyrin is also important for the chelation of iron on hemoglobin. Other groups are important in human and animal health due to their good chelating abilities. This is the case of EDTA (ethylenediaminetetracetic acid), which is a hexadent ligand used for the removal of heavy metals from animal tissues. Independently of their international classification, there are several characteristics that are important for the effectiveness of mineral chelates in the animal body. There are many research studies on chelates that have amino acids as ligands, whereas less is known on chelates using polysaccharides or other ligands with large molecular weights.

### Mineral utilization by poultry

Trace minerals have traditionally been supplemented in broiler feeds by the use of saline sources, which cost is usually very low. Saline sources are not frequently subjected to technical criticism as to their quality, especially because they are required in very low amounts and are found as components of other feed



ingredients. Variable availability, as well as the presence of contaminants, are important considerations when supplementing trace minerals. For instance, zinc oxide and copper sulfate are sources commonly utilized in animal feeding, but as they are often derived from residues of the steel industry, they can potentially carry high levels of contaminants, such as cadmium, fluorine, and lead to the feed. Market regulations, such as those issued by the European Union (CEC, 1999), have added new concerns into the meat production business with extended limitations in terms of heavy metals and other contaminants in animal feeds.

The determination of trace mineral requirements has also been a secondary concern in poultry nutrition as compared to other nutrients. Minerals have, however, a series of limitations in terms of utilization by animals. Source solubility and previous nutritional status of the animals are extremely important factors when measuring mineral requirements. Investigations on mineral requirements should take in account their physiological functions within the biological systems where they are expected to work. Those functions can be generally divided in construction and maintenance of hard and soft tissues, as well as in the regulation of biological processes (Underwood, 1977). The greatest contributions of macro minerals, such as calcium, phosphorus, and magnesium, are expected in hardening bones and teeth by the formation of complex inorganic matrices. Therefore, ash percentage in bones is used as corresponding measurement of Ca and P requirement levels. Soft tissues, however, also have large contributions from minerals, such as zinc, in addition to phosphorus, magnesium and sulfur. In the regulation of biological processes, essential minerals function as catalysts in enzymatic and hormonal systems as integral components of metalloenzymes, but they can also activate metalloenzymes.

Organic equilibrium needs also to be taken in consideration in each substances formed from minerals, especially trace minerals. These measurements are usually more difficult to be made as compared to live performance parameters and must involve metalloenzyme levels and other organic substances, which are usually in the form of chelates.

Mineral absorption can suffer many interferences, such as mutual antagonisms, which potentially reduce absorption and metabolism rates of some minerals. These interrelationships are traditionally expressed as shown in Figure 1, which simplifies how the interactions among minerals can potentially affect each other. Insoluble precipitates can be formed through the

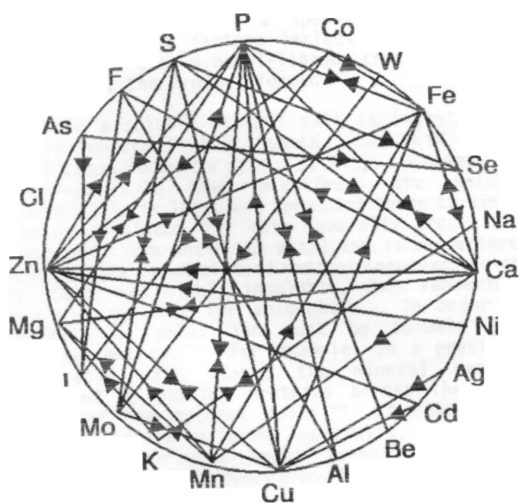
competition with organic and inorganic ligands. Examples are phytic acid and phosphates. Both can reduce or completely inhibit mineral availability (Cabell and Earle, 1965; Vohra *et al.*, 1965). Phytates reduce zinc uptake (Hempe & Cousins, 1989) and calcium impairs the absorption of copper and zinc (Lowel *et al.*, 1994; Wedekind *et al.*, 1994). Copper and molybdenum are strongly antagonistic, whereas manganese and iron compete for similar absorption mechanisms. Manganese supplementation to solution containing iron depresses iron intake (Sandstrom, 1992). It was demonstrated that phytase supplementation improves trace mineral availability in monogastric animals; however, action of this enzyme can be limited by dietary calcium level (Angel *et al.*, 2002).

Ashmead (1993a) summarized six groups of interactions that actively interfere with mineral absorption. Firstly, mineral absorption requires previous solubilization of the original mineral source in the intestinal lumen. Ionized metals can be further transported through the cell membrane using protein carriers. This process is pH-dependent, and therefore, the acidic environment in the proventriculus improves solubilization, whereas the neutral or alkaline pH of the small intestine reduces it. Solubilized metals in the gastric environment can potentially form insoluble precipitates in their course through the small and large intestine. The presence of active ligands, such as phytic acid, can intensify the formation of precipitates. This situation is significant in high phytate diets, such as those with high levels soybean meal or rice bran, and can strongly affect minerals availability. Phytate content is very variable in soybeans and can reach values as high as 3% in soybean meals (Cheryan, 1980; Mason *et al.*, 1992).

