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STABILITY OF TRACE MINERALS: DOES THE SOURCE MATTER?

"In the animal nutrition industry, various chelated Zn sources have been marketed for their claimed superior bioavailability over traditional sulfates. However, it is crucial to highlight that EFSA's opinions have not definitively concluded on this matter. To date, EFSA's scientific evaluations consistently challenge these claims of higher bioavailability for chelated Zn sources compared to standard sulfates."

Nutrients such as carbohydrates, fats, proteins, including minerals and vitamins, are primarily absorbed within the intestine. Before reaching this organ, the ingested bolus has to pass through an acidic environment: the stomach. The gastric milieu plays a crucial role in the digestion process across animal species, facilitating the activation of enzymes and the pre-digestion of proteins and lipids.

TRACE MINERALS AND THE ACIDIC GASTRIC ENVIRONMENT

As the feed enters the digestive system, pepsinogen and hydrochloric acid will be secreted to assist in the breakdown of feed and facilitate digestion. Across swine, poultry and ruminant species, the pH conditions in the gastric chyme usually range between 2.0 to 5.0 (Figure 1), before it reaches the small intestine. The acidic environment aids in the mineral solubilization for their absorption in the intestinal tract (Broom et al., 2021). Among the trace mineral sources in the market, we can find sulfates, oxides, chlorides, chelates, among others. In this article, we are going to focus on positively charged metals (cations).

Regardless of the source, the first stage of trace mineral absorption is the exposure to the acidic environment of the stomach, where gastric juices and low pH promote the solubilization of trace minerals. During this stage, the physical-chemical properties of trace mineral sources play an important role: sources with large particles which are not completely soluble in mild acidic conditions, such as low-quality metal oxides, are

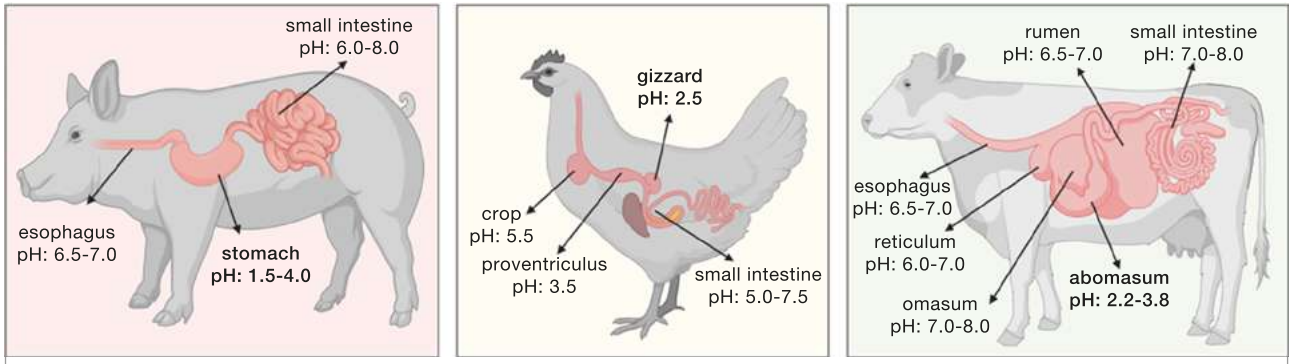

Figure 1. The digestive system of swine, poultry, and ruminant with certain pH conditions along the GI tract (authors).

not fully dissociated and thus have very poor bioavailability (Cardoso et al., 2021). In the stomach, most trace minerals dissociate from ligands to which they are bound, allowing the resulting free metallic ions to enter the small intestine. However, if not absorbed, the increase in pH conditions leads to a *de novo* chelation processes (Vacchina et al., 2010). Moreover, the bioaccessibility of zinc (Zn) and other metal ions is drastically dependent on chemical interactions in the small intestine. A higher degree of dissociation in the small intestinal lumen enhances phytic acid's capacity to chelate cations. Consequently, in conditions characterized by high phytate and low phytase activity, trace mineral sources that are less soluble in water may be less susceptible to interact with phytate. The acid-induced solubilization and dissociation set the stage for the subsequent absorption of metallic ions in the small intestine, ensuring the effective utilization of trace minerals. Then, in the small intestine, specific transporters aid the uptake of metallic ions into the intestinal cells, which are transported by their chemical ligands. These transporters recognize and enable the absorption of different trace minerals (Richards et al., 2010).

