

Chelates, complexes, simple salts and oxides

The many faces of mineral supplementation

By: Giovanni Predieri¹, Roberto Barea², and Silvia Peris²

¹ Department of Chemistry, Life Science and Environmental Sustainability, University of Parma (Italy)

² Technical Services, Novus Europe NV (Belgium)

I. Introduction to mineral supplementation

Specific amounts of main-group and transition metals such as calcium, iron, zinc, and other trace elements are essential for the normal growth of poultry and livestock, which is a major source of proteins for humans. Metals in biological systems (called *minerals*) function in a number of different ways: group 1 and 2 minerals (i.e., calcium, sodium, magnesium, potassium) operate as structural elements and in the maintenance of charge and osmotic balance. Transition metal ions that exist in a unique oxidation state, such as zinc (II), function as structural elements in enzymatic proteins - triggers for protein activity. Transition metals exhibiting multiple oxidation states behave as electron carriers, like iron and copper ions in cytochrome and plastocyanin respectively (Williams and Frausto da Silva, 1996). All of these metals are supplied as dietary minerals to poultry and livestock from external sources, i.e., in properly assorted animal feedstuffs containing various categories of nutrients for promoting faster growth, improving fertility, and offering greater resistance to diseases (Underwood, 1999; McDowell, 2003).

Until the end of the last century, minerals were supplemented in the salt forms of chlorides, sulfates, nitrates, phosphates, and carbonates, or in the form of oxides. However, the absorption rate of these inorganic minerals in a living body is so limited that significant amounts of unabsorbed minerals are excreted through feces, damaging the soil – and thereby the environment - through contamination. The first proposals to use mineral chelates in place of inorganic salts to improve mineral absorption by living organisms were received with skepticism, as their use was estimated to be economically disadvantageous (Underwood, 1999).

II. The rise of the “chelates era”

During the first decade of the new century, extensive *in vivo* experimental work showed that the bioavailability of inorganic minerals could be greatly improved by supplementing them in the “organic” form of chelate complexes. It was demonstrated that the use of chelated minerals led to significant improvements in growth, reproductive capacity, and general disease resistance when compared to simple inorganic salts (Wedekind et al., 1992; Spears, 1996; Jondreville and Revy, 2003; Ballarini and

Predieri, 2007; Yi et al., 2007; Suksomba et al., 2011; Liu et al., 2014). These beneficial effects are attributed to: (a) absence of free active metal cations (e.g., Fe^{2+} , Mn^{2+} , Cu^{2+}) responsible for feed deterioration through the formation of free radicals; (b) protection of minerals from phytates and oxalates sequestration and from competition with other minerals in the feed which results in improved mineral bioavailability; and (c) less mineral excretion into the environment, which makes animal production more environmentally sustainable.

Owing to these enhancements in environmental management, feed quality and nutritional properties of the diet, previous assumptions were reconsidered and suddenly products were marketed claiming to contain effective mineral chelates. The classification of *chelates* according to regional regulations for placing feed additives on the market normally are not totally satisfactory since they do not take into account the real nature of the active chemical species formulated as *chelates*. In fact, not all amino acid + mineral aggregations lead to chelated compounds (Ballarini and Predieri, 2007).

Hence, the “organic” forms of minerals should be rigorously classified based on the nature of the chemical bond connecting metal cations to organic molecules. This leads to a net distinction between general mineral **complexes** and **genuine mineral chelates** and this distinction can be adequately appreciated by recalling the basic principles of chemical bonds.

III. The nature of the chemical bond

The **chemical bond** is the mutual attraction between atoms that enables the formation of chemical compounds as **molecules** or crystals. All chemical bonds are due to electrons interacting simultaneously with atoms. Atoms will form a bond if their orbitals become lower in energy when they interact with each other. Depending on the nature of the atomic interactions, three categories of chemical bonds exist in nature: (i) *ionic bond*, (ii) *covalent bond*, (iii) *metallic bond* (*metallic bonds not described in this context*), corresponding to different types of substances with different physicochemical properties. The type of bond between two atoms is defined by their electronic configuration and **electronegativity**, which is a measure of an atom's ability to attract shared electrons to itself.

