RELATIVE TRACE MINERAL BIOAVAILABILITY

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RESUMO

BIODISPONIBILIDADE RELATIVA DOS MINERAIS-TRAÇO

Para determinar a eficiência de utilização de elementos minerais dietéticos, deve-se conhecer a biodisponibilidade relativa de cada elemento de um determinado ingrediente ou de uma ração completa. Análises químicas da dieta ou de um determinado ingrediente não indicam a efetividade biológica de um nutriente. Existem muitos fatores que influenciam a biodisponibilidade dos minerais, especialmente dos minerais-traço, tais como: nível de consumo do mineral, forma química, digestibilidade da dieta, tamanho da partícula, interações com outros minerais e nutrientes, agentes quelantes, inibidores, estado fisiológico do animal, qualidade da água, condições de processamento ao qual ingredientes individuais ou uma dieta completa foram expostos e, é óbvio, a idade e a espécie animal. Quando um mineral-traço é ingerido, sua biodisponibilidade é influenciada por propriedades específicas do mineral da maneira como está incluído na dieta. Por exemplo, sua valência e forma molecular (orgânica versus inorgânica) são importantes. Por causa dessas propriedades específicas, o mineral pode formar complexos com outros componentes no intestino, o que pode dificultar ou facilitar a absorção pela mucosa, o transporte ou o metabolismo do mineral no organismo. É bem conhecido que certos minerais em sua forma inorgânica competem com outros minerais por sítios de ligação e por absorção no intestino. O conhecimento sobre a biodisponibilidade dos minerais-traço nos ingredientes e fontes suplementares é importante para a formulação econômica de uma ração para garantir ótimo desempenho animal. A biodisponibilidade deve ser entendida como um valor "estimado" que reflete a absorção e a utilização do mineral sobre condições de um experimento específico e não de uma propriedade inerente e específica de um ingrediente ou suplemento de ração. Com a tecnologia disponível, a determinação da biodisponibilidade dentro de uma definição estrita é impossibilitada para alguns elementos, pois alguns ajustes devem ser feitos. Em outras palavras, não existe um valor percentual de biodisponibilidade que reflita todas as condições a que essa ração ou elemento mineral estão sujeitos.

PALAVRAS-CHAVE: Biodisponibilidade, minerais, animais

SUMMARY

In order to determine how efficiently an animal utilizes dietary mineral elements, one must know the relative bioavailability of that element from a feed ingredient or complete diet. Chemical analysis of the diet or an individual feed ingredient(s) does not indicate the biological effectiveness of a nutrient. There are many factors that influence the bioavailability of minerals, especially the trace elements. A few include level of mineral intake, chemical form, overall diet digestibility, particle size, interactions with other minerals and nutrients, chelators, inhibitors, physiological state of the animal, water quality, processing conditions to which the individual ingredients or complete diet have been exposed and, of course, the age and species of the animal. When a trace mineral is ingested its bioavailability is influenced by the specific properties of the mineral as it is in the diet. For example, the valence state of the mineral and its molecular form (inorganic versus organic) are important. Because of these specific properties the mineral may be inclined to form complexes with other components in the gut which may either hinder or facilitate the mucosal absorption, transport or metabolism of the mineral in the body. It is well known that certain minerals in the inorganic

form will compete with other minerals for the necessary binding and absorption sites in the gut.

Knowledge of the bioavailability of trace minerals in feedstuffs and supplemental sources is important for economic feed formulation to support optimal animal performance. Bioavailability should be thought of as an "estimated" value which reflects absorption and utilization of a mineral under of a feedstuff or supplemental compound. With current technology, determination of bioavailability within its strictest definition is often difficult to impossible for some elements, so acceptable compromises must be made. In other words, there is no one percentage bioavailability under all conditions.

KEY WORDS: Bioavailabity, minerals, livestock.

INTRODUCTION

Natural feedstuffs such as corn, wheat, soybean meal, etc. contain essential trace elements which are required by animals. However, these trace elements are often in a form which renders them unavailable to the animal. Also, even if the elements were totally available, in many cases, they would not be in adequate concentrations to meet the animal's requirement.

Therefore, when deficiencies of one or more of the trace mineral elements exist in a diet, they are usually provided to the animal in an inorganic or organic supplemental form. It is advantageous for nutritionists to know the bioavailability of any element in the natural feed ingredient or mineral source used as a dietary supplement. With this knowledge the proper amount of the trace element can be supplied to the animal.

At the present time, more information exists on the utilization of minerals commonly used as supplements than on those in practical dietary ingredients. The term "bioavailability" has been defined as the degree to which an ingested nutrient in a particular source is absorbed in a form that the nutrient is "available" at the tissue level rather than just at the dietary level. Other terms which have been used to express this degree of utilization include "biological availability", "bioactivity", "biopotency" and "bioefficacy" (Ammerman et al., 1995).

Bioavailability should not be considered as an inherent property or characteristic of the material being assayed, but, rather, an experimentally determined estimate which reflects the absorption and utilization under conditions of the test (Fairweather-Tait, 1987). Attempting to determine a simple, unique bioavailability value of a source applicable under all conditions can be considered somewhere between frustrating and misleading.

The definition of mineral products that are able to be sold in the United States as organically-bound mineral compounds is defined by the Association of American Feed Control Officials (AAFCO, 1997). 1) A *Metal Amino Acid Chelate* (57.142) is the product resulting from the reaction of a soluble metal salt with amino acids with a mole ratio of one mole of metal to one to three (preferably two) moles of amino acids to form coordinate covalent bonds. The average weight of the hydrolyzed amino acids must be approximately 150 and the resulting molecular weight of the chelate must not exceed 800. 2) A *Metal Amino Acid Complex* (57.150) is the product resulting from complexing of a soluble metal salt with an amino acid(s). 3) A *Metal (Specific amino acid) Complex* (57.151) is the product resulting from complexing of a soluble metal salt with a specific amino acid. 4) A *Metal Proteinate* (57.23) is the product resulting from the chelation of a soluble salt with amino acids and/or partially hydrolyzed protein. 5) A *Metal Polysaccharide Complex* (57.29) is the product resulting from complexing of a soluble salt with a polysaccharide solution declared as an ingredient as the specific metal complex. Simple, easily used methodology for verification of the speciation and degree of binding strength of organic mineral compounds has not been available and this has hindered progress in research with these products (Ammerman et al., 1997, 1998a, b).