Competition for the same or similar carriers is a major interference source when metals are transferred from the lumen into the enterocyte (Hill & Matrone, 1970). Carriers are small proteins, which bear high capacity of chelating free cations present in the intestinal solution when proceeding to transport. Therefore, physical-chemical competition between cations can potentially involve micro or macro minerals or both (Starcher, 1969). The competition between iron and copper is typical: they share at least two membrane proteins, transferrin and metallothionein. Excessive copper bound to those protein may lead to iron deficiency (El Shobaki & Rummer, 1979).

Heavy metals play a different role in antagonizing mineral absorption. Lead, for instances, inhibits the

enzymatic synthesis of carrier proteins, e.g., the porphyrin fraction of hemoglobin, inducing anemia (Hoffbrand & Konopka, 1977). In other situations, the replacement of one metal by another in metalloenzyme molecule modifies their efficiency (Ashmead, 1993a). After being absorbed by the enterocyte, some metals return to the intestinal lumen by excretion or mucosal sloughing and resume the competition for carrier proteins. One last possibility of interference in mineral absorption is related to the impact of reduced uptake of one specific metal on the cascade reactions involved in the uptake of other minerals (Ashmead, 1993a).



**Figure 1** - Potential interrelationships between minerals in intestinal lumen and metabolism.

With a few exceptions, mineral absorption depends on the capacity of the elements to bind to transport proteins present in the enterocyte membrane. Bound minerals are then transported into the cell cytoplasm through passive diffusion or active transport. Mineral absorption can occur in any region of the intestines, but metals are usually absorbed in the duodenum provided ions are still soluble after gastric treatment (Ashmead, 1993b). As stated before, any factor antagonizing the metal capacity to bind to the ligand will reduce its absorption. Each mineral absorption rate varies with its chemical source; therefore, it is not possible to establish a single absorption rate that would fit all metals (Ashmead, 1985). Chemical reactions between the metal present in the diet and any other dietary component are directly related with their degree of absorption. Similarly, there is more than one single mechanism available for the absorption of each metal, which also depend on its chemical form at the moment of contact with the intestinal mucosa.

Metal amino acid chelates are chemically inert due to the covalent and ionic bonds between the mineral and the ligand, and therefore are not affected by factors that lead to precipitation, as it happens to minerals ionized after salt solubilization (Ashmead, 1993b). Due to their stability and small size, most chelated minerals are not altered during their passage through the digestive tract, and are completely absorbed with no break down of their amino acids. Some minerals can be chelated to two or three amino acids and absorbed as di or tripeptides. After absorption, the final separation of the metal from the amino acid is expected only when the final physiological site is reached. Therefore, chelated minerals are absorbed and transported as integral part of an organic molecule until effectively used (Ashmead, 1993b).

Nutritional body balance of each specific mineral can directly affect the absorption of this very same mineral (Patton, 1997). Differences between zinc bioavailability may not be evident until its level in the diet becomes limiting (Rojas *et al.*, 1994). Under normal dietary supply, the passage of zinc through the enterocyte membrane depends on a carrier that is associated with it; however, rate of absorption is highly increased under zinc depletion (Menard & Cousins, 1983). Zinc previous nutritional status influences the genetic transcription of metallothionein (Sullivan & Cousins, 1997).

### Performance of poultry fed chelated minerals

Mineral chelates have been the subject of a growing number of investigations in the past few years. Results demonstrate a clear trend for a better utilization and higher bioavailability for this type of mineral supplements. Differences between traditional trace mineral sources are due to the salt source and, at least for zinc and copper, oxides have minimal contribution for poultry and swine (Baker *et al.*, 1991; Cromwell *et al.*, 1989; Han & Baker, 1993). Therefore, evaluations aiming at comparing metal chelates to salts should target those that present higher availability, such as sulfates.

Other dietary constituents play important roles when comparisons between chelates and salts are made. For instance, it is well known that phytate seizes positive charged metals and, consequently high phytate diets tend to confound the effects of the salt sources being tested. Zinc-methionine supplemented to corn-soybean meal diets presented 206% bioavailability, but only 117% when included in a purified diet without



phytate (Wedekind *et al.*, 1992). In all-vegetable feeds, which bear a high proportion of phytate, the advantage of mineral chelates tends to be easier observed as compared to diets with animal by-products inclusion.

Another confounding effect that appears when metal salt sources are compared result from the simultaneous interferences of metals with similar charge. Broilers receiving excess inorganic zinc accumulate a higher proportion of zinc in their tissues, and seem to present reduced iron turnover, as well as lower iron and copper concentrations in the liver and pancreas, and lower iron in tibia (Stahl *et al.*, 1989).

