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Measuring trace mineral bioavailability key

It is important to measure several indicators of trace mineral bioavailability in order to predict the outcome of feeding a particular mineral source.

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ZINC, copper, manganese and a number of other essential trace minerals are required for the proper development, maintenance and health of animals (Underwood and Suttle, 1999).

Collectively, these minerals support such diverse functions as immune development and function, structural integrity of bones and tissues, reproduction and defense against oxidative stress. As such, these trace minerals are supplemented in virtually all animal diets either as inorganic trace mineral (ITM) salts — such as sulfates and oxides — and/or as organic trace minerals (OTM).

While ITMs are relatively inexpensive, it is generally accepted that they suffer from relatively poor bioavailability compared to some chelated minerals, primarily due to the numerous antagonisms and interactions among ITMs and other components of the digesta (Leeson and Summers, 2001; O'Dell, 1989; Underwood and Suttle, 1999).

Phytic acid, for example, binds to trace minerals and precipitates at the neutral pH of the small intestine, thereby rendering the minerals unavailable for absorption (Cheryan, 1980; Leeson and Summers, 2001). Many other types of antagonisms have been reported (O'Dell, 1989; Underwood and Suttle, 1999).

It has been accepted that a higher plane of nutrition is required to achieve the full genetic and economic potential of today's animals. Optimizing mineral nutrition is part of this package.

So, how is this done, given the limitations in bioavailability of ITMs? The solution is not simply to feed higher levels of minerals, as this in itself can lead to even lower mineral bioavailability, increased environmental burden and potentially even decreased animal performance. The key is to feed a more bioavailable source of trace minerals.

What does bioavailability mean? Mineral bioavailability is simply the degree to which an animal can absorb and utilize a mineral (for example, zinc) from one particular source (such as a chelate). In practice, bioavailability of a mineral from a given source is usually measured relative to the same mineral in a second, or "standard," source.

In bioavailability experiments with OTMs, the standard source is typically an ITM salt like zinc sulfate or manganous oxide. Chelated minerals are widely reported to be more bioavailable than ITMs, presumably due to their ability to avoid feed ingredient antagonisms and deliver the trace mineral to the small intestine for absorption (Cao et al., 2000; Fly et al., 1989; Guo et al., 2001; Predieri et al., 2005; Wang et al., 2007; Yan and Waldroup, 2006).

Still, not all OTMs are more bioavailable than ITMs. While chelation is important, just because a mineral is chelated does not guarantee that it is more absorbable than an ITM salt. For this reason, it is important to examine how bioavailability is estimated.

Measuring bioavailability

People have tried to measure or predict mineral bioavailability with a wide variety of assays, only some of which are relevant or useful.

Furthermore, while it is tempting to focus on only one or two particular measures of bioavailability, it is important to realize that this would give only a partial understanding of OTM bioavailability and function.

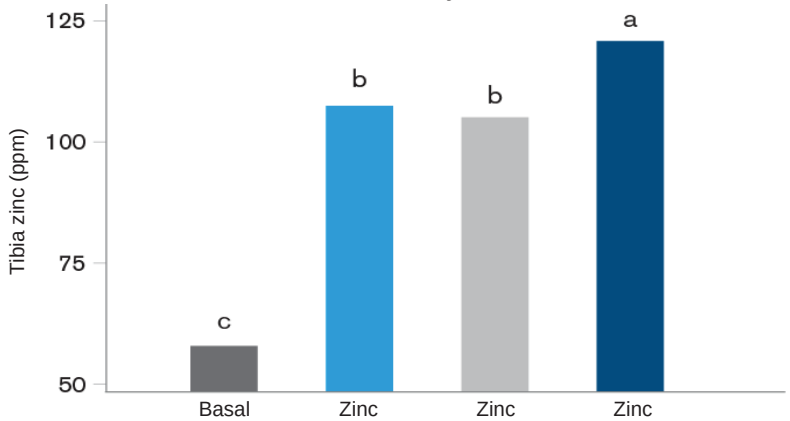
Rather, it is important to measure several indicators of mineral bioavailability in order to be able to understand and predict the outcome of feeding a particular mineral source.

Several labs have run chemistry-based experiments that attempt to define the physical characteristics that lead to high mineral bioavailability or have developed elaborate *in vitro* model systems of absorption to predict bioavailability *in vivo*, most of which have proven to be relatively uninformative.

For example, one test that has been proposed is solubility of the mineral source in water or buffered solution. Initially, the notion that solubility would predict bioavailability seemed to make sense in that in order for a mineral (or any nutrient) to be absorbed, it has to be in a soluble form at the absorptive surface of the small intestine. In addition, there are data to indicate that bioavailability and degree of solubility of OTMs at specific pH levels can be related (Guo et al., 2001).

