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Experimental designs to study organic trace mineral sources in animal nutrition

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Summary

Organic trace minerals are widely used in animal nutrition and play an important role in today's high performing farming animals. Their accepted high bioavailability contributes to reduce constant or time limited trace mineral deficiencies having a negative impact on productivity.

The industry offers a wide variety of organic trace mineral forms. Those are mostly difficult to identify and differentiate to each other due to lacking official analytical methods. Animal studies are therefore highly important to verify the quality and benefits for a given organic trace mineral source.

The goal of this paper is to resume the main in-vivo methods used to study organic trace minerals (Cu, Fe, Mn, Zn). Those have been classified as:

- Bioavailability studies:
  - Bioavailability below requirement
  - Bioavailability highly above requirement
  - Bioavailability through a single oral application
- Studies at adequate mineral supply (for performance data).

Trace mineral bioavailability, including absorbability and retention data in specific organs or tissues is the best information to clearly differentiate the quality of different supplemental sources. The study method "below requirement" is the most appropriate bioavailability method for this.

Data from "performance studies at adequate mineral supply" are related to the bioavailability studies: Supplemental sources with a proven increased bioavailability limit clinical or sub-clinical trace mineral deficiencies, which often occur in practice due to limited mineral supply, mineral antagonism or mineral imbalances. This even when trace minerals are supplied at recommended

levels. Reduced clinical or sub-clinical trace mineral deficiencies improve animal performances.

In veterinarian use, the short-term bioavailability studies "through single oral applications" are most appropriate for their direct compatibility to practice.

Market and identity of organic trace minerals

Today's high performing farming animals are very sensitive to the concentration and quality of each dietary nutrient. Essential trace minerals are part of those nutrients, affecting animal performance, fertility and health. The natural dietary trace mineral content and their availability are highly variable; and parallel to this, the individual trace mineral requirements fluctuate depending on animal age, production stage, … which mostly exceed the natural available dietary supply. Additional trace minerals such as iron, zinc, copper, manganese, cobalt and selenium are therefore supplied to the feed in either salt (oxides, sulfates, chlorides, carbonates) or in organic forms, primarily to guarantee an adequate trace mineral supply. Another common reason to supply trace minerals is the addition of zinc oxide and/or copper sulfate at pharmacological levels to monogastrics (3000 ppm, 250 ppm respectively) for antimicrobial reasons at feed and intestinal level.

Organic trace minerals have been supplemented into the animal feed industry for several years and represent today a world market evaluated between 100 and 200 million Euros. In Germany, 2% of the dairy herd was supplied with organic trace minerals in 1999 (1). Up to now, the German organic trace mineral market has increased, and its value represented in 2004 about 20% of the total estimated 12.9 million Euros trace mineral market (2).

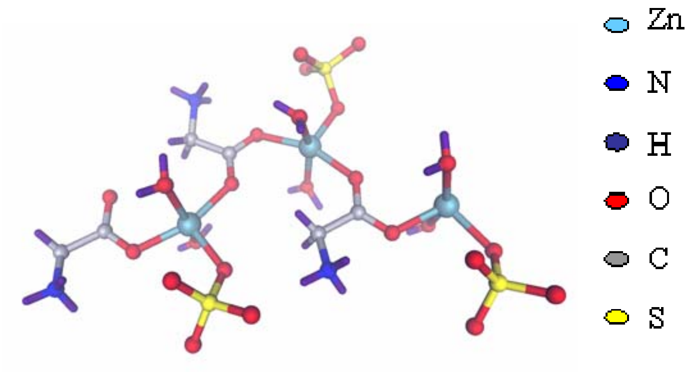
Parallel to the organic trace mineral market development, numerous studies have been conducted in the past 30 years to evaluate their potential benefits. Studies were reviewed by several authors (3, 4, 5, 6) and general conclusions on the benefits of organic trace minerals were positive, but highly heterogeneous. Based on various animal age, production stage or body weight; based on various mineral inclusion rates and measurement parameters, based various mineral reference source and based on the variety of organic sources, this heterogeneity is most probably explained.

Categories for the commercially available/authorized organic sources have been defined (table 1) by the Association of American Feed Control Officials (7) and, specifically for the E. U., by the European Commission (8).

