Bioavailability and Antagonists of Trace Minerals in Ruminant Metabolism

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Introduction

Modern analytical techniques and instrumentation make it possible to accurately determine trace mineral concentration in feed and animal tissues. Unfortunately, however, such determinations provide no information on utilization of the minerals by animals. Utilization involves the concept of bioavailability and is critical in the selection of a suitable source of the mineral. In terms of trace minerals, bioavailability may be defined as the proportion of an ingested mineral that is absorbed, transported to its site of action, and converted to the physiologically active species (O'Dell, 1983). Traditionally, trace mineral bioavailability studies have been conducted at deficient dietary levels, usually with purified or semi-purified diets. The mineral sources are then added at graded levels and response criteria measured. In general, the bioavailability of a mineral element in a particular source is determined relative to its functional availability from a standard source. Use of a standard source allows expression of bioavailability in terms of relative biological availability (Miller, 1983). Bioavailability can be affected by a number of factors including animal species, physiological state, previous nutrition, interactions with dietary nutrients and ingredients, choice of response criteria, choice of standard source, and chemical form and solubility of the mineral element. For more detailed information on the effects of each of these variables and their effects on specific minerals the reader is referred to a recent Academic Press publication on bioavailability of nutrients for animals by Ammerman *et al.* (1995).

A new method to determine bioavailability of mineral elements was developed at the University of Florida, and described in detail by Henry *et al.,* (1987). With this method, tissue uptake of the mineral element following high-level, short term supplementation is used as the criteria to determine bioavailability. This method appears to offer several advantages over the traditional approach including use of natural diets which are less expensive, and which allow the animals to grow to their maximum genetic potential. Other advantages cited include fewer concerns with respect to mineral contamination of diets and tissues, and the fact that it takes fewer animals to detect statistically significant differences. This new method has proven to be as effective as the traditional method in determining copper and zinc bioavailability from inorganic and organic sources of copper and zinc.

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Traditionally, trace minerals have been added to livestock diets to supply the animal's requirement for these minerals. Supplementing diets with trace mineral mixes is a common practice in the industry since it is assumed that diets may not always contain adequate amounts of minerals to meet requirements, or the trace minerals in feed ingredients may not be in a form that is biologically available to the animals. Initially, diets were supplemented with inorganic sources of the mineral elements. However, recently organic mineral sources have begun to gain popularity because of a number of perceived benefits to their use. In a recent review, Miles and Henry (1999) listed the following perceived benefits reported in the popular press: 1) the ring structure protects the mineral from unwanted chemical reactions in the gastrointestinal tract; 2) chelates easily pass intact through the intestinal wall into 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 the chelate facilitates the 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 low pH; and 11) chelates can be absorbed by the amino acid transport system. It remains to be seen how many of these perceived benefits will be validated experimentally. Trace mineral bioavailability from inorganic sources of the elements has been extensively reviewed by Ammerman *et al*. (1995), and therefore, will not be covered in any detail in this review. The current presentation will be limited to a discussion of factors that influence bioavailability, potential applications for organic copper and zinc sources, and a brief discussion of trace mineral antagonists of practical significance to ruminants.

AAFCO DEFINITIONS

Mineral products that can be sold in the United States as organically bound compounds are defined by the Association of American Feed Control Officials (AAFCO, 2000) as follows: 1) *Metal Proteinate* (57.23) is the product resulting from the chelation of a soluble salt with amino acids and/or partially hydrolyzed protein. It must be declared as an ingredient as the specific metal proteinate e.g. copper proteinate, zinc proteinate etc.; 2) *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 e.g. copper polysaccharide complex, zinc polysaccharide complex etc.; 3) *Metal Amino Acid Chelate* (57.142) is the product resulting from the reaction of a metal ion from 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. The minimum metal content must be declared. When used as a commercial feed ingredient it must be declared as a specific metal amino acid chelate e.g. copper amino acid chelate, zinc amino acid chelate etc.; 4) *Metal Amino Acid Complex* (57:150) is the product resulting from complexing a soluble metal salt with an amino acid (s). Minimum metal content

must be declared. When used as a commercial feed ingredient, it must be declared as a specific metal amino acid complex e.g. copper amino acid complex, zinc amino acid complex etc.; 5) *Metal (specific amino acid) complex* (57.151) is the product resulting from complexing a soluble metal salt with a specific amino acid. Minimum metal content must be declared. When used as a commercial feed ingredient, it must be declared as a specific metal, specific amino acid complex e.g. copper lysine, zinc methionine etc.