However, if solubility were the key

1. Relative bioavailability of zinc sources



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to bioavailability, then ITM salts would be extremely bioavailable. Copper, manganese and zinc sulfates are all highly soluble in water (CRC, 1981-82), yet they are not as bioavailable as a high-quality OTM. In fact, the majority of data show that there is little relationship between mineral solubility and mineral bioavailability (Guo et al., 2001; Ledoux et al., 1995; Miles et al., 1998).

Some peer-reviewed data even suggest that for zinc chelates, high solubility is inversely related to bioavailability in both poultry and ruminants (Cao et al., 2000). Thus, one should be cautious about using solubility — or any *in vitro* test — to predict the relative bioavailability of a trace mineral source.

Measuring the deposition or storage of minerals into selected tissues is the most common output in trace mineral relative bioavailability experiments (Underwood and Suttle, 1999).

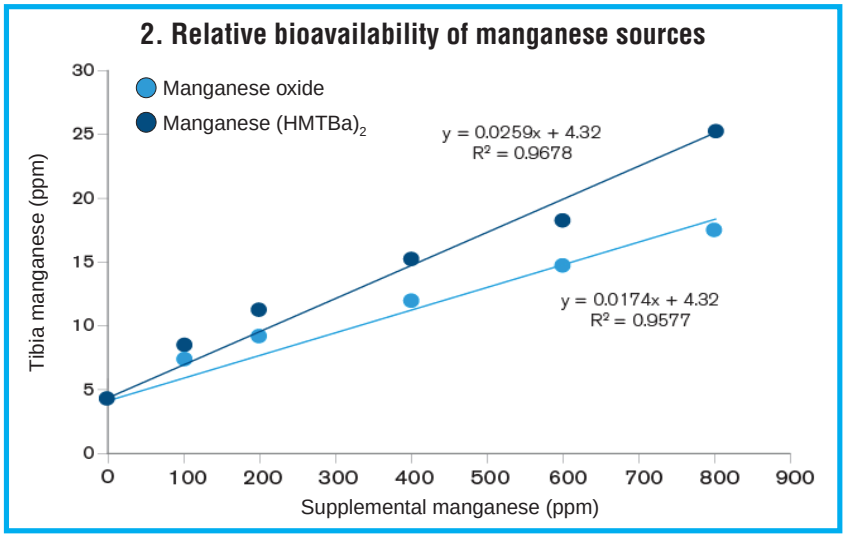
Figures 1 and 2 are from experiments that compared the relative bioavailability of zinc sources and manganese sources, respectively, by measuring tibia zinc or manganese content. Figure 1 shows that while all supplemented treatments (added at 40 parts per million of zinc) increased tibia zinc, zinc $(HMTBa)_2$ (zinc chelated by two molecules of the methionine hydroxy analogue) was more bioavailable than the other two sources.

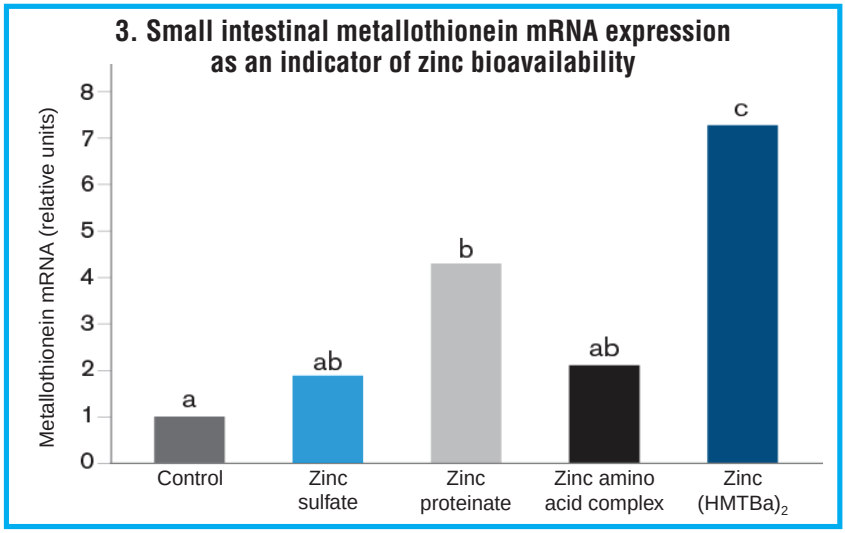
Figure 2, which is from a slope-ratio design, demonstrates that manganese $(HMTBa)_2$ was about 150% as available as the manganese from manganous oxide (Dibner, et al., 2004; Yan and Waldroup, 2006).

