

The Impact of Essential Minerals on Food Stability

A Mineral's Chemical Form Dictates its Rate of Interaction

Most higher life forms have evolved to utilize a wide range of mineral nutrients available from the crust of the earth. As farming intensity has increased with associated higher yields per acre, trace minerals provided by natural processes have been depleted from soils, leading to the need for supplementation. A number of metals, including cobalt, copper, iron, manganese, molybdenum and zinc have been proven to be essential nutrients for both plants and animals (Asher, 1991). In animals, an inadequate supply of such essential trace minerals (ETM's) will result in poor health and can, in the extreme, lead to death. Recent work illustrates that optimum health may require even higher levels of these nutrients than those needed to avoid clinical disease symptoms (the traditional criterion for essentiality), (Combs, 1998).

Unfortunately, these metals can also bring undesirable side effects by interacting with other ingredients in a prepared food or animal feed. If added to the mixture in the wrong chemical form, they can trigger formation of free radicals. These in turn lead to breakdown of vitamins and ultimately to degradation of fats and oils (Miles, 1998), decreasing nutrient value and palatability. In a packaged wet product, interactions can cause discoloration (Shields, 1998) and conversion to metal compounds that are not bioavailable.

Bioavailability of Metal Compounds

Recognition of the need for supplementation with ETM's occurred decades ago and was soon followed by research on the bioavailability of various sources. The term bioavailability refers to the ease with which a nutrient can be metabolized by organisms.

The concept of bioavailability is surprisingly complex. As it turns out, there is no absolute value that can be ascribed to a source since each result depends on the method used to measure it (Ammerman, 1995). Bioavailability values are only meaningful in the context of the test method, the species, and the conditions tested. Nevertheless, relative values for different sources can be obtained under one set of specified conditions. Then the results from many such trials can be evaluated to form a general impression of how various sources compare. Materials with low availabilities are less efficient, meaning that more must be purchased, fed, and excreted to achieve the intended result.

The inorganic acid salts of metals typically have high bioavailabilities. Examples are the sulfates, chlorides and nitrates of cobalt, copper, iron, manganese, molybdenum and zinc. The sulfates tend to be lowest in cost and therefore most widely utilized. This is due, at least in part, to the fact that sulfuric acid is the lowest-cost strong mineral acid. Oxides and hydroxides tend to be poorly available and are no longer considered to have much value as nutritional sources. Carbonates are typically intermediate. It is noteworthy that the ease with which nutrients can be dissolved seems to roughly correspond to their bioavailability. For example, acid salts of metals are typically much more soluble in water than alkaline salts - hydroxides and oxides.

Oxidation Reactions

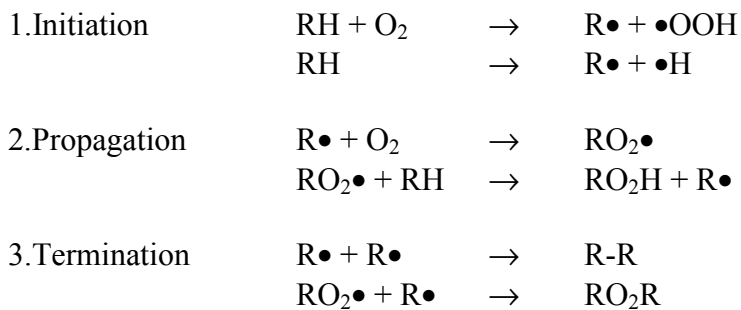
One negative aspect of many metal compounds is their promotion of oxidation and thereby destruction of critical components in food. Oxidation is defined as the loss of electrons. An "oxidizing agent" is therefore a substance with high electronegativity - an electron acceptor. The

most common engine of oxidation under natural, ambient conditions is oxygen from the atmosphere. It corrodes metals to oxides (such as rust) and converts organic materials to carbon dioxide during both combustion and metabolic processes. More subtle, but also ubiquitous, are room-temperature chemical reactions where electrons are transferred to oxygen from electron-rich sites such as the double bonds found in fats, oils and vitamins. These fragile compounds need only a gentle nudge to initiate the transfer. In fact, after the process is initiated, it is a self-propelling chain reaction, being propagated by intermediates known as free radicals (see side bar).