Nowadays, some of the trace minerals are being chelated to improve their bioavailability and minimize antagonistic interactions with phytase, calcium, or other dietary components. This process helps them resist degradation in the stomach's acidic conditions, thereby sustaining mineral homeostasis. Some advocates of chelation assert that these chelated trace minerals utilize active transport pathways supported by amino acid and peptide transporters across enterocytes, thereby optimizing mineral absorption and tissue deposition. However, according to recent European Food Safety Authority (EFSA) opinions, studies have shown that Zn deposition in animal tissues from chelates of glycine, hydroxy analogue of methionine, or amino acids hydrate have demonstrated no significant differences when compared to that of Zn sulfate or inorganic Zn. Additionally, previous studies using radioisotope labeling have shown varying ratios of Zn to C and S isotopes from Zn-methionine at the gut barrier and within enterocytes (Beutler et al., 1998, Hill et al., 1987a, and Hill et al., 1987b). These studies have also revealed distinct time kinetics of absorption of these labelled Zn ions compared to C and S ions in enterocytes, suggesting differing absorption pathways for Zn and methionine. Similar findings have been observed with other chelated sources. In addition, studies applying X-ray absorption structure spectroscopy determined identical Zn-speciation within intestinal cells of sheep and broilers fed either inorganic or organic Zn, respectively, providing further evidence that entry routes for Zn into the organism do not differ between feed Zn sources (Sui et al., 2011 and Liu et al., 2014). Recent research in pigs and poultry have illustrated that chelating agent alone significantly reduces phytate antagonism with Zn from Zn sulfate. This suggests that the occasional superiority of chelates under high phytate conditions is mainly attributable to altered chemical interactions within the gastrointestinal lumen rather than by alternative, molecular transport mechanisms (Windisch et al., 2002 and Boerboom, 2021). Under semi-synthetic conditions, denoted by the absence of phytic acid in the diet, the superiority

of chelated Zn as opposed to Zn sulfate diminishes, causing even numerically lower true absorption rates due to a small intestinal solubility of the chelates.

CHELATED MINERALS STABILITY UNDER DIFFERENT PH CONDITIONS

The stability of chelates can be severely affected by pH. An *in silico* simulation (Figure 2) represents a model of the copper-glycinate complex behavior in water at varying pH levels. Initially, before any interaction with water, copper is entirely bound to glycine in a stable complex. However, when the pH is lowered to 3, approximately 80% of copper becomes free ions (Cu^{2+}), indicating reduced stability of the complex. On the contrary, at high pH levels, both the complex and precipitated forms equally make up 50% of copper, with no free ions present, suggesting a less reactive complex. The figure also shows that at pH 5.25, only 70% of the chelate is available, highlighting its decreased proportion as the pH decreases. This emphasizes the partial dissociation of chelates in acidic stomach conditions and potential chelate reformation or formation of other complex with organic molecules present in the higher pH environment of the small intestine.

Based on the *in silico* simulation, our observations align with the findings reported by Byrne et al. (2021) for copper proteinates and Vacchina et al. (2010) for glycinates. For instance, Vacchina et al. (2010) illustrated an increase in metal-free glycine at acidic pH levels (below pH 5), with Fe-Gly appearing more stable during acidification compared to other glycinates (Cu, Zn, Mn). The graphs also indicated significant dissociation of these glycinates at pH below 4 to 5. The presence of glycinates in the intestinal tract leads to dissociation under acidic pH conditions, followed by chelation at basic pH, resulting in more than 100% of the initially added trace minerals in glycinates being present in the feed. Similarly, Byrne at al. (2021) revealed that proteinates are dissociated as soon as the pH drops below 6.5, with less than 50% of Cu-proteinates in the chelated form at pH <3.5. The key takeaway is that chelates, like other trace mineral sources, undergo dissociation at low pH and re-chelation at high pH. In the presence of free ions, there exists the potential for either the reformation of chelates or the creation of another complex with organic molecules in the higher pH environment of the small intestine.