METHODOLOGY

Bioavailability values can be influenced greatly by method of mathematical calculation used to derive the estimate. Data must meet certain criteria for valid tests. As examples, data must be linear or

transformable to linear for a multiple linear regression to yield valid results; whereas, for a parallel line assay, data must conform to parallelism as well as linearity. A control group displaying either no response or a limited response should also be included in an experiment to determine whether some measurable response to a test source has, indeed, occurred. An exception to this would be when a control diet is so deficient in an element that very high mortality would result and, consequently, no useful data would be derived. In a situation where feed intake differs among treatments, either because the control diet was deficient in the element or some diets had elevated concentrations of the element, the total amount of the element consumed rather than dietary concentration should be used as the independent variable in regression. Most importantly, values obtained must make sense biologically. Experimentation is not perfect, and sometimes unknown factors will radically influence results. Accepting results of all experiments may lead to costly errors in diet formulation.

A bioavailability method suitable for one element may be totally unsuitable for another element and yield erroneous results. An example would be comparison of tissue retention of selenium from selenomethionine *vs*. sodium selenite. Selenomethionine can be incorporated directly into body proteins, whereas selenite is metabolized through a different pathway and is not stored in this manner.

Absorption, either apparent or true, of a mineral has been used as an indicator of its bioavailability. Absorption, however, cannot always be equated to bioavailability as is the case with the selenoamino acids. For the trace elements, requirement levels are generally small and/or absorption so limited as to render balance experiments unreliable. For some elements in various forms, however, apparent absorption, or especially true absorption, can provide very useful information with regard to bioavailability.

Animal body weight gain and bone development have been used as primary criteria for functional assays of several trace mineral elements. The young chick with its limited nutrient store and its genetic potential for rapid growth is an ideal choice as an assay animal for several trace minerals. In the case of iron or zinc fed at elevated dietary concentrations, feeding should be delayed until 3 to 5 days of age to avoid severe growth depression. Metabolically essential compounds or enzymes have been used as criteria in several mineral bioavailability studies. Perhaps one of the earliest uses of such a compound is that of hemoglobin and its response to iron. More recently, measurement of glutathione peroxidase activity has been used to assay selenium sources.

Accumulation of a mineral element in various target organs has been used as a response criterion for bioavailability. Early observations, including those by Nesbit and Elmslie (1960) with rats and Bunch et al. (1961) with swine, indicated that biological availability of iron and copper from various compounds was related to tissue concentrations of the elements. Watson et al. (1970) fed semipurified diets (4 ppm manganese) to chicks and reported that bone manganese concentrations were more directly related to supplemental levels than were growth rate or leg development and suggested that a biological assay for manganese be developed using manganese sulfate as a reference standard and bone manganese levels as the response criterion. Years later this suggestion was followed to the extent that Black et al. (1984 a, b) used bone manganese concentrations along with concentrations of the element in other tissues as indicators of bioavailability.

Traditionally, bioavailability values have been determined at dietary levels below the requirement. These studies technically do not meet the levels below the requirement. These studies technically do not meet the definition of bioavailability in that the animal is no longer "normal" if consuming a deficient diet. Some dietary mineral deficiencies can affect other areas of metabolism indirectly through numerous complex interrelationships. A method to estimate the biological availability of the micro mineral elements for poultry based on tissue uptake of the element following high-level, short-term supplementation has been developed at the University of Florida (Henry et al., 1986). As stated by Combs and Combs (1986), with regard to the availability of selenium, this method measures the element in both its "adventitious" forms as well as in its "critical" or metabolically active form. One of the biggest advantages of this method is that it takes far fewer animals to detect statistically significant differences when greater dietary concentrations of the

particular element are fed and the degree of response is proportionately greater. The use of high-level, short-term supplementation suggests that levels of the mineral element fed can approach what would be considered the toxic range for longer term supplementation. It is important, however, that significant reductions in feed intake and weight gain do not occur. The use of elevated dietary concentrations allows formulation of diets with natural ingredients which meet the animal's nutrient requirements and allow them to grow at their maximal genetic potential. Diets containing such ingredients are less expensive and, in general, more palatable than are diets containing purified ingredients. Also, contamination of either diet or tissue samples with the mineral element being tested is of less consequence when high dietary levels of the element are being fed as opposed to having the diet as completely free as possible of the element. In studies of this kind, slope ratios are used in comparing responses from test sources with that of the standard source.

A comprehensive review on the bioavailability of the micromineral elements was prepared by Ammerman and Miller (1972). Much of the current information on the relative bioavailability of the organic forms of the trace minerals was summarized by Ammerman et al. (1997, 1998a, b). The following text contains some of the information excerpted from their review as well as other pertinent citations on the organic as well as the inorganic forms of the trace minerals.

MANGANESE

Except for recognition that rhodochrosite is an ineffective dietary source of manganese, few differences in bioavailability among manganese sources have been observed when chicks were fed purified diets and body weight change and leg abnormalities served as criteria. More recently, with supplemental dietary levels of 1000 to 4000 ppm, manganese carbonated and manganese monoxide have been found to be less effective than manganese sulfate as a source of the element for poultry and manganese dioxide was quite unavailable. Researchers contributing to these findings include Southern and Baker (1983); Black et al. (1984b); Henry et al. (1987a); and Wong-Valle et al. (1989). The relative biological availability of the monoxide form of manganese has been shown to be less than that of the sulfate and chloride forms, which are the two main forms used today as ingredients of foods for human consumption. Miles et al. (1986) reviewed the bioavailability and safety of manganese sources and these authors concluded that manganese monoxide is a very safe form of manganese to use in animal diets and should remain a GRAS feed additive.