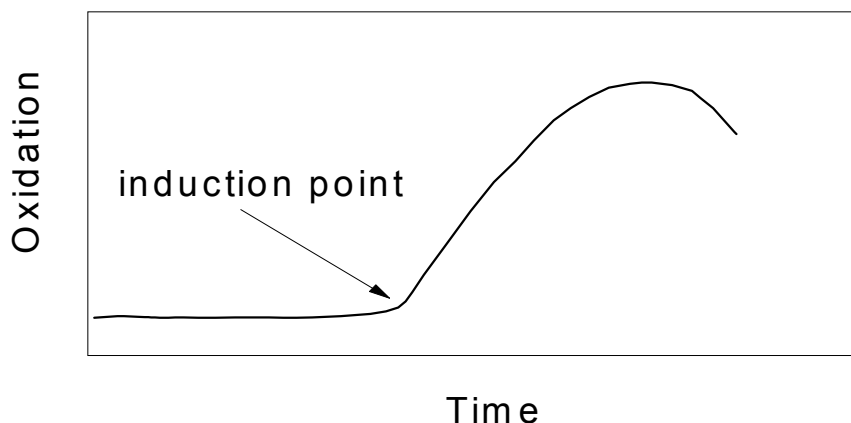
SIDE BAR

Oxidation Reaction Mechanisms

Researchers on food stability have extensively probed the mechanisms by which fats and oils are oxidized to cause rancidity. Three steps are recognized in the autoxidation (or free radical) process and the following are two different reactions typical of those that can occur in each step:



The rate of these reactions depend on the presence of prooxidants, antioxidants, energy inputs (light or heat), enzymes that catalyze lipid oxidation, the nature of the fat and the level of moisture in the food. Metals can participate by promoting both initiation and propagation reactions. In general, the rate can be described as shown below.

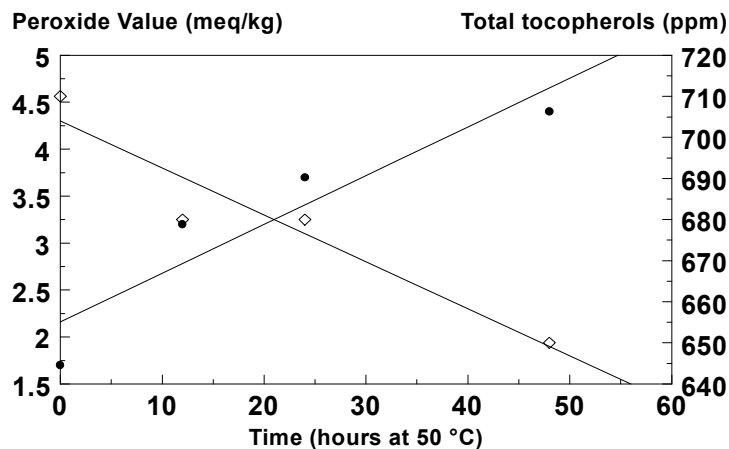


There is an initial phase where oxidation occurs very slowly, or not at all. After initiation and under favorable conditions, free radical reactions increase in rate very rapidly because they are self-catalyzing. This rapid phase continues until the substrate is depleted or, until conditions are changed to quench the reactions. Examples would be drying or chilling the food.

The measurement of lipid oxidation is generally done by measuring the first relatively stable products of the reactions – the peroxides and hydroperoxides. The “peroxide value” measurement is used to determine the quantity of these species in foods and is one of the most sensitive techniques during the early stages of lipid oxidation. However, in later stages of the process, the peroxides themselves degrade via a series of complex reactions to organic acids, alcohols and aldehydes. Many of these secondary products are strongly flavored (painty or cardboard-like) and lead to stale tastes that decrease palatability. During the later stages of oxidation, other analytical methods such as TBARS (thiobarbituric acid reactive substances) are used to measure the secondary reaction products.

Addition of antioxidants such as ethoxyquin, TBHQ, BHA or BHT will usually delay induction time by “soaking up” free radicals and converting them to inactive forms. These phenolic compounds are generally called “chain breaking” antioxidants since they interrupt the propagation step by removing free radicals, thus breaking the chain reactions.

While loss of valuable essential fatty acids is the main toll of these reactions, they also destroy sensitive vitamins such as A, C, and E as well as sulfur amino acids and other ingredients. The effect on Vitamin E is especially important since tocopherols act as chain breaking antioxidants. The following graph illustrates an example of this effect where the peroxide value of palm oil increases with corresponding decreases in total tocopherol levels.



The nature of a fat has a profound effect on the rate of oxidation, and some fats such as coconut and palm oil are very resistant to oxidation, while others such as fish oil, soybean oil, lard and tallow are less so. It has been well recognized that incorporating relatively unstable fats in an animal feed can result in loss of tocopherol with resultant nutritional diseases.

Prooxidants

Triggering the initiation of the chain reaction generally needs a nudge from either a prooxidant substance or the input of energy or both. Transition metals and acids are both common prooxidants.

