WHITE PAPER

ORGANIC TRACE MINERALS

Enhancing mineral bioavailability through chelation

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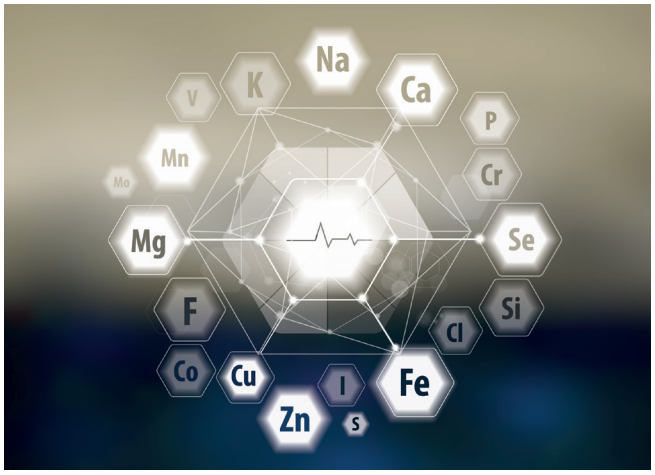
OVERVIEW

Organic trace minerals (OTMs) are recognized globally as being a more bioavailable mineral source than their inorganic counterparts. While there are many forms of mineral products available in the marketplace for use in animal nutrition, these have unfortunately been generically entitled 'organic trace minerals' by virtue of the fact that the trace elements in question are complexed or otherwise associated with organic molecules.



Typically speaking, OTMs can be produced through numerous mechanisms depending on the trace mineral product being manufactured. The process of complexing or chelating elements such as copper, iron or zinc, for instance, typically involves reacting inorganic mineral salts with a suitable bonding group, such as a peptide or amino acid, after which the mineral becomes part of a biologically stable structure. Numerous production processes have been developed, ranging from highly specific and controlled reaction processes to more involved chemical synthesis routes.

Given the vastly different products that exist in the marketplace, the importance of understanding the physical differences between them cannot be understated. A greater understanding of the basics behind these products will allow end-users to differentiate between them not only in terms of their physical make-up but also in terms of likely behavior *in-vivo*.

COMPLEXES OR CHELATES?

The chemistry of complexation or chelation, as it is commonly known, has created a great deal of confusion in the animal feed industry. Terms such as metal amino acid complexes, metal amino acid chelates, metal polysaccharide complexes and metal proteinates abound, yet official definitions remain vague and unhelpful.

Table 1: Official terminology for organic trace minerals (AAFCO and EFSA).

AAFCO		EFSA	
Metal proteinate (57.23)	The product resulting from the chelation of a soluble salt with amino acids and/or partially hydrolyzed protein.	**Metal chelate of protein hydrolysates**	A powder with a minimum content of x% metal where x = 10% copper, iron, manganese and zinc. Minimum of 50% copper, iron, manganese and 85% zinc chelated.
Metal amino acid chelate (57.142)	The product resulting from the reaction of a metal ion from a soluble metal salt with amino acids.	**Metal chelate of amino acids hydrate**	Metal amino acid complex where the metal and the amino acids derived from soya protein are chelated via coordinate covalent bonds.
Metal amino acid complex (57.150)	The product resulting from complexing a soluble metal salt with an amino acid(s).	**Metal chelate of glycine hydrate (liquid)**	A liquid with a minimum content of 6 % copper or 7 % zinc.
Metal (specific amino acid) complex (57.151)	The product resulting from complexing a soluble metal salt with a specific amino acid.	**Metal chelate of glycine hydrate (solid)**	A powder with a minimum content of 15 % copper, iron, zinc and manganese.

As an example, definitions of the most common organic trace minerals used in agricultural practice as laid down by the Association of American Feed Control Officials (AAFCO, 1998) are illustrated in Table 1. In deference to the AAFCO definitions, Table 1 also gives an overview of the EU classification of organic mineral products from which one can appreciate the stark differences between the official terminologies used for regulatory control and the obvious confusion that can occur when comparing products.

Typically speaking, chelates are prepared by reacting inorganic mineral salts with, for example, enzymatically prepared mixtures of amino acids and small peptides, under controlled conditions. Such amino acid and peptide ligands bind the metal at more than one point, ensuring that the metal atom becomes part of a biologically stable ring structure. Amino acids and protein digestion products, such as small peptides, are ideal ligands because they have at least two functional groups allowing for stable mineral bonding.

NOT ALL ORGANIC TRACE MINERALS ARE EQUAL.

Many different assertions are made as to the relative merits and suitability of amino acids versus peptides in forming mineral chelates, with an even greater number of arguments existing in relation to the so-called bioavailability of such products.

STABILIZING A CHELATE: THE EFFECT OF LIGAND

When trying to compare chelates or complexes based on "which is best under this set of conditions," one really needs to consider many different factors. However, it can be useful to compare products in terms of the strength of the bond between the mineral and the ligand using so-called stability constants.

A stability constant (also known as a formation constant or a binding constant) is an equilibrium value for the formation of a complex or chelate in solution. The overall stability constant is the product of all stepwise stability constants. For example, if K1 and K2 are the stability constants for the addition of the first and second ligand, respectively, then the overall stability constant (β_2) is K1 x K2.

This value is a relative measure of the strength of the interaction between a metal and the ligand in a chelate or complex. We can derive this value by measuring the relative proportions of metal ([M]), ligand ([L]) and chelate ([ML]):

$$[L] + [M] \leftrightarrow [ML]$$

Note the several influencing factors that play a role in this equilibrium:

- pH significantly influences the equilibrium between ML and L+M.
- Additional factors also influence this, such as the type and makeup of ligand, relative proportions of [L] to [M], etc.