Patton (1997) suggested that the use of mineral chelates should made after further observations of their actions on animal metabolism. The mere observation of their digestibility and absorption may not allow a correct understanding of their influence on metabolism. In fact, the determination of nutrient requirements is directly related to the type of response. Weight gain and feed conversion are traditionally measured when nutrient requirements are studied; however, micronutrient evaluations should also take in consideration cell involvement for fuller understanding of their effects.

Mineral chelates are currently more expensive than the traditional mineral supplements. However, there are indications that, at least in some situations, chelated minerals can achieve biological objectives better than inorganic sources (Patton, 1997). For instance, it is known that di- and tri-peptides are absorbed faster than free amino acids. Therefore, it could be considered that amino acids and small peptides are completely absorbed, and this is likely to have an effect on their physiological function.

Bone matrix is composed mostly of collagen, and skeletal mineralization is dependent on adequate collagen growth and final quality. Zinc, manganese, and copper, as well as vitamins (e.g., ascorbic acid), are directly related with bone matrix formation. Turkey diets supplemented with zinc and manganese-methionine improved feed conversion, and also reduced mortality and leg abnormalities (Ferket *et al.*, 1992). These diets had trace minerals in levels considered adequate (80 ppm zinc and 120 ppm manganese as sulfates), but there were improvements when 20 and 40 ppm zinc and manganese-methionine were supplemented.

There are few long-term investigations with organic mineral sources in literature, especially targeting broiler breeders. In broiler breeder studies conducted for short

periods (21 to 43 weeks of age), Barber *et al.* (2002) did not find any response when 150 ppm zinc sulfate was compared to the simultaneous use of zinc sulfate and zinc-amino acid. Hudson *et al.* (2004a) simultaneously supplemented zinc-amino acid and zinc sulfate, and observed eggshell improvements that led to the production of 3.6 extra chicks at the end of the laying period. Differences in total hatching period and chick weight at hatching were not observed (Hudson *et al.*, 2004b). Reduced early broiler mortality was found when zinc and manganese-amino acid were supplemented to broiler breeders throughout the laying period (Virden *et al.*, 2003). Recently, Tako *et al.* (2004) observed that injecting zinc-methionine *in ovo* at 17 days of incubation led to improvements in the morphological development and intestinal mucosa enzyme expression of hatching chicks.

Zinc benefits of on animal immunity were shown in the past (Chevalier *et al.*, 1996). Zinc-methionine usually has higher bioavailability than inorganic sources, and can be absorbed in its intact form, therefore potentially altering zinc balance in metabolism. Independently of the involved mechanisms, improvements were shown in some cell functions related to disease resistance when zinc-methionine was supplemented in broiler or breeder feeds (Kidd, 1996). Dietary supplementation with zinc and manganese chelates, when inorganic inclusions of these minerals where considered adequate, led to immune improvements in turkeys (Ferket & Qureshi, 1992). Disease resistance and the transference of antibodies to progeny can be improved when broiler breeders are fed zinc-amino acid alone or combined with zinc sulfate (Hudson *et al.*, 2004a). Synergistic effects are also expected when zinc-methionine is fed along with vitamin E. Cells deficient in zinc present better membrane structure when vitamin E levels higher than the traditionally used are supplemented to broilers (Bettger *et al.*, 1980). Protection against peroxidation and as well as better cell membrane integrity are the proposed modes of action of zinc during animal immune response. Skin scratches and other skin damages are frequent in broilers. *Escherichia coli* often invades the damaged skin tissue, leading to the development of cellulitis and to condemnation in processing plants. Mackling *et al.* (2000) observed improvements in the response to cellulitis-infected birds when higher vitamin E levels were added to the feed. Downs *et al.* (2000) found a synergistic effect when supplementing 48 IU vitamin E combined with 40 ppm zinc-amino acid in the same



feed, with reductions in cellulitis incidence and severity in broilers.

Studies with swine tend to show similar results as those found in broilers. Coffey *et al.* (1994) observed improvements in piglet performance when copper-lysine was used as compared with copper sulfate. On the other hand, Apgar *et al.* (1994) did not verify any response of this combination in growth. Hill *et al.* (1986) found higher feed intake with the use of zinc-methionine, but feed conversion was not influenced when that chelate was combined with copper sulfate. Higher nominal zinc absorption was found by Hill *et al.* (1987) for zinc-methionine as compared to zinc chloride, suggesting differences in transport rates between these two sources.

## CONCLUSIONS

Chelated minerals are increasingly used in animal nutrition. Growing restrictions to the use of antibiotics create opportunities to more public-friendly nutritional additives that can also improve performance, but using a different pathway. Mineral chelates have a different involvement in metabolism as compared to inorganic sources, promoting, among other benefits, better mineral uptake and enhancement of the immune response. These benefits of supplemental mineral chelates may resemble those already obtained with other organic minerals present in natural sources, such as iron in hemoglobin and cobalt in vitamin B12. The formulation of diets targeting precision nutrition will eventually demand the extensive use of mineral chelates, which could be added to the feeds to promote specific animals responses.

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