

Mineral supplementation: chelates, complexes, simple salts and oxides

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.

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Metals in biological systems (also called minerals here) 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

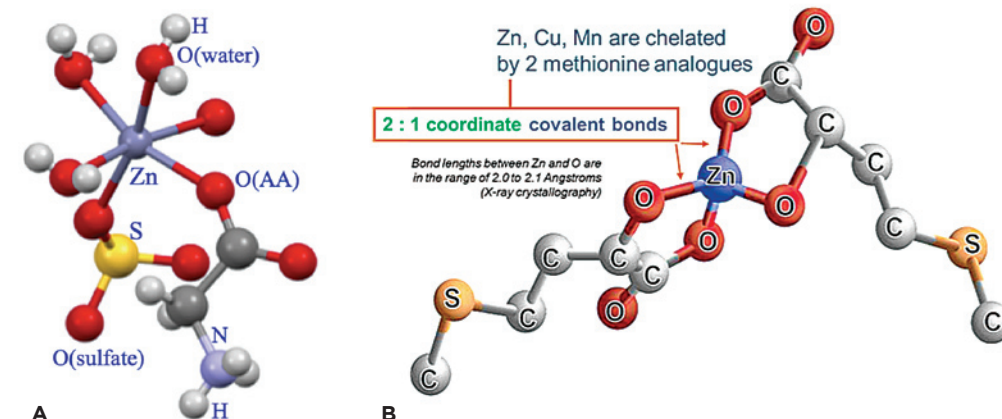


Fig. 2. Molecular structures in the solid state of (A) zinc sulphate glycine complex $Zn(HGly)(H_2O)_3SO_4$ (from Cambridge Structural Database) emphasising the non-chelating nature of glycine in this compound; (B) $Zn(HMTBa)_2 \cdot 2H_2O$ (coordinated water molecules omitted) showing the chelating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

plastocyanin respectively. 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.

Until the end of the last century, minerals were supplemented in the

salt forms of chlorides, sulphates, 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 faeces, 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 scepticism, as their use was estimated to be economically disadvantageous.

responsible for feed deterioration through the formation of free radicals.

- Protection of minerals from phytates and oxalates sequestration and from competition with other minerals in the feed which results in improved mineral bioavailability.
- Less mineral excretion into the environment, which makes animal production more environmentally sustainable.

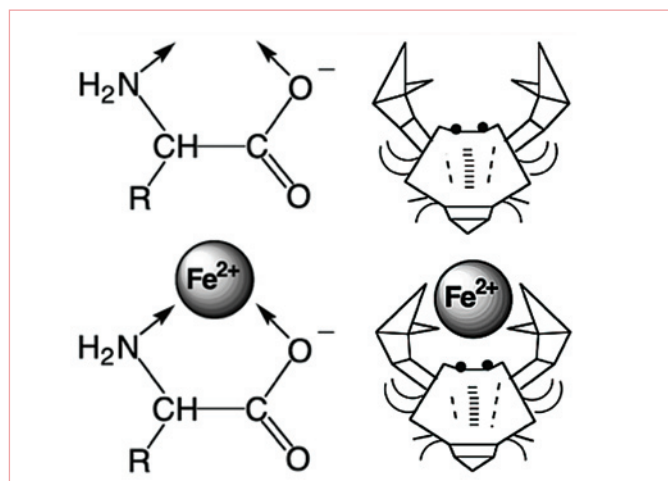
Owing to these enhancements in environmental management, feed quality and nutritional properties of the diet, previous assumptions were reconsidered and suddenly products were marketed claiming to contain effective mineral chelates.

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.

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

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Fig. 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).



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.

These beneficial effects are attributed to:

- Absence of free active metal cations (for example Fe^{2+} , Mn^{2+} , Cu^{2+})

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distinction can be adequately appreciated by recalling the basic principles of chemical bonds.

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:

- Ionic bond.
- Covalent bond.
- 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 respectively anions and cations. 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 characterised 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

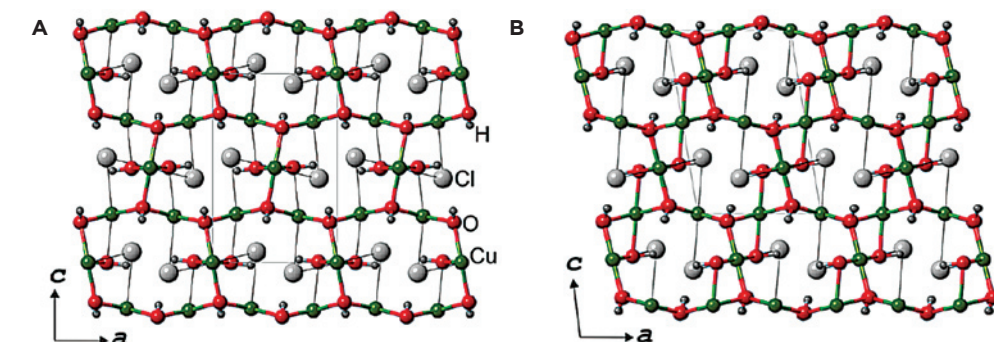


Fig. 3. Crystal structures of two polymorphs of copper hydroxychloride $\text{Cu}_2(\text{OH})_3\text{Cl}$: atacamite (A) and clinatoacamite (B). The pictures show the two ionic inorganic networks of the Cu^{2+} cations surrounded by hydroxide (OH^-) and chlorides (Cl^-) anions.

the origin of the molecular shapes (like that of the water molecule, H_2O , 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 cation forms a coordinate covalent bond between ammonia nitrogen (donor) and hydrogen ion (acceptor).