Table 1: Official definitions of organic trace minerals to be used in feedingstuffs

AAFCO (2001)	OJEC (2003)
Metal Amino Acid Complex Product resulting from complexing a soluble metal salt with an amino acid(s).	
Metal (specific amino acid) Complex Product resulting from complexing a soluble metal salt with a specifi amino acid.	
Metal Amino Acid Chelate Prduct resulting from the reaction of a metal ion from a soluble metal salt with amino acids with a molecular ratio of one mole to one to three (preferably two) moles of amino acids to form coordinate covalent bonds. The average weight of the hydrolized amino acids must be approximately 150 and the resulting molecular weight of the chelate must not exceed 800.	**Metal Chelate of Amino Acids Hydrate** $M\ (x)_{1\text{-}3} * n\ H_2O$ with x= anion of any amino acid derived from hydrolized soya protein. Molecular weight not exceeding 1500.
Metal Polysaccharide Complex Product resulting from complexing a soluble salt with a polysaccharide solution.	
Metal Proteinate Product resulting from the chelation of a soluble salt with amino acids and/or partially hydrolyzed protein.	

These defined categories would help to describe the identity of each source. But since most available organic trace minerals have so far, not been successfully analyzed on their chemical structure or at least on their “chelation” or “complexation” degree (9), their categorization remains fully theoretical. This lack of identification, as well as, the mostly imprecise product description in scientific literature contributes to obtain varying data in animal studies. Four new commercially available organic trace minerals can be routinely analyzed on their precise chemical composition and structure. These are crystalline complexes of iron glycinate, zinc glycinate, copper glycinate and manganese glycinate. Their stable crystalline form allows a structural analysis through official X-ray technique (10). As an example, the structure of zinc glycinate is described in graph 1.



Graph 1: Structure of zinc glycinate, dihydrated

The goal of this paper is to resume the different in-vivo experimental designs to determine the interest of organic trace mineral sources (Cu, Fe, Mn, Zn).

Experimental designs

Bioavailability studies:

“Bioavailability” is the most common term in mineral nutrition to evaluate different trace mineral sources. But its wide utilization has unfortunately led to numerous definitions, which end in sometimes misleading interpretations. It is therefore important to clarify which meaning is given to “mineral bioavailability” whenever used. Here, the term “trace mineral bioavailability” is defined as (11):

“The maximal trace mineral utilization for the biological functions in the metabolism, based on the amount of ingested trace mineral”

The term mineral bioavailability illustrates therefore the “quality” of a supplied form with data including:

- Maximal mineral absorbability
- Maximal metabolic utilization of the mineral (concentration in blood plasma, secretory fluids and tissues, activity of mineral dependent enzymes, performance).

The maximal mineral absorbability and the maximal metabolic use of a defined source can be studied in quantifying the trace mineral fluxes on laboratory

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animals (12, 13, 14). Zinc sulfate was recently compared to zinc glycinate on growing rats, previously labeled with ^{65}Zn (15). The true zinc absorbability of the organic source was significantly ($p<0.05$) increased by 14.9%, while endogenous losses were equal between both treatments. Zinc losses through urine were low and similar in both treatments. These results therefore indicate similar and high metabolic zinc utilization for the sulfate and glycinate source (95%, 96% respectively), but an improved zinc bioavailability with zinc glycinate (+16%) due to its better absorbability. Based on this study, the benefits in using organic zinc sources are therefore mainly due to their high absorbability.

In farming animals, the use of labeled minerals is highly limited for practical reasons, but the experience on quantitative zinc fluxes in laboratory animals helps to set up the experimental design of bioavailability studies in monogastrics or ruminants and would help their result interpretation.

The main complication in bioavailability studies consists in the fact that the animal naturally thrives to maintain a more or less constant trace mineral concentration in its metabolism with the help of homeostatic regulation processes, such as varying endogenous mineral excretion (16, 17, 18). The homeostatic regulation “softens” therefore the eventual mineral source difference when for example; absorbability or blood status is measured. Bioavailability studies must therefore be conducted in conditions limiting, as much as possible, the homeostatic regulations (19, 20). Those conditions are either created with the application of mineral dosages:

- Below requirement, more precisely below mineral homeostatic regulation.
- Highly above requirements, more precisely above mineral homeostatic regulation.
- Single oral application followed by short-term measurements not giving enough time to the animal to adapt its homeostatic regulation.