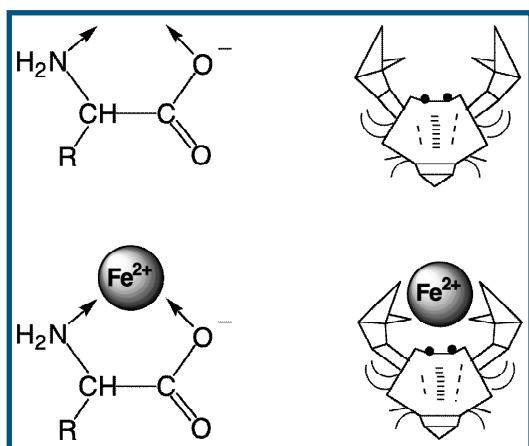
An **ionic bond** can be formed after two or more atoms gain electrons at the expense of other atoms, forming anions and cations respectively. These ions of opposite charge will attract one another, thus creating an ionic bond. This type of bond occurs between metals (Na, Mg, K, Zn, Ca, Mn, Cu, Zn, etc.) forming **cations**, and non-metals (N, O, S, etc.) forming **anions**.

A **covalent bond** is a form of chemical bonding characterized by the sharing of one or more pairs of electrons between atoms in order to produce a mutual attraction, which holds the atoms together in a molecule. Molecules are the exclusive products of covalent interactions between atoms. Unlike ionic bonds, covalent bonds between atoms take place in well-defined directions because shared electrons lie in external non-spherical, molecular orbitals. This is the origin of the **molecular shapes** (like that of the water molecule, H₂O, in that it is angular, not linear). A special type of covalent bond is the **dative covalent bond**, also known as a coordinate covalent bond, which occurs when one atom, called *donor*, gives both of the electrons to the other atom, called *acceptor*. As an example, ammonium cations form as a coordinate covalent bond between ammonia nitrogen (donor) and hydrogen ion (acceptor).

IV. Chelates are special complexes

A coordinate bond can also be found in **coordination complexes** involving metal ions (mineral complexes). In such complexes, substances donate their free pair of electrons to the metal ion, which accepts the electrons. In this case, the electron donors are called ligands. Molecules containing only one donor atom (N, O, S, etc.) are called monodentate ligands, those containing two or more donor atoms are called respectively bi- or polydentate ligands, which become **chelated (chele = pincers, crab claws)** if the arrangement of donor atoms around the mineral forms a **chelation ring**. The most stable rings are those with five atoms, which are formed by amino acids (AAs) or hydroxy acids and the mineral, (**Figure 1**). The resulting complexes are **genuine chelates**.

Figure 1. (top) Sketches of an amino acid (AA) acting as a chelated ligand (crab claws); (bottom) an iron (II) cation chelated by a deprotonated AA (the second AA anion of the neutral bis-chelate species is omitted for simplicity).



However, **AAs do not always behave as chelating ligands. It is only in** well-defined circumstances that this occurs, for example: (i) AAs are not involved in peptidic bonds (e.g., peptides or proteins) that prevent the formation of adequate chelation rings; (ii) no presence of certain anions (like Cl⁻ or SO₄²⁻) that compete with AAs for mineral complexation; (iii) pH values are adequately high to allow the deprotonation of the AAs. In this regard, it is noteworthy to remark that mineral sulfates/glycine 1:1 compounds, often called “mono-chelates,” are actually simple complexes wherein chelation cannot take place (see item **V. b** below).

V. Classification of “organic” minerals containing complexes or chelates

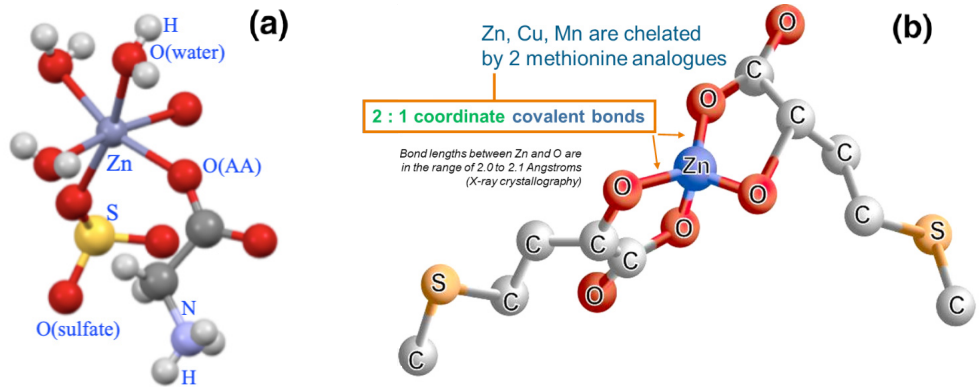
From the chemical point of view, four main classes of products embrace almost all commercialized “organic” mineral supplements containing complexes or chelates in the animal feed industry:

- (a) mixtures of mineral complexes with a pool of AAs and oligopeptides derived from protein lysates (e.g., from soybean meal), commercially defined as **proteinates**.
- (b) mineral complexes of a single AA with a well-defined formula (e. g. Zn(HGly)SO₄), called improperly **glycinates** or “**mono-chelates**.”
- (c) mineral chelates of a single AA (e. g. lysine or glycine) in its anionic form, with well-defined formula (e. g. Zn(Gly)₂), **AA bis-chelates**.
- (d) mineral chelates of a single α-hydroxyacid (methionine hydroxy analogue, HMTBa) of well-defined formula (e. g. Zn(HMTB)₂), **HMTBa bis-chelates**.

Class (a) contains mainly complexes since oligopeptides are not able to form stable mineral chelates owing to the presence of peptide bonds. They can only interact with mineral cations through terminal amino or carboxylate groups forming simple complexes. **Class (b)** refers to well-defined chemical species such as Zn(HGly)SO₄, whose crystal structure depicted in **Figure 2a** clearly shows the **non-chelating** behaviour of glycine bound to Zn only through the carboxylate oxygen. **Class (c)** contains the true chelates of deprotonated AAs, such as glycine in the form of bis-glycinates; they are genuine chelates whose stability decreases by lowering pH. Finally, **Class (d)** includes the true chelates of the deprotonated α-hydroxyacid HMTBa, which resist the low pH value found in the first digestive tract compartments better than AAs. This is due to the stronger acid character of HMTBa, whose anion is less prone to be protonated in acid media (Predieri et al., 2003; 2005) (**Figure 2b**).

In classes (b-d), Fourier-transform infrared spectroscopy (FTIR) allows univocal identification of the species, being able to distinguish a simple complex (b) from a chelate (c and d) (Predieri et al., 2009) and to quantify these species directly on the solid feedstuff. FTIR spectroscopy supplies rapid and non-destructive analytical methods with minimal or no sample preparation.

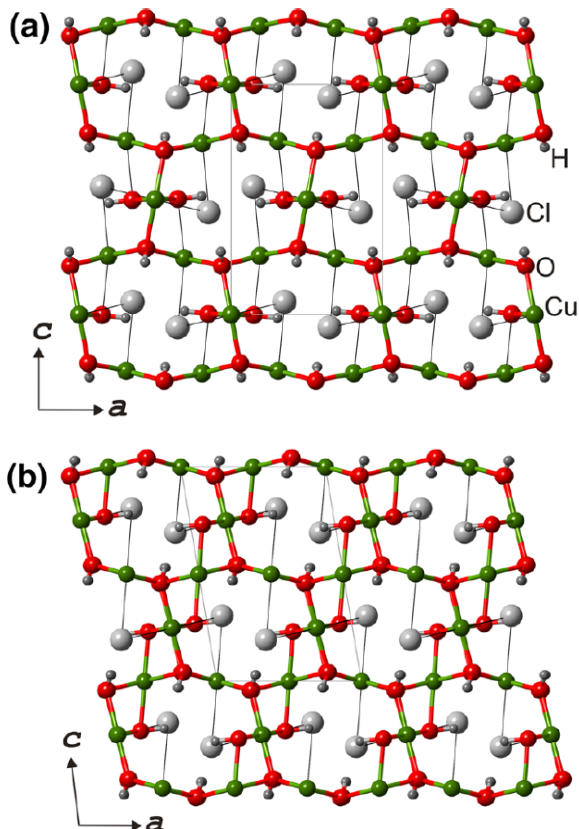
Figure 2. Molecular structures in the solid state of (a) zinc sulfate glycine complex $Zn(HGly)(H_2O)_3SO_4$ (from Cambridge Structural Database) emphasizing the non-chelating nature of glycine in this compound; (b) $Zn(HMTBa)_2 \cdot 2H_2O$ (coordinated water molecules omitted) showing the chelating behaviour of two HMTBa anions around zinc.