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 (Fig. 1).

The resulting complexes are genuine chelates. However, AAs do not always behave as chelating ligands. It is only in well-defined circumstances:

- AAs are not involved in peptidic bonds (for example, peptides or proteins) that prevent the formation of adequate chelation rings.
- No presence of certain anions (like Cl^- or SO_4^{2-}) that compete with AAs for mineral complexation.
- pH values are adequately high to allow the deprotonation of the AAs.

In this regard, it is noteworthy to remark that mineral sulphates/glycine 1:1 compounds, often called 'mono-chelates', are actually simple complexes, wherein chelation cannot take place.

Classification of 'organic' minerals containing complexes or chelates

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

- (a) mixtures of mineral complexes with a pool of AAs and oligopeptides derived from protein lysates (for example from soybean meal), commercially defined as proteinate.
- (b) mineral complexes of a single AA with a well-defined formula (for

example $\text{Zn}(\text{HGly})\text{SO}_4$), called improperly glycinate or 'mono-chelates'.

- (c) mineral chelates of a single α -hydroxyacid (for example lysine) in its anionic form, with well-defined formula (for example $\text{Zn}(\text{Lys})_2$), AA bis-chelates.
- (d) mineral chelates of a single α -hydroxyacid (methionine hydroxy analogue, HMTBa) of well-defined formula (for example $\text{Zn}(\text{HMTBa})_2$); HMTBa bis-chelates as in Mintrex Zn from Novus International, Inc).

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 $\text{Zn}(\text{HGly})\text{SO}_4$, whose crystal structure depicted in Fig. 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 better than AAs to the low pH value found in the first digestive tract compartments. This is due to the

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

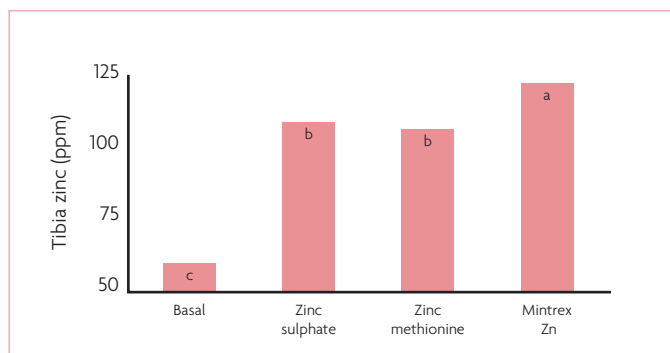
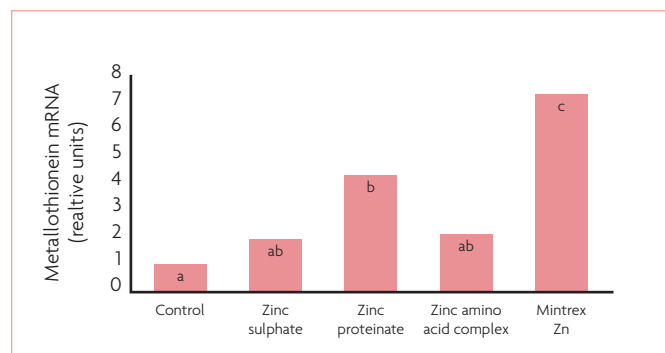


Fig. 5. Metallothionein mRNA expression in the intestinal mucosa as an indicator of zinc bioavailability.



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stronger acid character of HMTBa, whose anion is less prone to be protonated in acid media (Fig. 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) 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.

Other 'inorganic' mineral supplements

Recently, two already known hydroxy-chlorides of zinc and copper, namely copper hydroxychloride or TBCC (Tribasic copper chloride), $\text{Cu}_2(\text{OH})_3\text{Cl}$ (Fig. 3), and zinc hydroxychloride, $\text{Zn}_5(\text{OH})_8\text{Cl}_2$, have been re-proposed for supplementation in animal feed since they fit the request of feed stability, offered too 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. However, copper and zinc hydroxychlorides are reticulated solids, basic in nature, with an important ionic characteristic. This makes them subject to acid attack with complete solubilisation of the solid structure resulting in the Cu^{2+} or Zn^{2+} ions becoming unprotected in the solution. This occurs in the gastrointestinal compartments of the animals. In practice, after solubilisation, the chemical behaviour of copper and zinc hydroxychlorides are the same as copper and zinc simple salts.

Trace mineral bioavailability

The bioavailability of a mineral is generally defined as the proportion of the total mineral in feed that is utilised for normal body functions. The bioavailability of a given mineral source (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.

Measuring the mineral retention in select tissues has been the most common output in relative bioavailability experiments.

Fig. 4 shows the result of an experiment that compared the relative bioavailability of zinc sources by measuring tibia zinc content in broilers.

It shows that while all supplemented treatments (added at 40ppm 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 (Zn or Cu) status and by the amount of mineral absorbed.

Fig. 5 shows the use of metallothionein mRNA expression in the intestine as an indicator of zinc bioavailability.

In this experiment, broilers were fed a control diet or diets supplemented with 70ppm of zinc from different sources. The consistency of results, shown in Fig. 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.

Conclusion

Feeding a chemically well-defined, stable, and highly bioavailable trace mineral source is an important key to optimise the growth potential and well-being 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 are available from the authors on request