The transition metals - including cobalt, copper, iron and manganese - are nutritionally important but can also be good catalysts to promote electron transfers. The electron configuration in their atomic structure allows them to act as an effective shuttle by readily accepting and donating electrons in a suitable environment.

Acids are also electron acceptors as illustrated by the definition of acids and bases introduced in the early 1900's by G. N. Lewis. A Lewis acid is a material with a tendency to accept electrons from another (which is the corresponding Lewis base). Thus, any acid or acid salt of a metal will be a prooxidant. However, sulfate (SO_4^{2-}) and nitrate (NO_3^-) are among the strongest prooxidants because sulfur and nitrogen are nearly as electronegative as oxygen. Therefore those oxy-anions have a very high affinity for electrons. When a metal sulfate is included in food, the sulfate anion pulls electrons away from the metal leaving it depleted, and thus highly active as an electron acceptor. With elemental oxygen available as the ultimate "sink" for the transferred energy, the metal cation is able to act like a catalyst, continuously shuttling the charges without itself becoming depleted.

When there is moisture available, as in a food product, chemical reactions are inevitably accelerated. For oxidation reactions, this effect is increased up to the point where there is enough water to interfere with oxygen transfer to the target sites. This impact of water is particularly true when the water-soluble salts of metals (i.e. copper sulfate) are involved. They become extremely active since they are hygroscopic, drawing water even from humid air. A dry food product normally contains at least 10% moisture, so copper sulfate can pull water to its surface. It then dissociates into copper cations and sulfate anions in the film of moisture that collects on the surface of each particle, making the surface a "hot spot" for electron transfers.

Metal Chelates

There has been considerable recent interest in the use of metal chelates in both human and animal nutrition. This grew out of the observation that metals are transported and utilized within organisms in the form of organo-metallic complexes. These are formed naturally since an organism generates the appropriate organic "partners" – chemists call them ligands – to bond to the metal. An animal typically generates a number of distinctly different complexes with a given metal, each serving a specific purpose. Those who advocate chelated minerals in the feeding of animals believe that attaching the metal to some organic molecule will give nature a head start in these processes. Skeptics question the concept since the animal must convert any given starting form into the full range it needs and in the relative amounts needed at any given time. In this view, the animal will naturally manufacture the chelate(s) it needs, so long as the metal can be readily absorbed from the gut (i.e. is bioavailable).

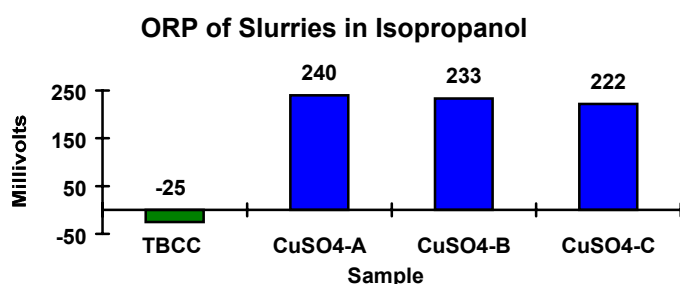
By definition, a chelate is a compound in which each metal atom shares coordinate bonds with at least two non-metal atoms in the same molecule. The name comes from the Greek word for crab because the two points of attachment to the organic molecule can be visualized as the pinchers of a crab holding the metal. True chelates are inevitably more expensive to manufacture than inorganic salts – typically causing a manifold increase in the cost of a unit of mineral. The purported advantages of chelated minerals are used in marketing to justify their necessarily higher cost.

Unfortunately, due to the higher selling prices associated with the term chelate, a wide range of metal-containing materials are marketed under that generic heading, leading to misunderstanding and confusion. Some commercial ingredients sold as chelates have well defined chemical structures, while others are only loosely defined, if at all. Some are salts of a specific amino acid while others are simply called “proteinate”. Some are water soluble (and therefore hygroscopic) while others are not. Bioavailability data for these products must therefore be considered as specific to the product and to the application tested. Similarly, predictions about their activity as prooxidants cannot be generalized. For example, a metal salt of a strongly electronegative organic acid will be a strong prooxidant.