Ultimately, the stability constant (β) can be defined as a measure of the ratio of the chelate concentration to the concentrations of the free metal and ligand under a given set of conditions.

For simplicity, it can be represented as follows:

$$\beta = [ML] / [L][M]$$

Essentially, what this tells us is that the greater the value of the stability constant β, the greater the proportion of the chelate or complex that is present relative to free ligand ([L]) or free metal ([M]) at a given pH.

The stability constants for a range of ligands, including single amino acids, dipeptides, tripeptides, etc., can be readily obtained from the NIST stability constants database, which calculates the value considering relative pH, ionic strength, temperature, ligand type, ligand and metal concentrations.

Typically, the stability constant is presented in log values and can serve as a useful guide when comparing different bonding groups, such as amino acids, dipeptides and tripeptides. In general, the higher the stability constant value, the greater the chelation strength and, thus, the relative proportion of bound mineral to free mineral and free ligand under a given set of conditions.

Obviously, it would be amiss not to state that there will be exceptions to this. Likewise, the very nature of the complex chemistry governing chelation dictates that additional factors will ultimately contribute to the bond strength and stability of ligand-mineral complexes.

THE ROLE OF **BOND STRENGTH** IN OTM STABILITY

Most amino acids and peptides bind metal ions through either nitrogen, oxygen or sulfur atoms. Individual amino acids exhibit a range of stabilities when complexed with minerals, and these can be assessed in a variety of databases. It is reasonable to expect that peptides that have a greater number of donor atoms and, hence, the potential to form a number of chelate rings when binding to a metal ion would have higher stabilities than simple amino acids such as glycine.

This is, however, dependent on the peptide being able to actually form more than one chelate ring. As in the case of amino acids, peptides also exhibit a range of stabilities. **In many instances, the stability of peptide chelates can be greater than single amino acid chelates.**

Arising from this, one would anticipate that proteinates will have the necessary physicochemical properties to ensure wide-ranging constancy under conditions of changing pH.

Consider the data in Table 2, which compares a range of ligands when complexed with copper under the same physiological conditions. For simplicity, the stability values (log data) have been transformed and compared on a relative basis to that of glycine. The molecular weight of each ligand is also indicated.

Table 2: Relative stabilities of organically bound copper complexes.

Bonding group	Relative stability
Propionic acid (74Da)	1x10-6
Methionine Hydroxy Analogue (150 Da)	2.63x10-6
Met (m.wt. 149Da)	0.5
Gly (m.wt. 75Da)	1
His-Ser (m.wt. 260Da)	2.5
His-Met (m.wt. 304Da)	2.5
Gly-Cys (m.wt. 196Da)	21
Gly-Lys (m.wt. 221Da)	2,818
Tyr-Trp (m.wt. 385Da)	3,235
Ala-Lys (m.wt. 238Da)	9,549
Tyr-Lys (m.wt. 327Da)	186,208
EDTA	5.6 x 1010

Adapted from: Critically selected stability constants of metal complexes, NIST Database 46.

What this indicates is that the size of the bonding group is not the most critical factor influencing bond strength and, ultimately, the stability of a chelate. Claims of superiority based on size clearly have little merit. However, simply increasing the number of amino acids in a ligand may not increase the stability of the metal complex and thus may not necessarily increase the relative proportion of bound mineral.

Ultimately, not only does the type of amino acid influence the stability of a given chelate, but the position of amino acids in a peptide can also significantly influence how the ligand and mineral interact.

This is illustrated in Table 3, where it can be appreciated that the most critical factors are the sequence and position of amino acids rather than the overall size.

Table 3: Role of amino acid sequence on chelate bond strength and stability

Bonding group	Relative stability
Gly-Gly-Gly (m.wt 225Da)	1
Gly-Gly-His (m.wt. 305Da)	270
Gly-His-Gly (m.wt. 305Da)	8,511

The substitution of a histidine into the tripeptide Gly-Gly-Gly to yield Gly-Gly-His, for instance, enhances the stability value and, thus, the relative proportion of bound mineral (copper in this instance). Furthermore, changing the position of this histidine within the tripeptide sequence (to form Gly-His-Gly, for example) can result in a further increase in the bond strength and, as such, an increase in the proportion of bound mineral.

In practical terms, simple changes in the configuration of amino acids in this tripeptide result in a greater proportion of bound mineral relative to free mineral and ligand.

Essentially, mineral chelate stability can be significantly influenced by not only the type of amino acid but also the configuration of amino acids in a peptide sequence.

From a production standpoint, it is important to note that the extent and type of hydrolysis of a protein source to form short-chain peptides can significantly influence the sequence of amino acids present in these peptides. The production of an 'optimal' protein hydrolysate for mineral chelation can be effected through careful selection of the hydrolysis conditions. This ensures that the final mix of hydrolyzed peptides will have the necessary properties to ensure constancy and mineral-binding stability under conditions of changing pH.

A recent study (Byrne *et al.*, 2021) using potentiometric-based techniques analyzed a range of commercial OTMs using a Cu ion-selective electrode to determine their in-vitro stabilities over a pH range reflective of physiological conditions (**Figure 1**). In this work, samples were reconstituted and suspended prior to titration of the supernatants, with subsequent measurement of the percentage of bound copper over a pH range of 3–8. This confirmed that notable differences exist in the pH-dependent stability of commercial OTMs, with the amount of bound copper varying considerably between samples.