More research has been conducted on bioavailability of the inorganic forms of the trace minerals. Research with the organic forms of the element has been rather limited. A manganese-protein complex was shown to have greater bioavailability than manganese sulfate as a source of manganese for chicks in one study by Baker and Halpin (1987). Manganese methionine was equal to manganese oxide for chicks in one study (Scheideler, 1991) and superior to manganese oxide in another study (Fly et al., 1989). The methionine form was equal to or more effective than manganese sulfate (Henry et al., 1989). Egg shell quality was reported to be improved in ISA brown layers when a proteinated form of zinc and manganese was supplemented to the diet (Klecker et al. 1997). Miles (1998) also reported an improvement in the amount of egg shell deposited on eggs from white Leghorn hens selected for low shell weight and fed a proteinated form of manganese, zinc and copper.

Luo (1989) investigated the bioavailability of several inorganic manganese sources in broilers and used percent ash in the middle toe as the response criterion. The manganese ores tested were lower in bioavailability relative to that in reagent grade manganese oxide. Smith et al. (1995), using two environmental temperatures, fed day-old broiler chicks 0, 1000, 2000, or 3000 ppm manganese from either sulfate, monoxide or proteinate forms for 21 days. From day 22 to 47, birds from each treatment were continued under thermoneutral (temperature cycled between 18 and 23.9 C) or heat distress (temperature cycled between 23.9 and 35 C) conditions. Based on bone manganese regressed on manganese intake, bioavailabilities were 100, 91, and 120 for sulfate, monoxide, and proteinate forms at 21 days, respectively. These values were 100, 83, and 125, for 7-wk-old birds under thermoneutral conditions and 100, 82, and 145, respectively, for

those under heat distress. Heat distress appeared to magnify the difference in bioavailability between manganese sulfate and manganese-proteinate.

Lambs were fed natural diets supplemented with 900, 1800, or 2700 ppm manganese from various sources for 21 days (Henry et al., 1992). The overall estimated bioavailabilities based on bone, kidney, and liver manganese depositions were 100, 121, 70, and 53 for manganese sulfate, manganese-methionine complex, and the two feed grade manganese monoxides, respectively.

COOPER

Nelson (1997) reviewed the benefits of feeding dietary cooper from various compounds in poultry. His review dealt mainly with those compounds that have historical significance or are presently supplemented to poultry diets at levels that result in responses in growth and feed efficiency. An early review of the literature (Ammerman and Miller, 1972) indicated that copper as cupric sulfate had been found to be the most available form for both domestic and laboratory animals. Copper as cupric oxide was absorbed to a lesser degree than that from cupric sulfate and cupric carbonate was intermediate in response between the oxide and sulfate forms. Considerably more information on the bioavailability of various sources has been generated in recent years. Most studies contributing to this information have used liver copper deposition as the criterion and, frequently, supplemental dietary copper levels have extended to 300 to 400 ppm.

Ledoux et al. (1989) fed high dietary copper concentrations and reported that liver copper accumulation was a useful bioassay criterion for estimation of copper bioavailability from inorganic sources. The cupric form of acetate was shown (Ledoux et al., 1991) to be equal to cupric sulfate as a source of copper. In general, cupric chloride has been shown to have a greater bioavailability than cupric sulfate (Norvell et al., 1974, 1975). Although cupric oxide has been used as a supplemental source of copper in the livestock and feed industry, more recent research suggested it has essentially zero bioavailability for poultry (Norvell et al., 1974, 1975; Baker et al., 1991; Ledoux et al., 1991). Baker et al. (1991) found that copper from cuprous oxide was well utilized by the chick. Supplemental cooper in the organic forms including cooper lysine, copper methionine and copper proteinate was absorbed equal to or greater than that from cupric sulfate (Kincaid et al., 1986; Baker et al., 1991). Aoyagi and Baker (1993b) reported the bioavailability of copper in copper-methionine complex for chicks was estimated as 96% by using a bile copper assay at low dietary intakes and 88% by a liver copper assay at elevated dietary intakes when compared relative to 100% for the copper in cupric sulfate.

Ledoux et al. (1991) fed cupric carbonate and cupric sulfate to chicks at supplemental copper levels of 150, 300, and 450 ppm. The basal diet contained 11 ppm copper. Liver copper concentrations were examined by the slope ratio method and relative bioavailability of the copper as cupric carbonate was 68%. Zanetti et al. (1991) conducted a similar experiment with chicks in which supplemental copper levels of 5, 10, 15, and 20 ppm were added to a diet containing 5 ppm copper. The relative bioavailability of copper in the carbonate form in this study in which dietary copper additions were much closer to requirement was very similar at 66%.

Spears et al. (1997) reported that the copper in tribasic copper chloride (TBCC) was more available to steers than that of copper sulfate when supplemented to diets high in the copper antagonists, molybdenum and sulfur. When evaluated in copper-deficient steers fed diets low in molybdenum, the two copper sources were of similar bioavailability. Miles et al. (1998a) studied cupric sulfate and TBCC as supplemental copper sources for broiler chicks. A corn-soybean meal diet containing 26 ppm copper was supplemented with either 0, 150, 300, or 450 ppm copper from sulfate or TBCC and fed for 21 days. Chicks fed 450 ppm copper from sulfate had lower ($P<0.05$) feed intake than those consuming other diets. Feeding supplemental copper increased ($P<0.0001$) liver copper concentration linearly with increasing dietary copper regardless of source. The slopes of regression of $\log_{10}$ liver copper on dietary copper intake did not differ between sources. Linear regression over nonzero dietary levels of $\log_{10}$ transformed liver copper concentration of analyzed total copper intake resulted in a slope ratio estimate

of 106% for bioavailability of copper from TBCC compared to 100% for that in cupric sulfate. Another study was conducted for 42-days with broiler chicks fed supplemental copper at 0, 200, 400, or 600 ppm from either feed-grade copper sulfate or TBCC. Liver copper increased linearly with increasing dietary copper. Based on $\log_{10}$ liver copper concentration, copper in TBCC was 112% available compared to 100% for the standard cupric sulfate. In a similar study, Miles et al. (1998b) fed broiler type chicks supplemental copper as either feed grade cupric sulfate or tri-basic copper sulfate at dietary concentrations of 0, 150, 300, and 450 ppm. Using liver copper accumulation, these authors reported that the copper in tri-basic copper sulfate was as bioavailable as the copper in feed grade cupric sulfate.