Other “inorganic” mineral supplements

Recently, two already known hydroxy-chlorides of zinc and copper, namely copper hydroxychloride or TBCC (Tribasic copper chloride), $Cu_2(OH)_3Cl$ (Figure 3), and zinc hydroxychloride, $Zn_5(OH)_8Cl_2$ have been re-proposed for supplementation in animal feed since they fit the request of feed stability, as offered by the chelates. They are non-hygroscopic and essentially water-insoluble crystalline solids, providing improved feed stability and less oxidative destruction of vitamins and other essential feed ingredients when compared to the corresponding simple salts (Cromwell et al., 1998). However, copper

Figure 3. Crystal structures of two polymorphs of copper hydroxychloride $Cu_2(OH)_3Cl$: atacamite (a) and clinoatacamite (b). The pictures show the two ionic inorganic networks of the Cu^{2+} cations surrounded by hydroxide (OH) and chlorides (Cl) anions. (Krivovicev et al., 2017)

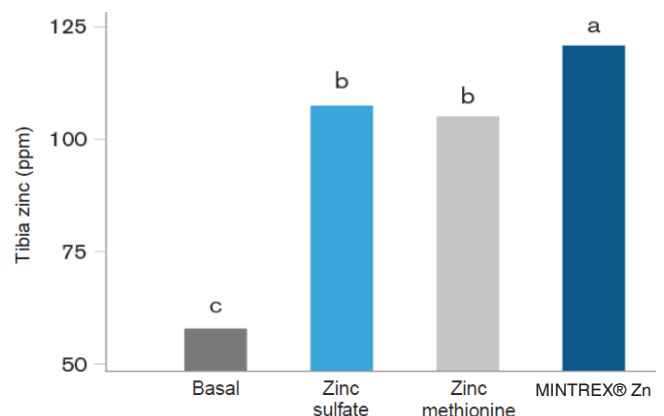


and zinc hydroxychlorides are reticulated solids, basic in nature, with an important ionic characteristic. This makes them subject to acid attack with complete solubilization of the solid structure resulting in the Cu^{2+} or Zn^{2+} ions becoming unprotected in the solution. This occurs in the gastro-intestinal compartments of the animals. In practice, after solubilization, the chemical behaviour of copper and zinc hydroxychlorides are the same as copper and zinc simple salts.

VI. Trace mineral bioavailability in livestock animals

The bioavailability of a mineral is generally defined as the proportion of the total mineral in feed that is utilized for normal body functions. The bioavailability of a given mineral source (i.e., an **organic trace mineral**, OTM) is usually measured relative to a “standard” source (i.e., an **inorganic trace mineral**, ITM). Several attempts have been made to define simple physical characteristics of minerals that can determine their bioavailability. One example is the link of a mineral’s solubility in water or buffered solution with its bioavailability. However, ITM salts are highly soluble but not necessarily highly bioavailable (Miles et al., 1998; Guo et al., 2001).

Figure 4. Relative bioavailability of zinc sources related to tibia zinc content in broilers



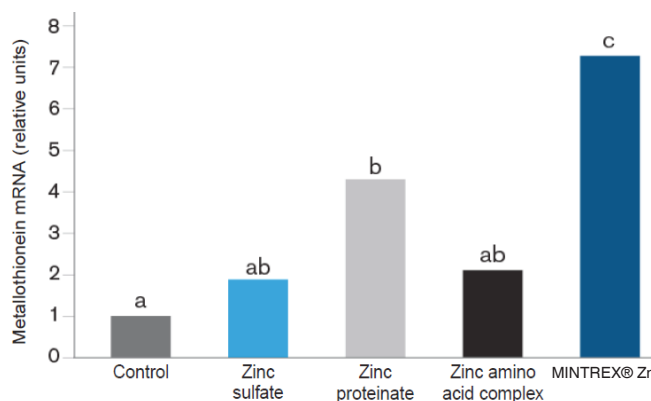
Measuring the mineral retention in select tissues has been the most common output in relative bioavailability experiments (Underwood and Suttle, 1999). Figure 4 shows the result of an experiment that compared the relative bioavailability of zinc sources by measuring tibia zinc content in broilers (Richards, 2010). It shows that while all

supplemented treatments (added at 40 ppm of zinc) increased tibia zinc content, the increase due to zinc chelated with α -hydroxyacid HMTBa ($\text{Zn}(\text{HMTBa})_2$, MINTREX[®]Zn from Novus International, Inc.) was higher, hence it is more bioavailable than the other two sources.