Furthermore, the data indicates that some OTMs have low or no capacity for stable mineral bonding at acidic pH, with obvious impacts on the bioefficacy of the products. These differences can be attributed to not only the type of bonding group used but also to the production process used to generate the same.

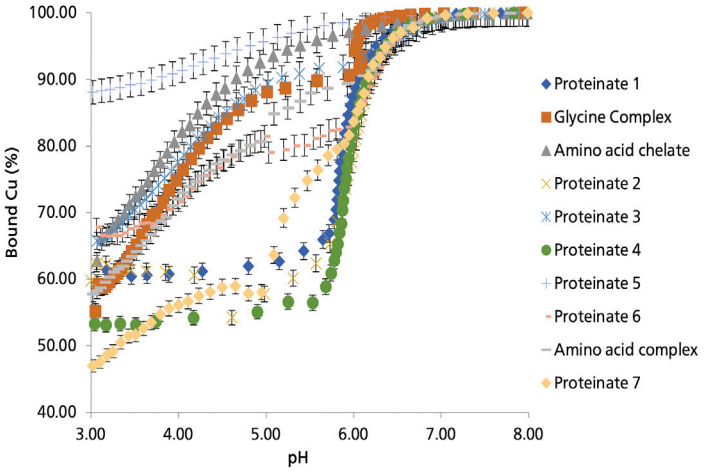
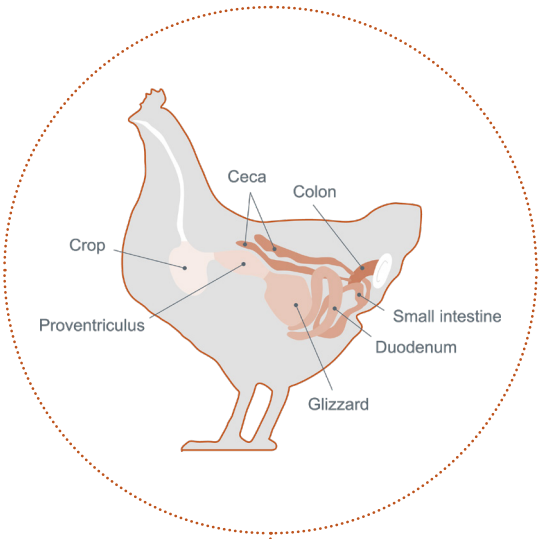


Figure 1: pH-dependent stabilities of copper chelates (Byrne *et al.*, 2021)

ULTIMATELY, THE STABILITY OF AN OTM IS OF PARAMOUNT IMPORTANCE TO ITS BIOAVAILABILITY.

During transit through the GI tract and as the pH decreases or acidifies, all OTMs are subjected to physiological forces, which can result in the bound mineral complex dissociating and releasing free mineral ions. There are several negative consequences to this pH-induced dissociation of OTMs.

For instance, the charged free mineral ion can react with negatively charged plant components, such as phytic acid, which may be present in the GI tract or, worse still, can form so-called hydroxides upon reaching the more alkaline environment in the intestine. This can lead to the phenomenon of pH-induced hydroxypolymerisation and result in precipitation of the mineral and, thus, lead to a very significant reduction in bioavailability.



Region		pH
Crop		4 – 6
Proventriculus		2.5 – 5
Glizzard		2.5 – 5
Duodenum		5 – 6.5
Small intestine	Upper	6 – 7
	Lower	7 – 7.5
Cecum		5.5 – 7
Colon		7

Essentially, complexes or chelates with low stabilities will not deliver the mineral to the sites of absorption in the intestine and reduce the effectiveness of the product to that of the corresponding inorganic salt. **Maximizing the pH-dependent stability of OTMs will increase mineral bioavailability and uptake in the intestine.**

IN ESSENCE, THE HIGHER THE STABILITY OF AN OTM, THE GREATER ITS BIOAVAILABILITY IS LIKELY TO BE.

INFLUENCE OF **OTM STABILITY** ON **RELATIVE BIOAVAILABILITY**

In addition to differing by virtue of the bonding group used in the chelation process, OTMs also differ greatly in terms of how well they are absorbed and utilized by an animal. Significant time and effort have been devoted by researchers to understanding the relative bioavailability of organic minerals, usually by comparison with inorganic mineral sulfates using a range of assessment parameters. There are numerous definitions of bioavailability, but in terms of trace minerals, this may be considered as the relative proportion of an ingested mineral that is absorbed and retained by the animal species under study. Table 4 highlights some examples of zinc-source bioavailability studies in poultry. It can be appreciated that, in general, organic zinc sources have greater bioavailability than their inorganic counterparts.

Table 4: Relative bioavailability of zinc sources in poultry (adapted)