Ewing et al. (1998) used commercial male broiler chickens to test the ability of three commercially available copper sources (cupric sulfate pentahydrate, copper oxychloride and cupric citrate) to improve the growth performance of broiler chicks. Even though this study was not designed to determine the relative bioavailability of copper in these sources, they were shown to increase weight gain. Copper oxychloride was shown to be a viable copper source compared to the industry standard copper sulfate when growth was used as the response criterion; however, the growth responses to copper sulfate or copper oxychloride were not comparable to those observed with cupric citrate, as reported earlier by Pesti and Bakalli (1996). Swine are able to utilize the copper in copper sulfate, but cupric sulfide is not utilized very well by swine. Bunch et al. (1961, 1965) reported low liver copper concentrations in weanling pigs when fed cupric oxide relative to cupric sulfate. In the studies with pigs, cupric carbonate was intermediate in bioavailability to the sulfate and oxide forms. In studies with swine, Apgar et al. (1995) fed weanling pigs 100, 150, or 200 ppm copper as either cupric sulfate or copper-lysine complex for 35 days. Average daily gain increased with supplemental copper, but did not differ between sources. Liver copper concentrations were similar for the two copper sources at 100 and 150 ppm supplemental copper. At 200 ppm added copper, liver copper was much greater for copper-lysine than for cupric sulfate suggesting greater absorption from the organic form. Further research from the same laboratory (Apgar and Kornegay, 1996), however, found that absorption and retention of copper were similar for pigs fed growth-stimulating levels of the element from either cupric sulfate or copper-lysine complex.

Ammerman et al. (1997, 1998a, b) reviewed the literature on the utilization of several of the forms of organic minerals in livestock. Numerous studies have been conducted in rats to determine the utilization of copper in organic compounds (Du et al. 1996b). These authors reported on the utilization of copper in copper lysine complex, copper-proteinate, and cupric sulfate. In two experiments, liver copper deposition was greatest from the proteinate and the lysine complex. These authors suggested that the copper in inorganic copper sulfate and that the copper supplemented to the diet in copper complexes was absorbed by different mechanisms. In another study, DeBonis and Nockels (1992) using calves found that copper as copper-lysine was utilized better than that in cupric sulfate.

Not all studies with ruminants have shown that the organic form is more available than the inorganic form (Ward et al., 1993). In their study both copper-lysine and copper sulfate were equal in their ability to furnish copper to steers. In a 7-month experiment, Rabiansky et al. (1998) found that heifers utilized the copper in copper sulfate and copper lysine equally and that in copper-lysine exhibited a greater utilization in heifers with low copper status. Du et al. (1996a) reported that Holstein and Jersey cows utilized the copper in a proteinate form as well as the copper in cupric sulfate. Similar results were found for a proteinate form fed to cattle by Ward et al. (1996a). When compared to the carbonate and sulfate forms the copper in the proteinate form was equal to that in the inorganic forms. However, in the presence of added molybdenum, a higher utilization of copper seemed to occur with the proteinate form. A copper-lysine complex was supplemented to diets of lambs and compared to copper sulfate (Luo et al., 1996). In the combined feeding regimes used by these authors the copper in the copper-lysine complex was 93% as available as that in copper sulfate (100%). Winn and Schalatter (1998) fed different organic sources of the trace minerals zinc, copper, manganese and cobalt to dairy heifers over a six week period along

with an unsupplemented control. The trace minerals were either complexed with an amino acid, glucoheptonate, or propionate. Generally, supplementation of the trace minerals increased uptake of the trace minerals from all forms. Copper propionate elevated serum copper levels greather than did copper-lysine. As discussed by Ammerman et al. (1997, 1998a, b), Attaelmannan and Reid (1996) suggested that copper-lysine "chelate" fed to a ruminant will dissociate quickly in saliva and exist mainly as carbonate and phosphate compounds in the gastrointestinal tract.

ZINC

The relationship between zinc deficiency and parakeratosis in swine is well recognized and the potential need for supplemental dietary zinc is not questioned. Zinc is typically supplemented as zinc sulfate, carbonate, oxide or zinc complexed to amino acids and proteins. Recently, Woodworth et al. (1998) used a total of 360 early-weaned barrows in a 36-day growth trial to determine the influence of added zinc from zinc oxide on starter pig performance. The nine treatments consisted of a control diet containing no added zinc, and eight diets containing increasing levels of zinc from zinc oxide (50, 100, 200, 400, 800, 1,600, 2,400, and 3,200 ppm zinc). Their results suggested that 3,200 ppm of zinc from zinc oxide can be added to starter pig diets to achieve maximum growth performance, but only 100 ppm of zinc from zinc oxide is required to meet the basal requirement for zinc.

Galvanized pipe enriches the water supply with zinc and in many instances has been shown to prevent a deficiency in animals. In fact, many captive animals, especially birds, will gnaw at their cages and may ingest enough zinc to cause a copper or iron deficiency. New cages are often a rich source of zinc, and it is not uncommon for captive birds, especially psittacines, to develop a fatal zinc toxicity within a few weeks of being housed (Howard, 1992). Several bioavailability studies were conducted in which inorganic zinc compounds were evaluated as sources of the element for chicks or poults (Ammerman and Miller, 1972). Zinc in the form of acetate, chloride and sulfate have commonly been used as standards in bioavailability research, and all three forms of the element are well utilized by animals. Most of the studies used growth as the response criterion and few differences were observed among the supplemental sources of zinc tested.

Zinc, in the organic form, has been studied for many years. Pensack et al. (1958), using chicks, compared a zinc-proteinate with several inorganic sources and reported no differences in the different forms of zinc to overcome a zinc deficiency. Pimentel et al. (1991) reported no difference in bone zinc content when zinc oxide was compared to zinc-methionine.

Aoyagi and Baker (1993) added graded levels up to 8 ppm zinc from either feed grade zinc sulfate or zinc-lysine to a semipurified basal diet containing 13 ppm zinc. These authors reported that relative bioavailability of zinc-lysine complex was 111% based on bone zinc accumulation for the chick. The bioavailability of zinc as either zinc-methionine complex or zinc sulfate as affected by dietary calcium concentration was studied by Wedekind et al. (1994). Chick diets were supplemented with 0, 5, or 10 ppm zinc in the presence of .60 or .74% calcium. Based on slope ratio comparisons of tibia zinc concentration, bioavailability of zinc-methionine was 166 relative to 100 for zinc sulfate when the dietary calcium concentration was .60% and 292 when the dietary calcium concentration was .74%.