EFSA WEIGHS ON THE BIOAVAILABILITY OF CHELATED TRACE MINERALS

Beyond considerations of pH stability, the bioavailability of chelated trace minerals has garnered atten-

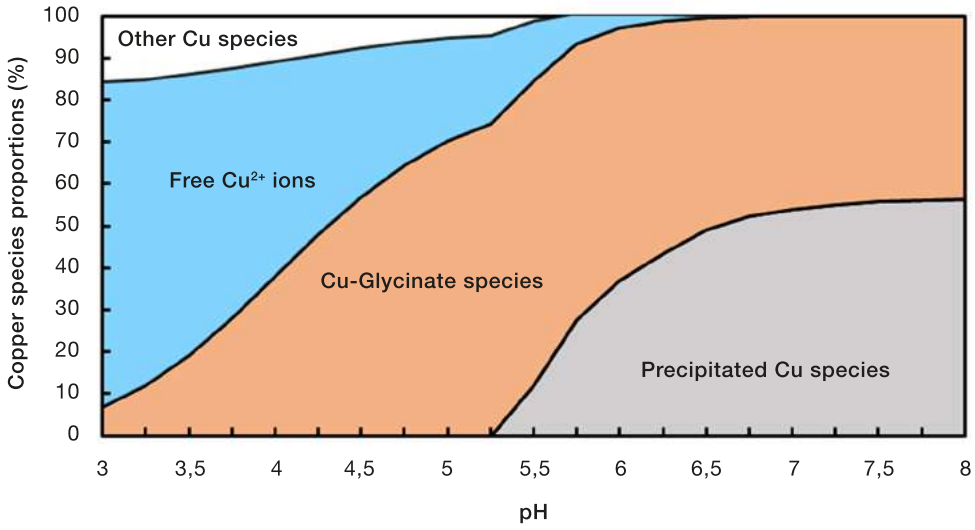

Figure 2. In silico simulation of the proportion of copper chemical species relative to solution pH. The software Visual Minteq was used to determine the copper chemical species present at 25°C in a solution of 4.2 mM Cu-Glycinate and 0.1M $NaNO_3$ at a pH range between 3 to 8 (authors).

tion due to its potential impact on animal nutrition and overall performance. EFSA is actively exploring the scientific evidence surrounding the utilization of chelated trace minerals in both food and feed. This investigation addresses aspects such as safety, bioavailability, and the potential health and nutritional effects of these minerals in humans and animals.

In considering the absorption mechanisms of chelates, there is a theoretical proposition that suggests their absorption via amino acid or peptide transporters. Taking Zn as an example, ZIP4 (Zrt/Irt-like Protein 4) serves as the major active apical transport mechanism from lumen to enterocyte. However, ZIP4 expression tends to be downregulated if there is a sufficient or oversupply of Zn to prevent excessive Zn absorption, thereby maintaining Zn homeostasis. When there is an excess of free Zn ions in the cytosol of the enterocyte, ZnT1 is activated as a response to maintain Zn balance. This activation is regulated by Metal Transcription Factor 1 (MTF1). ZnT1 works by transporting Zn across the basolateral membrane of the enterocyte, directing it toward the circulation and preventing an accumulation of Zn within the cell (Goff, 2018 and Windisch, 2002). If chelated Zn is indeed absorbed intact, it poses a risk of toxicity. A chelator with such strength that even the metal transporters at the apical gut mucosa (which are strong chelators themselves) cannot extract its metal would fail to dissociate within the enterocyte cytoplasm, leading to uncontrolled accumulation. However, all available data to date suggests that Zn from both chelated and inorganic sources is subject to homeostatic regulation, which strongly suggests that both deliver ionic Zn to the respective molecular machinery.