These experiments support the notion that certain chelated minerals can be more bioavailable than ITMs.

Still, tissue mineral experiments have some limitations. First, these experiments measure only a fraction of the mineral that is absorbed. Minerals are absorbed by the small intestine and then distributed via the bloodstream to other tissues. Therefore, tissue mineral levels only measure the mineral distributed to those particular tissues and, as such, may not reflect total mineral uptake.

A second limitation is that tissue mineral levels actually represent a storage pool of mineral rather than the total amount of mineral delivered to that particular tissue. In general, tissue mineral stores can be altered as a consequence of mineral excretion, use or stress (Underwood and Suttle, 1999). Therefore, measuring tissue minerals tells only part of the story. To truly measure bioavailability, it would be useful to measure mineral absorption in the small intestine where absorption occurs.





Biomarkers

Another method for estimating bioavailability is to measure mineral-responsive biomarkers, such as changes in gene expression, or the activity of a mineral-dependent enzyme. Biomarkers are particularly informative when measured in the small intestine.

Metallothionein is one such biomarker, because its expression is regulated by zinc status; the magnitude of metallothionein messenger RNA (mRNA) and protein expression depends on the amount of zinc absorbed (Davis and Cousins, 2000). Therefore, metallothionein mRNA or protein expression is often used as an indicator of the zinc status of humans and animals and to evaluate the bioavailability of different zinc sources (Blanchard et al., 2001; Cao et al., 2002; Huang et al., 2009; Lu et al., 1990; Martinez et al., 2004; McCormick et al., 1981; Reeves, 1995; Rojas et al., 1995; Sullivan et al., 1998).

Figure 3 shows an example of using small intestinal metallothionein mRNA expression as an indicator of zinc bioavailability (Richards et al., 2007; Richards et al., 2008b). In this experiment, broilers were fed control diets or diets supplemented with 70 ppm zinc from the indicated sources. Because zinc absorption occurs in the small intestine, differences in metallothionein expression here would be expected to more closely represent relative bioavailability than tissue zinc levels would.

These data are consistent with the data in Figures 1 and 2 in that they demonstrate that some, but not all, chelated zinc sources are more bioavailable than inorganic zinc. Consistent with the other experiments, $Zn(HMTBa)_2$ was the most available source.

Measuring tissue minerals or mineral-dependent biomarkers can be the easiest and most straightforward measures to generate a quantitative estimate of mineral bioavailability.

Fundamental roles

With that said, it is important not to stop there. Zinc, copper and manganese play fundamental roles in the biochemistry

of the cells of the animal, primarily by serving as essential components of hundreds of cellular enzymes and transcription factors (Underwood and Suttle, 1999).

If trace minerals are limiting in the diet, any of these processes can suffer.

So, by supplementing diets with more highly available forms of trace minerals, a producer will more effectively "feed" these enzyme systems, which should translate into a wide variety of biological benefits.

Indeed, recent results with trace minerals chelated with HMTBa have demonstrated benefits such as enhanced immune function (Dibner, 2005), reduced incidence of tibial dyschondroplasia (Dibner et al., 2007; Richards et al., 2006) and footpad lesions (Richards et al., 2006), reduced leg abnormalities (varus, valgus, shaky leg) and increased bone breaking strength (Ferket et al., 2009), reduced oxidative stress (Richards et al., 2008a) and improved performance (Ferket et al., 2009) even at lower levels of trace mineral inclusion (manuscript submitted).

While measures such as these are not traditionally considered to be indicators of trace mineral bioavailability, they can and should be used to confirm the information gained from assessing more customary measures, such as tissue minerals and genetic biomarkers.

Conclusions

Feeding high-quality, high-bioavailability trace minerals is an important key to maximizing the growth potential and well-being of production animals. 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 of the animal's cells and tissues.

This, in turn, leads to a wide variety of biological and performance benefits. Unfortunately, not all OTMs can do this. Only by truly understanding the structure and consistency of a given OTM source and by rigorously investigating its bioavailability through a variety of methods can one be assured of the predictability and consistency of the animal's responses to OTM supplementation.

Mineral bioavailability has been estimated in a wide variety of ways that vary greatly in their effectiveness. In general, techniques that rely on chemical assays and *in vitro* methodology are of limited utility and can be misleading. In contrast, *in vivo* methods can be very reliable methods on which to base nutritional decisions.

Finally, and perhaps most importantly for decision-making, we need to rely on not only one but many different *in vivo* techniques for assessing trace mineral quality and bioavailability. Only when we examine a variety of different readouts, including tissue mineral levels, mineral-dependent biomarkers and functional assays, can we make well-informed, confident decisions about trace mineral supplementation.

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