However, this method of measurement considers only a fraction of the mineral that is absorbed because it may not relate to the total pool of minerals in the animal body. To measure the mineral absorption at the intestinal level, where uptake occurs, provides supplementary insights about the behaviour of different mineral molecules in the organism, and additional reasons why they are more or less bioavailable.

For this reason, the use of biomarkers is definitively useful. This is the case of metallothionein content in the intestinal mucosa, whose expression is regulated by mineral (e.g., Zn or Cu) status and by the amount of mineral absorbed. Figure 5 shows the use of metallothionein mRNA expression in the intestine as an indicator of zinc bioavailability (Richards et al., 2007; Richards et al., 2008). In this experiment, broilers were fed a control diet or diets supplemented with 70 ppm of zinc from different sources. The consistency of results, shown in Figure 4, demonstrated that all OTM sources are more bioavailable than inorganic zinc, but according to this and other experiments, MINTREX[®]Zn was the most available source.

Figure 5. Metallothionein mRNA expression in the intestinal mucosa as an indicator of zinc bioavailability



In conclusion, feeding a chemically well-defined, stable, and highly bioavailable trace mineral source is an important key to optimize the growth potential and wellbeing of poultry and livestock animals. Real chelated minerals have the potential to deliver trace minerals more effectively to the tissues of the animal and, thus, to better support the biochemical functions, performance, and needs of the animal.

References available upon request from:
info.europe@novusint.com

Novus Research Shows Discrepancies in Amino Acid Recommended Requirements under Antibiotic-Free Swine diets

New research from Novus International, Inc. shows that swine producers operating without antibiotics in diets may be using the wrong ratio of amino acids in their feed formulations.

The research, published in Volume 11, Issue 11 of the peer-reviewed journal *Animals*, looks at how additive amounts in antibiotic-free (ABF) diets differ from some recommendations listed in the National Research Council's (NRC) nutrient requirement standards.

Ping Ren, Novus research scientist who led the study, said the study was both timely and necessary due to the shifting trend toward ABF diets for piglets around the world. Starting in 2006, the European Union banned antibiotic use in pig diets. In 2017, producers in the United States were banned from using medically important human antibiotics as growth promoters for monogastric and ruminant animals. There is limited data on how these reductions of antibiotic use impact the performance of feed additives.

"We know without antibiotics, pathogens cause immune stimulation and an increase in amino acid requirements – especially total sulphur amino acids since they are important for antioxidant status and immunity. Pathogen response causes the nutrients in total sulphur amino acids to be diverted from growth, instead being used to optimize immune function," Ren said. "Knowing this we saw a need to fill the information gap and determine the total sulphur amino acid requirement in nursery pigs raised without antibiotics."

The study included two parts. First, it looked at the effect of increasing the standardized ileal digestible (SID) total sulphur amino acid to lysine

(TSAA:Lys) on the growth performance of nursery pigs fed with or without antibiotics. Secondly, researchers set out to determine the optimal SID TSAA:Lys for nursery pigs fed without antibiotics. For the two studies, Novus partnered with swine genetic company Genus PIC North America. In the first study, researchers found that the nursery pigs fed diets without antibiotics had a greater SID TSAA:Lys requirement (> 66%).

Using Novus's MHA[®] feed supplement, a dry, granular source of methionine, in the second study researchers found the optimal SID TSAA:Lys for average daily gain and gain to feed in nursery pigs during the first 21 days post-weaning were 62% and 72%, respectively, indicating that SID TSAA:Lys under an antibiotic-free feeding regime in the early nursery period was 13% to 31% greater than the NRC (2012) recommendation.

Moreover, a SID TSAA:Lys of approximately 58% was required to maximize average daily gain and gain to feed for the late nursery phase, indicating that SID TSAA:Lys in ABF diets in the late nursery period was 5% higher than the NRC (2012) recommendation.

"While these findings aren't exactly surprising, the information is helpful for swine producers. Now that they're aware, those with ABF diets can make adjustments in precision feeding to maximize their profitability," Ren said.

The full article titled, "Optimal Standardized Ileal Digestible Total Sulfur Amino Acids to Lysine Requirements are Increased in Nursery Pigs Raised under Antibiotic-Free Feeding Regime" is available to view for free online.