There has been an increased interest recently in determining the bioavailability of zinc supplements. Chick zinc bioassays were conducted with a soy isolate-dextrose diet containing 13 ppm zinc to which 7.5 or 15 ppm were added from either feed grade zinc oxide or feed grade zinc sulfate (Wedekind and Baker, 1990). When weight gain was regressed on supplemental zinc intake the relative bioavailability of zinc as zinc oxide was 61%, when the sulfate form was assigned a value of 100%. A similar comparison based on tibia zinc gave a value for zinc oxide of 44%. However, zinc concentration of the basal diet and feed intake were not accounted for in this calculation. Wedekind et al. (1992) reported on studies with chicks in which the bioavailability of zinc methionine was compared to that of feed grade zinc sulfate using three different diets described as purified (crystalline amino acid), semipurified (soy isolate), and complex (corn-soybean meal) diet. Zinc from zinc methionine was absorbed significantly greater than that from zinc

sulfate with the purified diet and the difference in degree of bioavailability was even greater with semipurified complex diets. The authors suggested that metabolism of the zinc methionine complex differed from that of inorganic sources as influenced by phytate and fiber in the diet.

The potential for using elevated dietary levels of zinc with practical dietary ingredients was examined by Henry et al. (1987b). These investigators fed a basal corn soybean meal diet containing 74 ppm zinc to chicks and supplemented it with 0, 500, 1000, and 1500 ppm zinc as reagent grade zinc sulfate. Feed intake and body weight gains were depressed, especially at the greatest concentration of zinc, when chicks were fed for 1 week. Highly significant increases in tissue zinc occurred and the authors suggested that this technique might be useful as a measure of zinc bioavailability. Of the tissues examined, bone was most sensitive to dietary zinc followed by liver and kidney. Sandoval et al. (1997) used this technique with chicks and when zinc sulfate served as the control zinc carbonate, zinc oxide and zinc metal were lower in bioavailability in the order listed. Sandoval (1992) reported that the age of the chick at the start of plethoric dosing of zinc sulfate had a profound effect on feed intake. This may be related to structural integrity of the developing gastrointestinal tract of the newly hatched chick or establishment of gut microbial populations. Hahn and Baker (1993) reported that the relative bioavailability values for supplemental zinc sources calculated from serum data collected in studies in which plethoric levels were fed to swine were similar to those bioavailability values in which levels near the chick's zinc requirement were fed (Wedekind and Baker, 1990).

Sandoval et al. (1998) conducted four experiments with the purpose of identifying several factors that might be useful in improving the accuracy and reproducibility of zinc bioavailability assays for chicks. These authors reported that only one factor may refine the model used for zinc bioassays. Delaying the onset of high zinc feeding by several days may help alleviate feed intake problems observed with zinc sulfate. Use of either zinc gluconate or zinc acetate as a standard in assays or use of metallothionein synthesis as a bioavailability criterion was not found to be useful in improving the accuracy of zinc bioavailability estimates. Carlson et al. (1997) used nursery pigs to determine if metallothionein concentration in the intestinal mucosa was affected by organic and inorganic zinc sources. The sources of zinc were zinc oxide, zinc amino acid complex and zinc methionine complex. After feeding different dietary concentrations of these three zinc sources for 20 days the metallothionein fractions in the intestinal mucosa were analyzed for copper. Zinc concentrations in the metallothionein fractions of zinc oxide fed pigs were higher compared to the control and the two other zinc sources. Therefore, it appears that intestinal metallothionein responds differently to organic and inorganic forms of zinc.

Ammerman et al. (1997, 1998a, b) summarized the research reported by Cao et al. (1997, 1998) in which chicks were fed various dietary concentrations of zinc in the forms of sulfate, amino acid chelate and proteinate and solubility of the sources was determined. Zinc was found to dissociate from its ligand in the soluble fraction for the organic sources at pH 2 and 5. The organic products were also soluble in deionized water.

Ammerman et al. (1997, 1998a, b) in their review of the literature concerning organic forms of zinc have shown that, in general, the organic forms are just as available as the commonly used forms of inorganic zinc. In some cases the organic forms have been shown to be more available. The authors provide the following references from Ammerman et al. (1997, 1998a, b) for those wishing to become more informed about the use of organic forms of zinc in the diet of various animal species: Swinkels, et al. 1991; Kornegay et al., 1993; Wedekind et al., 1993; Hahn and Baker, 1993; van Heugten et al., 1995; Cheng and Kornegay, 1995; Cheng et al., 1995; Matsui et al., 1996; Schell and Kornegay, 1996; Ward et al., 1996b; Kornegay et al., 1996; Hoover et al., 1997a, b; Greene et al., 1988; Spears and Kegley, 1994; Spears, 1989; Chirase et al., 1991; Nockels et al., 1993; Brazle, 1994; Reiling et al., 1992; Rojas et al., 1996; Kincaid et al., 1997; Kegley et al., 1997.

Swinkels et al. (1996) conducted a 36-day experiment with 32 zinc depleted pigs and reported that serum and soft tissue zinc concentrations were clearly affected by the zinc status of the diet and zinc depletion. However these authors reported that when

zinc sulfate and a zinc amino acid chelate were used to replete the pigs, an effect of zinc source was not observed. In two zinc depletion/repletion experiments, Edwards et al. (1998) tested the relative bioavailability of two new by-products of the galvanizing industry. These two new zinc sulfate compounds contain iron and are produced when the galvanizing industry reclaims zinc from the zinc coating used on steel. Their data showed that both mixed sulfate products of iron and zinc are excellent sources of bioavailable zinc. More recently, Edwards et al. (1999) conducted two zinc depletion-repletion assays with chicks from 8 to 20 days of age to determine the zinc bioavilability of three sources of zinc oxide and two sources of zinc sulfate. Feed grade zinc sulfate, regardless of source, relative biological values that were not significantly different from 100%, but feed grade zinc oxide sources differed widely in their relative bioavailability.