Zn source	Zn indices	Relative bioavailability (%)	Reference
Zn sulfate (reagent grade)	Bone Zn	100	Cao *et al.*, 2000
Zn sulfate (basic)	Bone Zn	101	Cao *et al.*, 2000
Zn chloride (basic)	Bone Zn	107	Cao *et al.*, 2000
Zinc oxide (feed-grade)	Bone Zn	49	Cao *et al.*, 2000
Zn sulfate	Weight gain	100	Ao *et al.*, 2006
Zn proteinate	Weight gain	183	Ao *et al.*, 2006
Zn sulfate	Tibia Zn	100	Ao *et al.*, 2006
Zn proteinate	Tibia Zn	157	Ao *et al.*, 2006
Zn acetate	Bone Zn	100	Cao *et al.*, 2002
Zn proteinate	Bone Zn	110–124	Cao *et al.*, 2002
Zn methionine	Bone Zn	78–91	Cao *et al.*, 2002
Zn acetate	Mucosal MT	100	Cao *et al.*, 2002
Zn proteinate	Mucosal MT	99–130	Cao *et al.*, 2002
Zn methionine	Mucosal MT	77–94	Cao *et al.*, 2002
Zn sulfate	Weight gain	100	Batal *et al.*, 2001
TBZC	Weight gain	110	Batal *et al.*, 2001
Zn sulfate	Bone Zn	100	Cao *et al.*, 2000
Zn Aa chelate	Bone Zn	83–104	Cao *et al.*, 2000
Zn proteinate A	Bone Zn	116–139	Cao *et al.*, 2000
Zn sulfate	Mucosa Zn	100	Cao *et al.*, 2000
Zn Aa chelate	Mucosa Zn	64–104	Cao *et al.*, 2000
Zn proteinate A	Mucosa Zn	65–133	Cao *et al.*, 2000
Zn sulfate	Bone Zn	100	Wedekind *et al.*, 1992
Zinc-methionine	Bone Zn	117–177	Wedekind *et al.*, 1992
Zinc oxide	Multiple parameters	100	Pimental *et al.*, 1991
Zinc-methionine	Multiple parameters	100	Pimental *et al.*, 1991

Additionally, inter-study variability can be noted, which is not only dependent on the measurement index used to assess bioavailability but also on the source used. In essence, even within groups of organic trace minerals (e.g., proteinates), differences in bioavailability can be observed.

Many factors affect bioavailability, including (but not limited to):

- animal species
- sex
- physiological state
- existing mineral status
- choice of response criteria
- choice of standard source and chemical form
- solubility of the mineral element

With respect to the chemical form of the mineral, the chelation strength between the mineral and bonding group will define OTM stability and ultimately play a significant role in influencing relative bioavailability. A limited number of studies exist that compare OTM chelation strength with bioavailability. These studies are, however, very insightful and demonstrate a direct link between OTM stability and bioavailability.

TO ENHANCE OTM BIOAVAILABILITY, INCREASING THE STRENGTH OF THE BOND BETWEEN THE MINERAL AND THE BONDING GROUP USED WILL, THEREFORE, PROVE TO BE A VERY EFFECTIVE STRATEGY.

Table 5 highlights the key findings from a number of these studies, in which it is apparent that as chelation strength increases, so too does the relative bioavailability of the mineral source.

Table 5: Chelation strength is critical to relative bioavailability of OTMs (adapted)

Mineral source	Chelation strength (Qf)	Relative bioavailability (%)	Reference
Zn proteinate C	120	108	Cao *et al.*, 2000
Zn proteinate B	91	99	Cao *et al.*, 2000
Zn polysaccharide	3.8	94	Cao *et al.*, 2000
Zn proteinate A	944.02	111–196	Yu *et al.*, 2010
Zn proteinate B	30.73	107–187	Yu *et al.*, 2010
Zn Aa complex	6.48	106–162	Yu *et al.*, 2010
Zn glycinate	n/r	108–157	Yu *et al.*, 2010
Zn methionate	n/r	109–145	Yu *et al.*, 2010
Mn Proteinate	147	169	Liao *et al.*, 2019
Mn Aa chelate	61.9	141	Liao *et al.*, 2019
Mn Aa complex	2.35	112	Liao *et al.*, 2019
Mn Aa chelate C	115.4	93–114	Li *et al.*, 2004
Mn Aa chelate B	45.3	96–133	Li *et al.*, 2004
Mn methionate E	3.2	95–110	Li *et al.*, 2004
Fe proteinate ES	8,590	174	Zhang *et al.*, 2016
Fe proteinate M	43.6	143–164	Zhang *et al.*, 2016
Fe methionate	1.37	102–129	Zhang *et al.*, 2016

QUANTITATIVE ASSESSMENT OF CHELATION

Tests of varying scientific nature and credibility are widely claimed as having the ability to differentiate between good and bad OTMs.

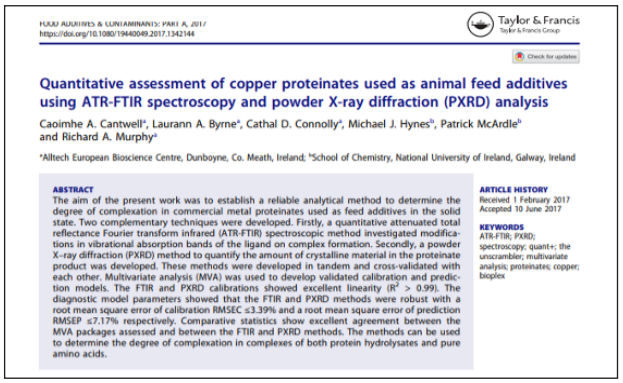
Basic parameters that can be analyzed include:

- percent mineral
- nitrogen-to-mineral ratio
- percent bound mineral
- molecular weight
- bioavailability
- stability

While some of the analyses in use can provide meaningful and valuable information on defined or individual products, understanding the limitations of these tests is critical if one is to apply them successfully in the assessment of OTMs. Historically, determination of the percent bound mineral utilized filtration through a low molecular weight membrane. The mineral retained behind the filter was assumed to be bound, while the mineral in the filtrate (solution) was assumed to be unbound. Such methods, however, are subject to manipulation, whereby changing the pH of the buffer can cause precipitation and lead to false estimates of the true level of bound mineral.

The only validated assays that fully quantitate the level of bound mineral in OTMs are based on the use of techniques known as ATR-FTIR and PXRD and were developed and validated by researchers at Alltech's European Bioscience Centre.