A New Class of Inorganic Metal Ingredients

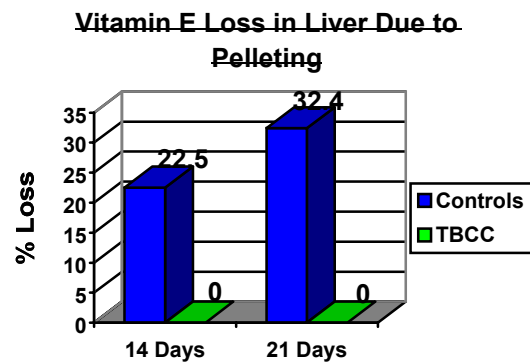
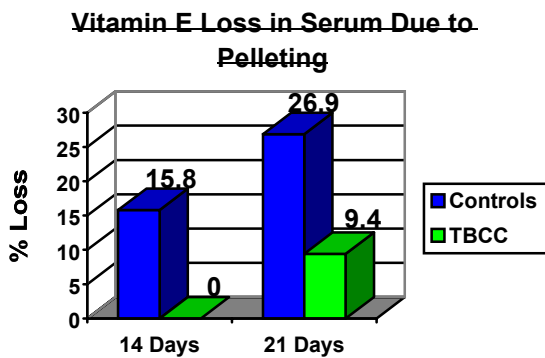
Neutral, inorganic salts of metals represent a relatively new class of mineral ingredients. One example that has been widely used in commercial animal production for several years is tri-basic copper chloride (TBCC) (see Miles, 1998). This compound can be thought of as a hybrid between copper chloride (strongly acidic) and copper hydroxide (strongly alkaline), in which three-fourths of the acidity has been neutralized. The result is a salt that is totally insoluble in water and yet very easily and quickly soluble in an animal’s gut (a low pH and complexing environment). Animal testing in swine, poultry and cattle has shown this salt to have bioavailability 7% to 21% higher than copper sulfate (the normal standard of comparison).

A key impetus behind development of tri-basic copper chloride as a feed ingredient was realization that it would be far less destructive, both because of its low water solubility (and thus hygroscopicity) and also because it would behave as a Lewis base in a food or feed mixture. The following graph compares it to several feed-grade copper sulfate samples in an ORP test. ORP stands for oxidation/reduction potential and is measured between a standard reference electrode such as calomel and a precious metal electrode in contact with the test sample. It measures the tendency of the specimen to either accept electrons (a prooxidant) or contribute them (a reducer or anti-oxidant).

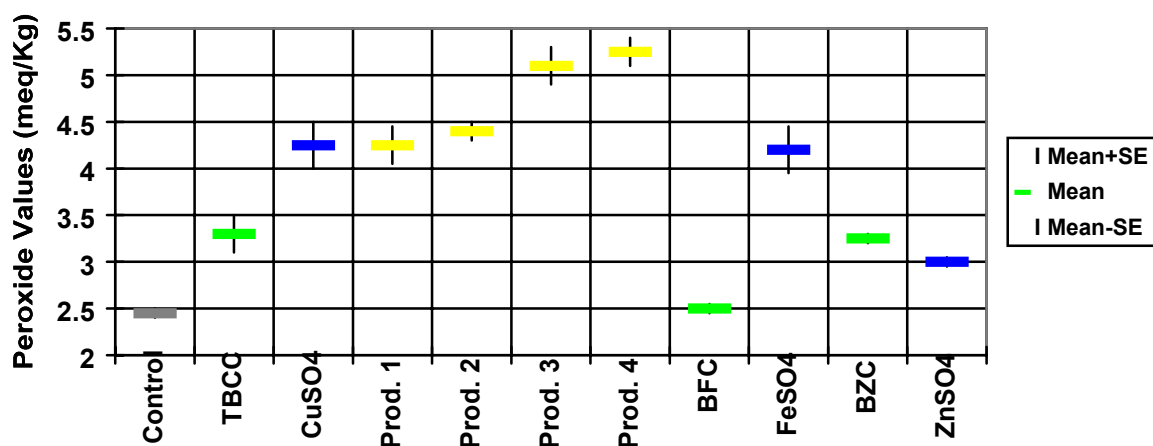


Work done at PARC Institute, Easton, MD in 1997 evaluated the effect of TBCC in protecting vitamins during pelleting of chicken feed. In the initial screening phase of the work, single samples of raw mash and pelleted feed for Starter, Grower and Finisher diets were analyzed for Vitamins A, D3, E and Riboflavin. The results were variable due to the inherent imprecision in assaying a complete feed, but did show a consistent pattern with the samples containing TBCC losing less of each vitamin during the pelleting process.

In a subsequent phase, 240 chickens were fed for 21 days and samples of serum and liver tissue analyzed on days 14 and 21 for Vitamin E. There were six replications of 10 birds per treatment. Controls had only the basal amount of copper (12 ppm Cu) from copper sulfate and were fed as raw mash or crumbled pellets. The other two treatments were mash and crumbled pellets with 250 ppm Cu from TBCC added to the same starter diet as the controls. The following graphs show the loss of Vitamin E activity caused by pelleting as measured in serum and liver samples and expressed as a percentage of the level in the unpelleted diet. It is normal to see higher differences in the liver because it serves a homeostatic function, striving to keep blood levels stable. The feed mixtures without TBCC lost as much as 32% of their Vitamin E potency as a result of oxidation initiated by pelleting. The mixtures containing TBCC lost little or none.