COBALT

Cobalt is an essential trace element, needed as a component of vitamin B_{12} produced by microorganisms. Elemental cobalt is of practical significance for the ruminant; however, when a highly available form of cobalt such as cobalt chloride is added to the diet of poultry at high levels it can result in cobalt toxicosis. If the diet is low in iron, the absorption of cobalt increases, aggravating the toxicosis (Hill, 1974). An interrelationship between cobalt and sulfur amino acids has been reported in chickens (Southern and Baker, 1981). Sulfur amino acids reduce cobalt toxicity (Baker and Czarnecki-Maulden, 1987).

Diaz et al. (1994) fed cobalt, as cobalt chloride, at levels of 0, 125, 250, and 500 ppm in the diet of broiler chickens. All levels of cobalt reduced feed intake, body weight gain, and gain: feed ratio and caused a dose-dependent increase in mortality. Chickens fed 250 and 500 ppm developed pancreatic fibrosis, multifocal hepatic necrosis, and lesions in skeletal and cardiac muscle and smooth muscle of the duodenum. The results demonstrated that excessive dietary cobalt has serious adverse effects on the health and performance of broiler chickens. Although excessive dietary cobalt is highly toxic for broiler chickens, cobalt toxicosis in unlikely to occur under practical conditions, since the normal cobalt concentration in most poultry diets does not normally exceed 5 ppm (Puls, 1988).

Only a few critical studies have been reported on the utilization of various sources of cobalt in ruminants. The sulfate, carbonate, chloride, and nitrate forms have been indicated to be highly available (Ammerman and Miller, 1972). The oxide compounds in the form of heavy pallets which remain in the reticulorumen for several months, have been effective in supplying cobalt to sheep and cattle. Henry (1995) reviewed the bioavailability of cobalt in animals.

As reported by Ammerman et al. (1997, 1998a, b), based on research conducted at the University of Florida, Ammerman et al. (1982) suggested liver accumulation of cobalt as an indicator of its solubility in the rumen and, thus, its availability to ruminal microorganisms for vitamin B_{12} synthesis. This research along with that conducted with the Rowett Research Institute has been published in a series of papers (Henry et al. 1997; Kawashim et al., 1997a, b). Cobaltous sulfate, cobaltous carbonate, and cobalt glucoheptonate were essentially equal in utilization when based on liver and kidney accumulation of cobalt in sheep fed dietary levels of 40 to 60 ppm cobalt for 16 to 20 days. The oxide forms were less well utilized. Serum and liver vitamin B_{12} concentrations increased with increasing dietary cobalt, but were not useful in separating the supplemental sources. Relative bioavailability values for cobalt sources based on production of vitamin B_{12} in ruminal effluent in a semicontinuous *in vitro* culture system were similar to those obtained with tissue cobalt accumulation.

IRON

Although iron deficiency is not generally a practical problem in poultry nutrition, the chick has been used extensively as a model for human bioavailability studies. Iron absorption has been reported to decrease with age (Forbes and Reina, 1972). Iron is commonly supplemented to animal diets as ferrous sulfate, ferric chloride, ferric citrate, ferric ammonium citrate, and iron amino acid complexes. Iron oxide is poorly available to birds (Henry and Miller, 1995).

Hemoglobin regeneration following feeding an iron-deficient diet for several weeks has generally been used to measure availability of iron sources in relation to that of ferrous sulfate heptahydrate. Clear evidence has been presented that proves blood hemoglobin is a better response parameter in iron depletion-repletion work than either blood hematocrit or weight gain (Chausow and Czarnecki-Maulden, 1988a, b; Biehl et al., 1997).

In general, the utilization of iron from organic sources including the citrate, fumarate, and gluconate forms is essentially equal to that of ferrous sulfate heptahydrate (Ammerman et al., 1995). Ferric iron as ferric oxide is very poorly utilized. Considerable differences have been observed in bioavailability of iron in the form of ferrous carbonate, without any obvious explanation. Ferric oxide was reported to be 17% as available as ferrous sulfate in very early studies (Elvehjem et al., 1933). Fritz et al. (1970) reported values of 107, 99, 98, 44, 37, 4, and 2% that of ferrous sulfate for ferric ammonium citrate, ferrous ammonium sulfate, ferrous chloride, ferric chloride, reduced iron, ferric oxide, and ferrous carbonate, respectively. Chausow and Czarnecki-Mauldin (1988b) reported relative iron bioavailability estimates based on hemoglobin regeneration and compared to ferrous sulfate of 96% for sesame seed meal, 77% for rice bran, 65% for alfafa meal, 45% for soybean meal and 20% for corn. Relative bioavailability of iron in feedstuffs of animal origin was poultry by-product meal 68%; meat and bone meal, 48%; feather meal, 39%; fish meal, 32%; and blood meal, 22%.

The major feed-grade phosphates have been found to range from 0.4 to 1.2% in iron content. Thus, when added to diets as supplemental sources of phosphorus, and with some compounds such as calcium, they also provide significant quantities of iron. Ammerman et al. (1993) conducted a study with chicks in which a three-day depletion period followed by a 14-day repletion period was used to determine the relative bioavailability of iron in commercial feed-grade phosphates and rock phosphate. The phosphate sources were compared with reagent-grade and feed-grade ferrous sulfate heptahydrate. When based on both hemoglobin and hematocrit, relative bioavailability values for samples of monocalcium/dicalcium phosphate, tricalcium phosphate and rock phosphate were 71, 50 and 49%, respectively.

The nursing pig was used by Spears et al. (1992) to compare iron-methionine with ferrous sulfate. As pointed out by Ammerman et al. (1997, 1998a, b), based on hemoglobin concentrations, the relative bioavailability of iron as iron-methionine was 180% when compared to that from ferrous sulfate. More recent data from Lewis et al. (1995), however, showed relative values of 81% and 68% for the iron in iron-methionine based on weight gain and hemoglobin, respectively, when compared with responses from ferrous sulfate for the weanling pig. Cao et al. (1996) fed chicks either an iron-methionine complex or ferrous sulfate heptahydrate at concentrations in the diet of 400 to 800 ppm iron for a period of 14 days. Relative bioavailability values of iron in iron-methionine was 88% when dietary iron concentration was used in slope ratio data and 83% when based on total iron intake during the trial.