The first assay uses a form of infra-red spectroscopy to measure the amount of bound mineral, whereas the second assay uses a form of crystallography to measure unbound mineral. Both are complementary to each other, and both are peer-reviewed and published (Cantwell *et al.*, 2017). In the case of the FTIR assay, this was independently validated and verified by the Central Reference Laboratory (CRL) and is accepted as an official control method by European Food Safety Authority (EFSA) in the EU.



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Quantitative assessment of copper proteinates used as animal feed additives using ATR-FTIR spectroscopy and powder X-ray diffraction (PXRD) analysis

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ABSTRACT

The aim of the present work was to establish a reliable analytical method to determine the degree of complexation in commercial metal proteinates used as feed additives in the solid state. Two complementary techniques were developed. Firstly, a quantitative attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopic method investigated modifications in vibrational absorption bands of the ligand on complex formation. Secondly, a powder X-ray diffraction (PXRD) method to quantify the amount of crystalline material in the proteinate product was developed. These methods were developed in tandem and cross-validated with each other. Multivariate analysis (MVA) was used to develop validated calibration and prediction models. The FTIR and PXRD calibrations showed excellent linearity (R^2 > 0.99). The diagnostic model parameters showed that the FTIR and PXRD methods were robust with a root mean square error of calibration RMSEC ≤3.39% and a root mean square error of prediction RMSEP ≤7.17% respectively. Comparative statistics show excellent agreement between the MVA packages assessed and between the FTIR and PXRD methods. The methods can be used to determine the degree of complexation in complexes of both protein hydrolysates and pure amino acids.

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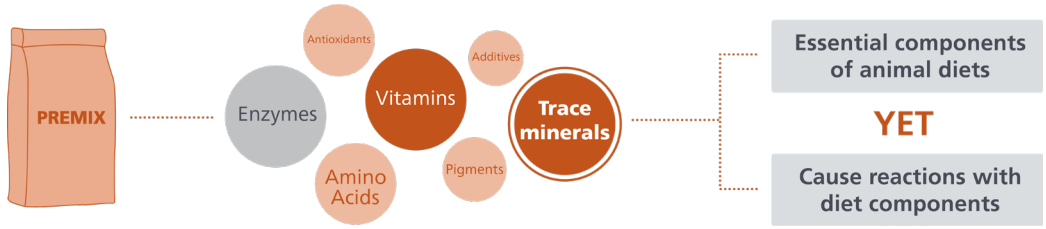
ATR-FTIR; PXRD; spectroscopy; quant+; the unscrambler; multivariate analysis; proteinates; copper; bioplex

PREMIX AND FEED ANTAGONISM

Increasingly, the agonistic and antagonistic effects of feed components have come under scrutiny, with choice of components gaining increasing importance in diet formulation. The possibility for negative interactions occurring between individual components within premixes and feeds is high and often overlooked, as are the underlying effects at a cellular level following digestion and absorption of the mineral source.

Recent studies have focused on assessing these potential antagonisms. The differential effects noted indicate that **not all chelates are created equal.** Moreover, they all differ in terms of their

stabilities, releasing mineral in a pH-dependent fashion based on the pH in the local micro-environment. This instability results in some chelates having a negative impact on premix and feed components.



EFFECT OF **MINERALS** ON **ENZYME ACTIVITY**

Very little information is available comparing the potential antagonisms that can occur between different mineral sources and enzymes within premixes, as well as the repercussions that this might have in terms of losing enzyme efficacy.

Santos *et al.* (2014) focused on assessing the potential in-vitro interaction between inorganic and organic chelated sources of Fe, Zn and Cu with three commercially available phytase preparations. The study also investigated if the degree of enzyme inhibition was dependent on the type of OTM used as a mineral source.

The authors demonstrated that a highly significant relationship between phytase inhibition and trace mineral type, as well as mineral source and concentration, existed.

Proteinates were consistently and significantly less inhibitory than the other mineral sources, and this was shown in the case of the *Escherichia coli* and *Peniophora lycii* phytases for Fe and Zn, as well as for Cu with *E. coli* and *Aspergillus niger* phytases. The dose-response curve illustrating the impact of Fe source on *P. lycii* phytase activity is shown in **Figure 2**.

The authors further highlighted the impact of mineral form on phytase activity by calculating the half-maximal inhibitory concentration (IC50), thereby allowing them to indicate how much of each mineral source was needed to inhibit phytase function by half, thus providing a measure of the potency of individual mineral sources. Table 6 summarizes the IC50 values for the individual Fe sources and phytase enzymes assessed, further illustrating that not all mineral sources are equivalent with respect to their inhibitory impact.

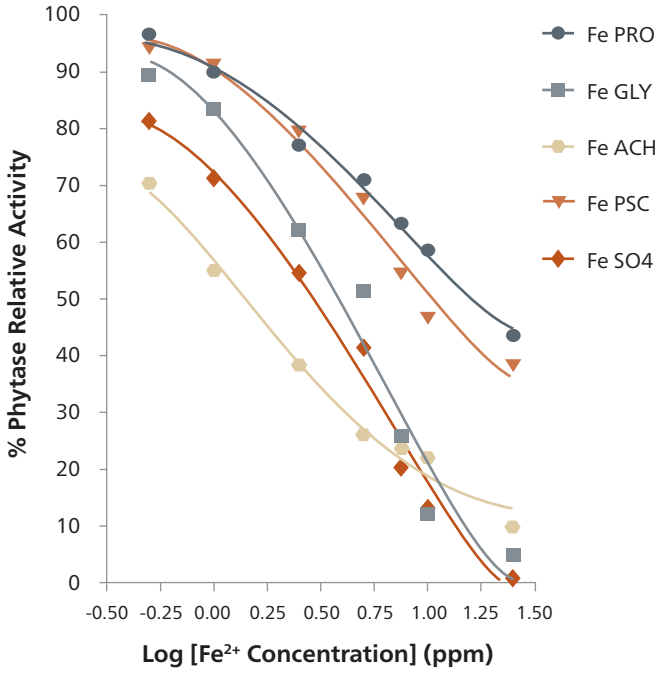


Figure 2: Sigmoidal dose–response curves representing the effect of Fe sources on *P. lycii* phytase activity (Santos *et al.*, 2014)