In the animal nutrition industry, various chelated Zn sources have been marketed for their claimed superior bioavailability over traditional sulfates. However, it is crucial to highlight that EFSA's opinions have not definitively concluded on this matter. To date, EFSA's scientific evaluations consistently challenge these claims of higher bioavailability for chelated Zn sources compared to standard sulfates (Table 1). An optimal chelating agent should possess a stability constant that can balance effective sequestration from feed materials and efficient uptake by the animal.

CONCLUSIONS

While chelating agents aim to improve absorbability, the animal has a pivotal role in downregulating transport pathways, especially when the trace mineral requirements are already met. Regardless of the source, all forms of trace minerals face instability at low pH, including chelates, as at least some of them are partially dissociated and then re-chelated at a higher pH environment. Moreover, in modern conditions with phytase-supplemented diets, bioavailability studies performed in the past decades without phytase should be reevaluated. Among trace mineral forms, sources with slow dissolution kinetics along the acidic segment of the GIT must be considered, as they can affect the bioavailability of trace minerals. Moreover, to ensure proper trace mineral availability determination, they were best studied under subclinical deficiency as the most common Zn-malnutrition phenotype.

Table 1. Zinc chelates and the summary of EFSA Opinions regarding their efficacy and bioavailability

Zinc chelates	Published date	Summary of EFSA Opinion
Zinc chelate of hydroxy analogue of methionine	27 November 2009	Studies involving piglets, laying hens, and dairy cows indicate this does not result in different Zn concentrations in muscle, liver, kidney, skin/fat, eggs, and milk compared to inorganic sources.
Zinc chelate of amino acids hydrate	23 March 2012	Tissue deposition of this zinc source is expected to be similar to that of zinc sulfate.
Methionine-zinc	23 January 2013	Data on Zn uptake and tissue deposition in species other than poultry do not suggest greater bioavailability than other authorized Zn sources.
Zinc chelate of L-lysinate-HCl	10 November 2015	Tolerance studies indicate safety as a zinc source, with no distinct zinc deposition in edible tissues/ products compared to the standard inorganic source, Zn sulfate heptahydrate.
Zinc chelate of methionine sulfate	8 June 2017	Bioavailability comparable to Zn sulfate or ZnO in poultry, pigs, and ruminants.
Zinc chelates of lysine and glutamic acid	25 July 2019	Based on Zn deposition across various tissues (e.g., tibia in chickens), it serves as a bioavailable Zn source, comparable to the standard inorganic Zn source, with no expected increase in zinc content in animal tissues and products.
Zinc chelate of ethylenediamine	22 March 2021	May contain multiple Zn species and not solely comprised of Zn mono-chelate of EDA; submitted combined tolerance, residue, or efficacy study in chickens for fattening was deemed invalid.
Zinc (II)–betaine complex	21 February 2023	Considering the deposition of Zn in edible tissues/ organs in chickens for fattening, the additive serves as a source of bioavailable Zn and is comparable to the standard inorganic Zn source.

About Dr. Yron Joseph Y. Manaig

Dr. Yron Joseph Y. Manaig, an animal nutritionist from Philippines/Spain, holds a master's degree in animal nutrition from University of the Philippines Los Baños. He earned a European Joint Doctorate in Molecular Animal Nutrition (MSCA-ITN) from Universitat Autònoma de Barcelona (Spain) and Università degli Studi di Milano (Italy). Dr. Manaig specializes in swine nutrition, feed ingredient evaluation and formulation, and OMICS. He joined ANIMINE in 2023 and has been working with the R&D group since.

About Marion Taris

Marion Taris is currently the analytical project engineer of Animine Precision Minerals. She is a scientist specializing in chemical analysis. She studied chemistry and physics before specializing in chemical analysis. She graduated from the University of Pau (France). Between 2017 and 2021, she worked on the development of new strategies for the analysis of enzymes immobilized on electrodes for the French national center for scientific research (CNRS). Marion joined Animine in 2023 and has been working with the research and development group since.