High dietary concentrations of soluble iron have been reported to depress feed intake and cause death in young animals, especially when fed prior to 5 days of age (van Ravenswaay, 1986; McGowen et al., 1992; Spears et al., 1992). In order to overcome the depressed feed intake associated with feeding high dietary iron at one day of age, Cao et al. (1996) delayed feeding chicks the high iron diets until 5 days of age.

Recently, two new sources of supplemental iron and zinc have become available to the feed industry. These products are popularly referred to as iron-zinc sulfate and zinc-iron sulfate. Both of these products are by-products of the galvanizing industry and are crystallized from the pickling fluid that is used for cleaning the scale from galvanized steel. All of the iron in these products is ferrous (+2 valence). Using young chicks, Boling et al. (1998) tested the relative bioavailability of these new sources of iron. Their data suggested that these two iron by-products are excellent sources of bioavailable iron, whereas ferric sulfate and cottonseed meal are relatively poor sources of usable iron by the chick.

IODINE

The iodine content of animal feedstuffs is extremely variable and iodine is commonly supplemented to animal diets. Plants grown in regions with iodine deficient soils are commonly deficient in iodine. Even if the concentration of iodine in natural feedstuffs appears adequate it is a good idea to use a supplemental source, since the iodine in the feedstuff is often not biologically available. High calcium levels in the diet or in the water supply may increase the need for iodine (McDowell and Parkey, 1995). Iodine is routinely added to animal diets as calcium iodate, potassium iodate, or potassium iodine. Ammerman et al. (1995) indicated that most sources of supplemental iodide were bioavailable and well utilized by animals. These sources included potassium iodide, potassium iodate, sodium iodide, calcium iodate, ethylenediamine dihydriodide (EDDI) and pentacalcium orthoperiodate. The exception to this list of compounds was diiodosalicylic acid (DIS). Research revealed that DIS was absorbed intact and cleared rapidly from the body of cattle without iodine ever being released from the compound. Rats apparently have a considerably greater capacity to remove iodine from the DIS compound. This is an excellent example of why experiments should be conducted in relative bioavailability of mineral sources with the animal to which the mineral is to be fed.

Stability of the iodine-containing compounds is always of concern, but is especially so when mineral mixtures containing iodine are stored under conditions of elevated moisture and heat, or exposure to sunlight. There are some organic forms of iodine, such as iodinated amino acids, and these seem to be available to the animal. McDowell and Parkey (1995) also emphasized that crystalline iodine salts are susceptible to heat, moisture and acidity. Segregation is an everyday problem due to extreme weight of iodine sources and minute dietary inclusion rates. Chemical interactions between crystalline iodine sources and specific microingredients along with ambient moisture are commonplace. To overcome these interactions, Dr. Robert Teeter of Oklahoma State University (McDowell and Parkey, 1995) indicated that iodine absorbate technology overcomes many of the above mentioned problems and offers over four

times more availability in the finished ration than EDDI. From extrapolation of studies with other species (Ammerman and Miller, 1972), it would appear that cuprous iodide would also be a highly available source of iodine for poultry. However, research data to confirm this are needed.

SELENIUM

The bioavailability of selenium is somewhat of a moot point as government regulations in numerous countries prohibit use of supplemental sources with the exception of sodium selenite and sodium selenate. Combs and Combs (1986) compiled a table which summarized the 291 inorganic and organic selenium sources which were evaluted by Schwarz and coworkers for their efficacy in prevention of liver necrosis in vitamin E-deficient rats. Ammerman and Miller (1975) reviewed the role of selenium in ruminant and what was known about is bioavailability up to that time. With regard to poultry, three approaches have been taken to estimate bioavailability or selenium in feedstuffs and supplements: (a) prevention of various selenium- responsive diseases; (b) functional assays measuring glutathione peroxidase (GSH-Px) activity, and (c) tissue accumulation of selenium. Unfortunately, estimates derived by these different methods are often dissimilar. For example, selenomethionine was not as well utilized as sodium selenite for prevention of exudative diathesis in the vitamin E-deficient chick, but was valued at approximately 350% compared with 100% for sodium selenite for prevention of nutritional pancreatic atrophy in vitamin E-fed chicks.

Extensive work at Cornell University using prevention of exudative diathesis in chicks indicated that availability of selenium in animal by-product sources was low, generally 9 to 25%, while that in plant feedstuffs was on the order of 79% that in sodium selenite. Ammerman et al. (1995) indicated that plant sources of selenium ranged in relative bioavailability from about 60 to 90% compared with sodium selenite. Selenium in sodium selenate has been estimated to be 120% that in sodium selenite (100%) based on tissue uptake in chicks from a 6 ppm addition of the element in a practical diet (Echevarria et al., 1988a). In a similar study (Echevarria et al., 1988b) the bioavailability of sodium selenite, sodium selenate and calcium selenite was high and the selenium in each source was well absorbed by chicks when fed at high dietary concentrations (0, 3, 6 or 9 ppm) for one week. In turkeys fed a selenium-deficient diet, relative availability of sodim selenate was 141% that of sodium selenite based on plasma selenium concentration and 220% based on plasma GSH-Px activity (Cantor and Tarino, 1982). Echevarria et al. (1988c), using thirty crossbred wethers, in a time-dose study reported that tissue selenium deposition is indicative of selenium bioavailability. It appeared from their studies that feeding selenium, as sodium selenite, at 3 to 9 ppm in the diet for ten days should result in sufficient tissue selenium deposition of determine differences among sources during selenium bioassay studies. Estimates for selenomethionine in another experiment with turkeys were 124 and 97%, respectively, based on the same variables (Cantor et al., 1982). High selenium yeast products contain most of their selenium in the form of selenomethionine and would, therefore, have similar bioavailability.

Organic forms of selenium such as selenocystine, selenomethionine, and a selenoyeast product were about equal to sodium selenite when compared on the basis of their ability to promote GSH-Px activity or overcome the incidence of exudative diathesis. When tissue deposition of body retention was used as the response, inflated relative bioavailability values were obtained, no doubt due to the metabolism and deposition of the selenium as an integral part of the amino acid (Ammerman et al., 1995).