Table 6: IC_{50} concentration values (ppm) of iron sources for inhibition of phytase

Phytase source	Proteinate	Glycinate	Amino acid complex	Polysaccharide complex	Sulphate
E. coli	$6.7 \pm 1.1^{A\,1}$	$3.4 \pm 0.3^{B\,C\,1}$	$0.9 \pm 0.1^{C\,1}$	$3.7 \pm 0.1^{B\,1}$	$4.1 \pm 0.4^{B\,1}$
P. lycii	$16.7 \pm 2.5^{A\,1}$	$3.8 \pm 0.8^{B\,1}$	$1.4 \pm 0.2^{B\,1}$	$10.4 \pm 1.3^{C\,1}$	$2.9 \pm 0.4^{B\,1}$
A. niger	$10.9 \pm 1.0^{A\,1}$	$6.0 \pm 0.9^{B\,1}$	$1.1 \pm 0.1^{C\,1}$	$12.0 \pm 1.3^{A\,1}$	$8.5 \pm 1.0^{A\,B\,1}$

Adapted from: Santos et al., 2014

Overall, different OTM sources displayed differential effects in their inhibition of exogenous phytase activity. The consequences that this mineral-induced inhibition of enzyme activity has for premix and feed formulation are tremendous and go some way towards explaining the variation noted in supplementation response.

IT MAY WELL BE THAT THE TREND TOWARD SUPER-DOSING OF PHYTASE ACTIVITIES IN DIETS IS AN UNINTENTIONAL CONSEQUENCE OF THE NEGATIVE INTERACTIONS OF PREMIX COMPONENTS.

EFFECT OF **MINERALS** ON **VITAMIN STABILITY**

Vitamin oxidation and antioxidant function are primarily caused by autooxidation of fats (a phenomenon that can be self-propagating) or by trace minerals through Fenton-type oxidizing reactions. In trace mineral premixes, oxidation-reduction reactions are the predominant cause of vitamin instability.

The type of trace mineral will influence its reactivity; copper, iron and zinc being the most reactive and having the greatest potential for vitamin destruction.

THE FORM THAT THE TRACE MINERAL IS PRESENTED IN, HOWEVER, HAS AN EVEN MORE SIGNIFICANT ROLE TO PLAY IN INFLUENCING VITAMIN STABILITY.

A recent study (Concarr *et al.*, 2021) illustrates these effects nicely. The study, which examined vitamin E stability following short-term inclusion in mineral premixes containing inorganic sulfates or different forms of organic minerals, demonstrated that mineral form significantly influenced the stability of α-tocopherol (**Figure 3**).

Vitamin E stability in the premixes containing proteinated chelates was not significantly different when compared to the vitamin control. The two premixes that had the highest α-tocopherol acetate loss were those containing the amino acid complex (25.7% decrease) and the glycinate (31.9% decrease). Both of these premixes were noted to have higher losses than were found within the vitamin control and the proteinate source. This data demonstrates the importance of carefully choosing premix components.

An additional study by Concarr *et al.* (2021b) further examined the destabilizing impacts of mineral form on vitamins in premix. The author found that both retinol acetate and cholecalciferol stabilities significantly increased ($P \leq 0.05$) within

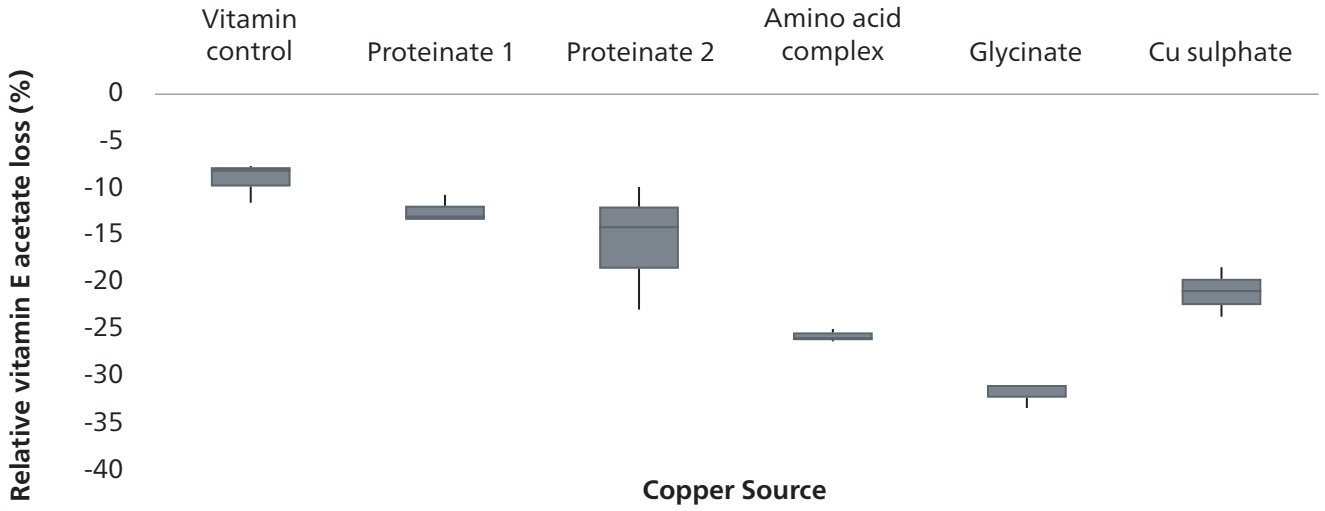


Figure 3: Vitamin E instability in premixes is influenced by mineral form (Concarr *et al.*, 2021)