Depending on the specie, the metal and the applied study method, measurable parameters are not all equally important (5). These authors suggested weighing them differently in order to define one final average bioavailability value for a given source. When studying the bioavailability of mineral sources, those are usually compared to an iso-dosed reference source (metal sulfate) and thus described as a relative bioavailability value (RBV). As example, table 2 lists the importance of each parameter (increasing importance with increasing value) for zinc bioavailability in ruminants and iron bioavailability in pigs (5). The RBV of a source is calculated as an average of all weighed parameter values. Table 3 resumes the calculated RBV of several organic zinc sources to $ZnSO_4$ in ruminants and organic iron sources to $FeSO_4$ in pigs (5). It can finally be noticed that the number of compatible bioavailability studies on organic trace minerals is limited. This last observation is also noticed in the bioavailability review written by Ammerman et al (3).

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Table 2: Ranking of importance of various response parameters

Parameter	below requirement		above requirement	
	Zn (ruminant)	Fe (pig)	Zn (ruminant)	Fe (pig)
Apparent absorbability	2	4	1	1
Liver	4	1	2	2
Kidney	3	n.a	1	n.a.
Plasma	2	n.a	1	n.a.
Haemoglobin	0	2	0	2
Enzyme activity	2	n.a	2	n.a.
Performance	0	1	0	0

n.a. : ranking not mentioned

Table 3: RBV of different zinc sources in ruminants and iron sources in pigs

	Zn in ruminants		Fe in swine	
Organic source	n° studies	RBV	n° studies	RBV
Metal proteinate	1	102	0	n.a.
Metal chelate	2	97	1 [3)]	102 [3)]
Metal amino-acid complex	1	102	1 [1)]	109 [1)]
Metal methionine	6 [2)]	101 [2)]	0	n.a.
Metal lysine	2	107	0	n.a.
Metal methionine + lysine	1	105	0	n.a.
Metal glycinate	1 [2)]	146 [2)]	1 [3)]	124 [3)]

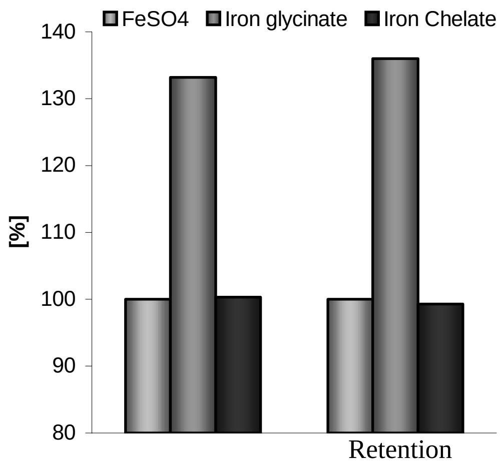
Recent data added from: [1)] 21 [2)] 22 [3)] 23

Another method to evaluate the RBV of mineral sources consists in regression analysis when several mineral dosages were used (3, 24)**.**

Bioavailability below requirement:

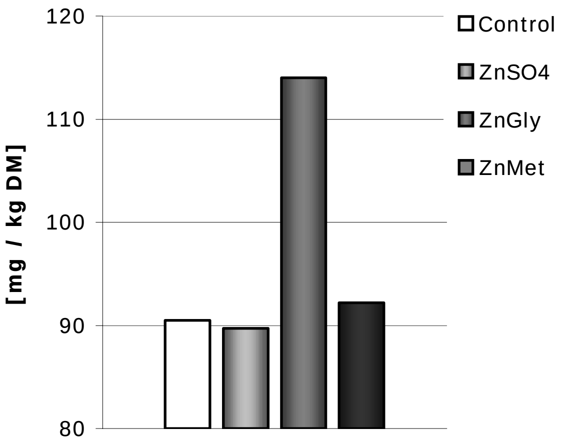
This method consists in using animals, which are deficient in the studied mineral. Achieved by either feeding a diet low in the content of the specific mineral or high in the content of the mineral's antagonists such as phytate in monogastrics, for example. Their metabolism and homeostatic regulation is then set to absorb, retain and use the maximum possible mineral quantity provided through the diet. Endogenous mineral excretion is minimized and mineral storage in tissues maximized. An eventual difference in apparent absorption or tissue concentration data is therefore directly related to the tested mineral source. This method requires an important attention to eventual mineral contaminations (through installation, water, …) and a control treatment (negative or positive) is recommended. Two studies are briefly described below:

A recent study (23) in weaned piglets permitted to measure the apparent absorbability of 28 ppm supplemental iron (as iron sulfate, iron glycinate and iron European chelate) added to a control diet naturally containing 33.7 ppm Fe. Fecal iron concentration was reduced with iron glycinate when compared to iron sulfate (-11.9%, $p<0.10$) or to iron chelate (-15.8%, $p<0.05$), which resulted in improved ($p<0.05$) iron absorbability and retention for iron glycinate (graph 3).