Mahan and Parrett (1996) evaluated the efficacy of selenium-enriched yeast and sodium selenite on tissue selenium retention and serum GSH-Px activity in swine. Pigs fed the diets supplemented with yeast had greater liver, loin and pancreas selenium concentrations than those pigs fed the inorganic form of selenium, thus indicating greater bioavailability of the selenium from yeast. Mahan and Kim (1996) in a similar study using swine found that the selenium yeast was superior over the inorganic form. Pehrson et al. (1989), using heifers, also reported that the selenium in yeast was more available than inorganic selenium. Koenig et al. (1997) used ^{77}Se enriched yeast and ^{82}Se-selenite and found that sheep utilized the stable isotope in selenium yeast more than that in the selenite

form.

Drip loss in poultry and swine meat is of major economic importance in each industry. Torrent (1996) discussed the advantages of using organic selenium in a yeast form over inorganic selenium in helping to minimize drip loss in swine. Edens (1996) discussed the advantages of using selenium yeast to minimize drip loss in poultry and in the same article discussed the improvement in feathering observed in broilers fed diets supplemented with selenium from selenium yeast compared to sodium selenite. Torrent (1996) and Edens (1996) discussed the bioavailability of selenium from different sources and proposed and discussed the possible mechanisms of how the yeast product was able to minimize drip loss as compared to inorganic selenium.

CHROMIUM

Since the late 1980's there has been considerable research interest in the utilization of chromium in animal feed. Chromium is an essential trace mineral with importance in the metabolism of carbohydrates and lipids. The beneficial effect of chromium in animal health is well documented for its role as an integral component of the glucose tolerance factor (GTF). The GTF consists of one atom of trivalent chromium (Cr^{34}) bound to several molecules of niacin and amino acids found in glutathione (glutamic acid, glycine and cystine). Without chromium at the center of the GTF molecule, it is inactive (Mertz, 1969). The GTF potentiates the action of insulin, which functions in the animal as one of the most important anabolic hormones. Insulin regulates rate of glucose entry into body cells, energy metabolism, muscle tissue deposition, fat metabolism and cholesterol metabolism. Because of its role as part of the GTF, which influences overall cellular utilization of carbohydrates, fats and proteins, chromium has been shown to affect carcass composition and meat yield in animals.

Chromium is normally supplemented to animal diets as either chromium chloride, chromium picolinate or chromium yeast. Up to this point in time there have not been any detailed comparison studies of the relative bioavailability of the chromium from these sources reported in the literature. What has been reported, however, are the data on animal performance which are a result of using these three sources as the form of supplemental chromium in the diet. The authors of these reports have presented an extensive list of other excellent references on chromium and animal performance. We have, therefore, provided the following references so that those that wish to do so may learn more about the influence that chromium has on animal performance References cited are: Chang and Mowat (1992); Mowat (12996, 1997); Mowat et al. (1995); Page et al. (1993); Lien et al. (1996); Hossain et al. (1998); Hossain (1998); Lindemann (1996, 1998); Campbell (1998); Trout (1995).

ORGANIC MINERAL SUPPLEMENTS: AN OVERVIEW

Inorganic minerals in feed an digestive juices present in chyme as ions interact with available ligands and become bound as various organic complexes or form low solubility salts which are unabsorbable. It is currently unknown if chelated or complexed mineral supplements dissociate to form free ions at low pH, which are in turn sequestered in a manner similar to inorganic free ions and enter the glycocalyx of the intestinal mucosa for absorption. Alternatively, depending upon stability constants and conditions in the gastrointestinal tract, the organic forms may be absorbed intact by amino acid or peptide transport pathways, as well as mineral pathways. This may account for the modest increases in availability observed in animal trials. Some of the factors affecting uptake of chelated minerals include the water-lipid partitioning coefficient, site of absorption for the particular mineral and stability of the metal-ligand complex. However, if chelates have strong stability constants, the mineral cannot be released from the ligand for used in metabolism after absorption. Mineral chelates differ from amino acids in several ways which may affect their ability to be absorbed by amino acid pathways. These included geometry of the molecule, charge density, molecular weight, size binding site affinity, charge of functional group and heterogeneity of amino acids in the complexes. In order to determine the absorptive characteristics of organic minerals, it would be necessary to manufacture the chelates with an intrinsic radiolabel

for the element in question, then follow uptake of the element in the living animal which is consuming commercial-type diets in amounts similar to that used under practical conditions. Experiments with simple extrinsinc labeled amino acids in ligated gut sections in purified media cannot truly be relied upon to characterize absorption of these compounds in a living animal. Considering the expense and difficulty of obtaining the necessary information, it is unlikely that scientists will be able to determine the true nature of the absorption of organic chelated and complexed mineral supplements any time in the near future.

Chelates form a ring structure around the metal ion and have coordinate covalent bonds with the nitrogen (amino) or oxygen (carboxyl) donor groups of amino acids and/or proteinates. Gluconates, fumarates and citrates have only ionic metal-ligand bonding and are less stable than chelates. All chelates are complexes, but not all complexes are chelates.

Some of the suppositions which have been made in the popular press during the past few years about the organic mineral supplements are:

1. The ring structure protects the mineral from unwanted chemical reactions in the gastrointestinal tract.

2. Chelates easily pass intact through the intestinal wall to the blood stream.

3. Passive absorption is increased by reducing interactions between the mineral and other nutrients.

4. The mineral is delivered in a form similar to that found in the body.

5. Chelates are absorbed by different routes than inorganic minerals.

6. Each mineral in a chelate facilitates absorption of other minerals in the chelate.

7. Chelates carry a negative charge so they are absorbed and metabolized more efficiently.

8. Chelation increases solubility and movement through cell membranes.

9. Chelation increases passive absorption by increasing water and lipid solubility of the mineral.

10. Chelation increases stability at a low pH.

11. Chelates can be absorbed by the amino acid transport system.

However, no definitive studies have been reported in the scientific literature supporting any of these claims, although at least one has been shown not to be true. None of the chelated zinc products reported by Cao et al. (1998) and copper products (Henry, personal communication) were stable at a pH of 2.0. It should also be pointed out that during the past few years chelated and complexed products have been improved by the manufacturers often without changing the original product name(s). Because of this, the data presented in the literature, in many instances, do not represent the present day products available to the animal feed industry.

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