vitamin-trace mineral premixes through the inclusion of chelated mineral. The data indicated that enhanced levels of trace mineral increased retinol acetate and cholecalciferol degradation in line with the duration of time in storage, but that losses could be minimized by switching from inorganic to organic forms. This is nicely illustrated in Figure 4, where the impact of mineral form and level on vitamin D3 stability is apparent.

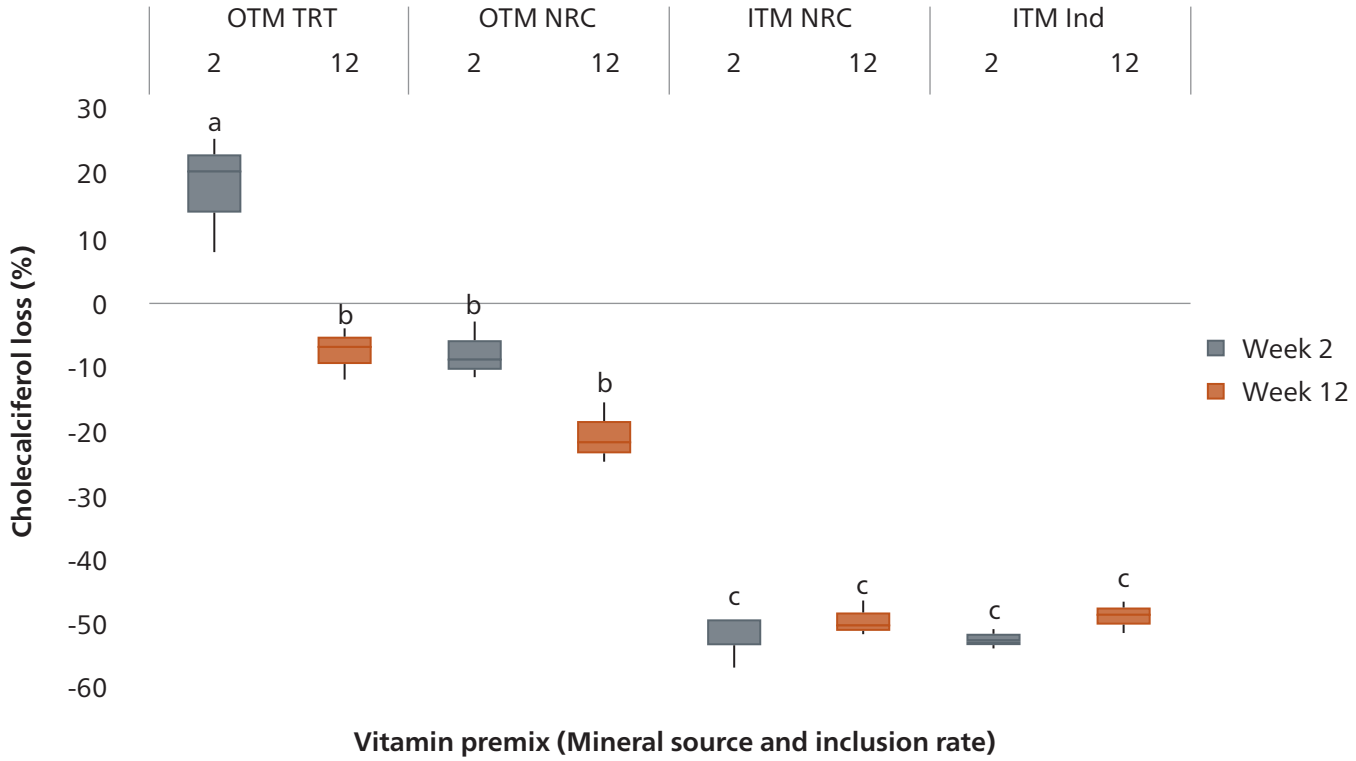


Figure 4: Impact of mineral form, level and storage time on vitamin D_3 stability in premix (Concarr *et al.*, 2021)

IMPACT OF **MINERAL FORM** ON **ANTIOXIDANT EFFICACY**

Additional research by the same author assessed the effect of mineral form in reducing the efficacy of recognized feed antioxidants such as BHT (butylated hydroxytoluene). This study compared inorganic copper sulfate to different organic mineral sources of copper (glycinates, amino acid chelates and proteinates). The results further indicate that the efficacy of commonly used premix components, such as antioxidants, can be compromised by the use of inorganic trace elements (**Figure 5**).