Recently, preliminary experiments were done at the Food Science and Human Nutrition department at the University of Florida to compare the prooxidant activity of neutral salts of copper, zinc and iron to the corresponding acid salts normally used as feed ingredients and to four commercial products marketed as copper chelates. A conventional poultry feed formula was supplemented with each of the test sources to give 100 ppm of the respective metal. The resultant mixes were analyzed for peroxide values after controlled storage for two days at 37°C. Although none of the observed values are yet high, the speed with which peroxides are initially formed is known to be a strong predictor of the rate at which substrate will be degraded over time.



Control – Poultry diet with no metal supplementation

TBCC – *Micronutrients TBCCTM* - $\text{Cu}_2(\text{OH})_3\text{Cl}$

BZC / BFC – Basic zinc chloride / basic ferrous chloride made by Micronutrients.

Acid Salts – $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot \text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$

Products 1,2,3 and 4 – Proprietary copper / organic feed ingredients

Implications in Petfood

Petfood manufacturers have a much more difficult challenge than commercial animal producers in controlling mineral interactions for several reasons.

- Processing is often done at higher temperatures to destroy pathogenic organisms. The additional energy input increases the likelihood of initiating oxidative reactions.
- Shelf storage of dry processed feeds allows destructive reactions to proceed albeit at a slow pace.
- Wet or semi-moist products contain enough water to allow other chemical interactions to proceed even when oxidation is effectively avoided by excluding oxygen.

Choosing a source for an essential metal under these conditions is a difficult compromise.

Insoluble alkaline salts such as copper oxide or zinc oxide have less potential for interactions in the product, but also have very low bioavailability and therefore, do not provide the proper health-sustaining nutrition. Acid salts, such as the sulfates of the metals, are more available to the animal, but they are highly water soluble, allowing reactive metal ions to promote free radical formation or to interact with the food in other ways.

The use of antioxidants has been getting increasing scrutiny and acceptable levels of some common compounds such as ethoxyquin have been lowered from 150 to 75 ppm. Thus, choice of mineral ingredients that are less active in initiating oxidation reactions will be helpful in providing high nutritional quality and excellent palatability.

Interactions with water-soluble minerals are particularly acute in medium or high moisture products. As an example, under the anoxic, reducing conditions found in a canned product, copper, zinc and iron sulfates can be reduced and form the corresponding sulfides, which are

completely unavailable. This almost certainly contributed to the discoloration observed when some formulations were switched from copper oxide to copper sulfate (Shields, 1998), because copper sulfide is a black compound.

A neutral, water-insoluble inorganic salt such as tri-basic copper chloride has excellent bioavailability but greatly reduces interactions with other food ingredients, thus protecting vitamins, and improving shelf life, appearance and palatability.

A highly refined form of tri-basic copper chloride has recently been developed by Micronutrients specifically to meet the needs of the petfood industry, and is available under the trade name Greenshield TBCC™. This product is manufactured with a high level of documented consistency and quality assurance in an ISO 9002 registered facility. Development of a copper product was undertaken first because copper is recognized as the essential metal that is most antagonistic to food stability. Eventually an entire family of highly bioavailable, neutral, inorganic metal salts will be available to petfood manufacturers, further improving the stability and nutritional efficiency of their products.

REFERENCES

Ammerman, C. B., D. H. Baker & A. J. Lewis (eds.) (1995) Bioavailability of Nutrients for Animals: Amino Acids, Minerals and Vitamins. Academic Press, New York, NY.

Asher, C. J. 1991. Beneficial elements, functional nutrients, and possible new essential elements. p.703-723. *In* J. J. Mortvedt et al. (ed.) Micronutrients in agriculture, 2nd Edition. SSSA, Madison, WI.

Combs, G.F. Jr., 1998. Adequate vs. optimum. *Petfood Industry* 40/ 4: 31-43. Optimum vs. Essential – *Petfood Industry*

Miles, R.D., O'Keefe, S.F., Henry, P.R., Ammerman, C.B., Luo, X.G., 1998. The effect of dietary supplementation with copper sulfate or tribasic copper chloride on broiler performance, relative copper bioavailability, and dietary prooxidant activity. *Poultry Sci.* 77:416-425.

Shields, R.G. Jr., 1998. Vitamin / mineral update. *Petfood Industry* 40/3: 4-14.