Graph 3: Iron absorbability and retention relative to sulfate

Organic zinc sources are mainly used in beef and dairy cattle. Only a limited number of studies have been conducted to clearly assess their bioavailability (table 3). The source assessed with the best relative zinc bioavailability (zinc glycinate, with RBV of 146%) was based on the following study (22): 20 ppm of zinc sulfate, zinc glycinate or zinc methionine were compared to each other in zinc depleted beef cattle. Apparent absorbability was numerically increased with zinc glycinate inducing an increased plasma zinc ($p<0.05$) and liver zinc ($p<0.05$) concentration compared to zinc sulfate at the end of the 42-day repletion study (graph 2). Zinc methionine was not clearly differentiated to zinc sulfate.



[1)] Means adjusted using day 0 liver Zn as a covariant

Graph 2: Liver Zinc concentrations on day 42 [1)]

When conducting bioavailability studies below homeostasis, with diets naturally high in mineral antagonists, organic trace minerals are supposed to be less affected (chelation with unabsorbable antagonist or competition on absorption site) than inorganic sources. As example, copper – zinc antagonism was studied with inorganic and organic sources in growing pigs (25). Organic copper did not lead to any benefits compared to inorganic copper, but organic zinc improved ($p<0.05$) the apparent absorbability of both minerals.

Other possibilities consist in studying the effect of phytate rich diets on different zinc and copper sources in monogastrics or to study the effect of high dietary molybdenum and sulfur on the bioavailability of several copper sources in ruminants.

Numerous suggestions have been written down on the possible reasons for improved organic trace minerals absorbability. Additional measurements on intestinal tissues (semi-in vivo) with the help of using chambers or Caco-2 cells for example, would permit to investigate further the potential different mode of absorption between inorganic and organic minerals.

Bioavailability highly above requirement:

This study method consists in creating a mineral “overflow” to the animal, which saturates its homeostatic regulation capacity. The collected data illustrates the animal’s capacity to eliminate mineral in excess or its mineral tolerance. Mineral concentrations in bone or liver are therefore meaningful indicators, whereas mineral absorption or mineral plasma concentration are less appropriate (table 2). The compatibility of this method with the definition of bioavailability is questionable since mineral absorbability is useless due to the “overflow” and its utilization is saturated.

The potential on increased growth promotional effects in piglets with the use of organic trace minerals instead on inorganics or the possible reduced inclusion level with organic sources for equal performances has been of interest since the ‘90s. Studies were conducted above homeostatic regulation with organic minerals and compared to zinc oxide (1500 - 3000 ppm) or copper sulfate (150 - 250 ppm). Jondreville (4) reviewed the published studies according to that theme and concluded that organic sources of copper and zinc would not improve growth in a greater extent than pharmacological levels of copper sulfate or zinc oxide. Since pharmacological dosages of zinc and copper have antimicrobial properties as well as in the feed as in the intestine, it can be questioned if such above described studies really contribute to the definition of mineral bioavailability to the animal.

The interest in reduced pharmacological levels of zinc and copper with organic sources compared to inorganics, permitted to maintain animal performances, while, as expected, reducing their mineral excretion (4, 26). Reduced supply with organic zinc and copper sources is therefore positive in term of environmental mineral load while keeping performances constant.

Bioavailability through a single oral application:

This study method consists in the supply of a high single oral dose of trace mineral, followed by the monitoring of the mineral blood status (“peak serum response”, “area under curve”, etc.) over a few hours after the supply, before the mineral homeostatic regulation is activated. This method especially illustrates the use of minerals in veterinarian applications.

As an example, the zinc homeostatic regulation needs 3 to 4 days to adapt its metabolism to a drastically changed quantity in zinc supply (27, 28). Different zinc sources (Zinc oxide, zinc sulfate and zinc glycinate) were compared on ponies (29). Plasma Zn concentrations 2, 4, 6, 8, 10 and 24 hours after the single 20 mg Zn/kg BW supply, were increasing compared to a negative control treatment ($p<0.01$). The peak serum response was highest with zinc glycinate compared to zinc oxide ($p<0.05$) and zinc sulfate ($p>0.05$). Zinc glycinate was therefore considered as more bioavailable than the other sources.