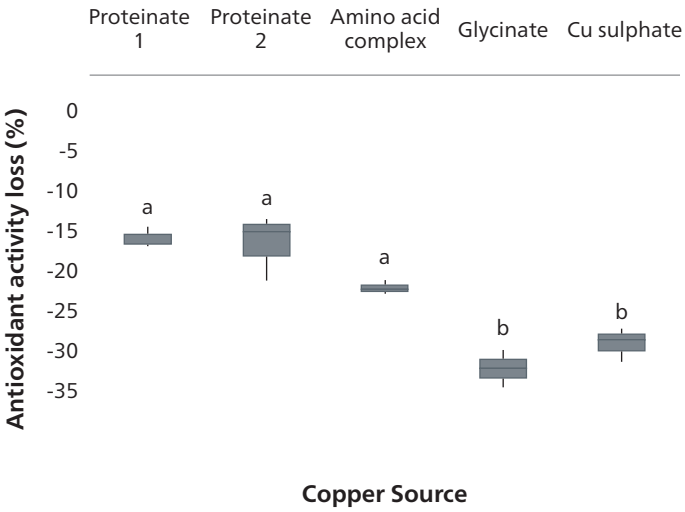
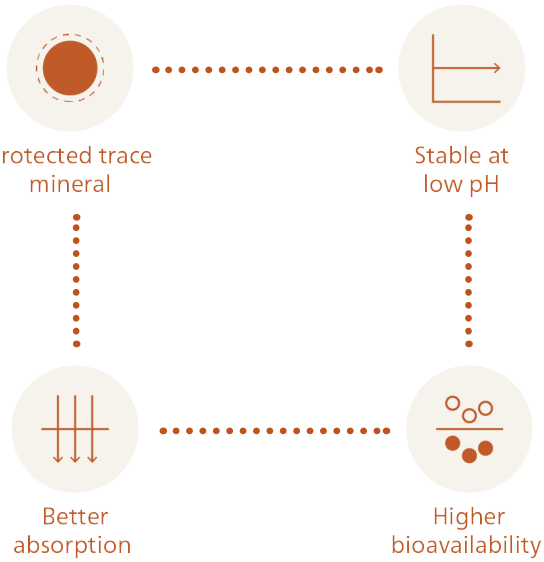


Figure 5: Impact of mineral form on antioxidant (BHT) efficacy (Concarr *et al.*, 2021)

The data further indicates that, in some cases, organic trace elements also had a significant destabilizing impact on antioxidant function. Essentially, weakly bonded minerals may result in the liberation of free mineral ions, causing reactive oxygen species generation, which leads to greater oxidation and a reduction in the efficacy of feed antioxidants such as BHT.

IMPACT OF **SELENIUM SOURCE** ON **SELENOMETHIONINE STABILITY**

Selenium can be included in premixes as inorganic sodium selenite, organic selenium-enriched yeast and chemically synthesized forms, such as L-selenomethionine (L-SeMet) or Hydroxy-selenomethionine. Concarr and colleagues recently compared the stability of selenomethionine source in inorganic mineral premix by assessing the SeMet recovery from premixes containing organic selenium-enriched yeast or chemically synthesized L-SeMet.



The data on the effect of source on the stability of SeMet in the presence of ITM demonstrated that the chemically synthesized L-SeMet was significantly (P≤0.05) less stable than organic selenium-enriched yeast when formulated into a premix containing ITM sources (**Figure 6**).

A report by EFSA (2013) stated that synthesized L-SeMet is unstable in premixtures containing

compounds of trace elements, with a SeMet loss of 45, 46 and 63% seen after three, six and nine months, respectively. The instability of chemically synthesized L-SeMet within a premix may be due to the fact that its purified form can be easily oxidized. The presence of pro-oxidative minerals increases the formation of ROS, which subsequently oxidizes SeMet. While the current experiment correlated with the EFSA data in relation to the instability of chemically synthesized L-SeMet in the presence of trace minerals, it showed that **the organic selenium-enriched yeast source was significantly more stable (P≤0.05).**

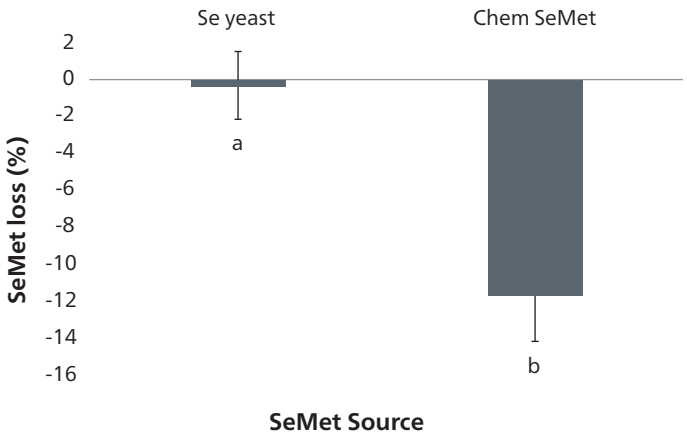


Figure 6: Influence of selenium source on selenomethionine stability (Concarr *et al.*, 2021)

Given the stabilizing influence of the chelation process on mineral reactivity, one would expect that the use of chelated trace elements would reduce the negative impact of trace elements on selenomethionine stability.

CONCLUSIONS

Despite the confusion and often contradictory information that exists, mineral chelation is a relatively straightforward process governed by some fundamental chemistry basics. By carefully considering factors important in mineral chelation, one can begin to distinguish between the products on the basis of their biological stabilities and thus biological bioavailability.

ULTIMATELY, THE STABILITY OF AN OTM IS OF PARAMOUNT IMPORTANCE TO ITS BIOAVAILABILITY.

During transit through the GI tract and as the pH decreases or acidifies, all OTMs are subjected to physiological forces that can result in the bound mineral complex dissociating and releasing free mineral ions. Organic trace minerals with optimized stability and bond strength will have far less potential for reactivity compared to inorganic sources. However, different forms of organic trace mineral will react differently and cause greater or less inhibition of enzyme activity, vitamin stability and antioxidant function depending on source.

Ultimately, diet formulators may well need to pay greater attention to their choice of individual component to minimize the financial costs associated with negative interactions, which could be significant.

**References available on request.*

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