Studies at adequate mineral supply (for performance data):

Most organic trace mineral studies available in the scientific literature have been conducted under a wide range of practical conditions with inclusion levels compatible to official nutritional requirements or local recommended supply. The main interest in such studies consists in indirect criteria, such as animal performances. The direct criteria (minerals status) are rarely taken into account. Comparisons of organic sources were made either with unsupplemented

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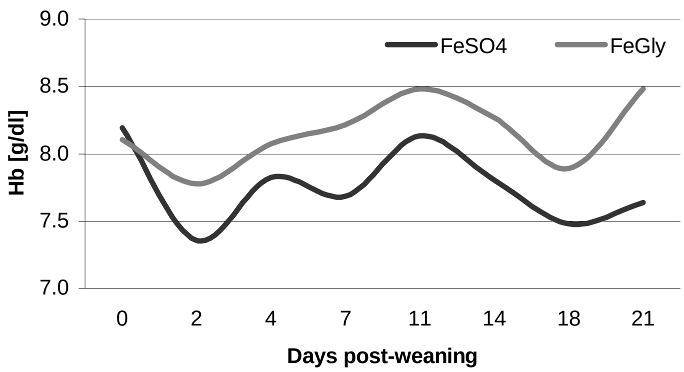
treatments, unequally dosed inorganic sources or more rarely iso-dosed inorganic sources. It is therefore highly important to verify the identity of the “control” treatment. Below, two studies are presented using two sources which were equally supplemented.

Organic iron sources are mainly used in swine nutrition. Young and fast growing piglets are very sensitive to insufficient iron supply and based on a recent French investigation on farms (30), the average blood hemoglobin (Hb) content of 21-day old piglets is adequate (10.6 g/dl), but the high variability is problematic (range between 3g/dl and 14.6 g/dl). Anemia (Hb <8 g/dl) reduces growth performances, especially in heavy piglets (29). In this context, 384 weaning piglets were supplied during 42 days with 100 ppm Fe via the diet in either sulfate or glycinate form (31). Piglet’s Hb level developed positively over the first 21 days (graph 3). At the end of the study, piglets fed iron glycinate were 10.8% higher ($p<0.05$) in Hb level than those fed iron sulfate. 41.1% of the piglets fed iron sulfate were still considered as anemic (Hb<8 g/dl), while for zinc glycinate, only 10.7% were anemic. In situations of sub-clinical anemia, iron glycinate is therefore more efficious to improve piglet’s iron status. The reason of the increased Hb status with iron glycinate was probably due to its proven high iron absorbability (23).



Graph 3: Hb evolution over the first 21 days post-weaning.

In ruminant nutrition, several investigations permitted to measure reduced mastitis (32) illustrated in table 4, improved hoof quality (32, 33), increased antibody titers (34) and increased performances (32) through the replacement of inorganic zinc with organic sources**.** Improved fertility was measured, as well, through addition of organic zinc onto a normally supplied diet (35).

Table 4: Effect of trace mineral source on dairy cow productivity

		+1200 mg Zn, Mn /cow/day		
		Inorganic	E.U. Chelate	P
Milkproduction	[kg / d]	33.0	33.8	<0.0001
Urea	[mg/l]	267	280	<0.0001
Somatic cell count	[1000/ml]	201	147	0.02
Mastitis	[n° of cases]	81	22	
Lameness	[n° of cases]	13	8	

Such long-term studies, covering for example an entire lactation, the risk in individual or time limited sub-clinical mineral deficiencies is high. Reasons would be various, but the addition of several of such occasional mineral deficiencies or mineral imbalances would, in long term, affect important economical criteria such as milk productivity or fertility. Organic trace minerals, proven to be more bioavailable through bioavailability studies, would reduce the risk of momentarily mineral deficiencies and guarantee in the end, a better productivity.

Conclusion

In conclusion, there are numerous methods to study organic trace minerals (Cu, Fe, Mn, Zn) in animal nutrition, but those can be classified in two groups:

- Bioavailability studies in which, the mineral homeostatic regulation capacity is suppressed.
- Performance studies at adequate mineral supply.

Trace mineral bioavailability, including absorbability and retention data in specific organs or tissues is the best information to clearly differentiate the quality of different supplemental sources. The study method “below requirement” is the most appropriate bioavailability method for this.

Data from “performance studies at adequate mineral supply” are related to the bioavailability studies: Supplemental sources with a proven increased bioavailability limit clinical or sub-clinical trace mineral deficiencies, which often occur in practice due to limited mineral supply, mineral antagonism or mineral imbalances. This even when trace minerals are supplied at recommended levels. Reduced clinical or sub-clinical trace mineral deficiencies improve animal performances.

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