FACTORS AFFECTING BIOAVAILABILITY

A review of the data summarized in Tables 1 and 2, indicates that in most studies, organic mineral sources were at least as available as the standard inorganic sources, and in some cases were more available. In ruminants, the well known interaction among copper, molybdenum, and sulfur has been shown to influence the availability of organic copper sources in some studies but not in others. Kincaid *et al.* (1986) reported a higher bioavailability of copper from copper proteinate compared with copper sulfate in calves fed diets containing molybdenum (Cu:Mo ratio near unity). Similarly, Ward *et al*. (1996) found that copper proteinate and copper sulfate were equally effective in supplying copper to cattle fed low dietary Mo. However, when the diet contained higher levels of Mo, copper from proteinate was more bioavailable. In contrast to these studies, Ward *et al*. (1993) found no difference in copper bioavailability between copper sulfate and copper lysine regardless of dietary Mo and sulfur levels.

One of the hypothesized reasons for increased bioavailability of organic minerals is that this form of mineral is protected from unwanted interactions in the gastrointestinal tract. Support for this hypothesis was provided by Wedekind *et al.* (1992). In an elegantly designed study, Wedekind *et al.* (1992) compared the bioavailability of zinc-methionine relative to that of zinc sulfate using three different diets: purified; semipurified; and a practical corn-soybean meal diet. Bioavailability estimates for zinc methionine relative to zinc sulfate were 117, 177, and 206% for the purified, semipurified, and corn-soybean meal diet, respectively. The authors concluded that the higher phytate and fiber content of the semipurified and corn-soybean meal diet reduced the bioavailability of zinc from zinc sulfate, whereas the zinc in zinc-methionine was protected from the negative effects of phytate and fiber. Dietary calcium concentrations have also been demonstrated to differentially affect zinc bioavailability from organic and inorganic sources of zinc. Bioavailability of zinc-methionine was 166% relative to zinc sulfate at a dietary calcium concentration of 0.60% calcium, and 292% at 0.74% calcium (Wedekind *et al*., 1994a).

One obvious reason for variable bioavailability associated with organic sources is undoubtedly due to the fact that these products are produced by processes that can potentially result in products with similar names (e.g. metal amino acid chelate) but containing variable concentrations of minerals and organic ligands (amino acids). The definitions used by AAFCO (2000) clearly demonstrate the potential for considerable variability during the manufacturing process of these products. The absence of standardized methods to chemically characterize mineral chelates and to relate those characteristics to in vivo bioavailability has been considered a major hurdle to the

acceptance of these products (Leach and Patton, 1997). Early attempts to characterize mineral chelates included microbial degradation (Kerley and Ledoux, 1992), chromatography (Brown and Zeringue, 1994), and solubility (Leach and Patton, 1997). Recently, a comprehensive evaluation of organic copper (Cao *et al.,* 2000) and zinc sources (Guo *et al.,* 2001) was conducted by the group at the University of Florida. Evaluation procedures included chemical analysis (mineral and N content), polarographic analysis (chelation effectiveness), solubility (in various fluids), stability to different pH buffers (pH 2 and 5), gel filtration chromatography (for evaluation of structural integrity of the chelate structure), and in vivo bioavailability assays. Results indicated that although there were numerous differences in the chemical characteristics of these organic mineral sources, the in vivo studies failed to distinguish among products with regard to absorption or tissue mineral deposition. Bioavailability of zinc sources was most related to negative solubility of zinc in pH 5 buffer in chicks, and pH 2 buffer in lambs. In contrast to zinc, bioavailability of copper sources was most closely related to solubility of copper in pH 2 buffer. Results of these studies suggest that development of standardized techniques to evaluate organic mineral sources will continue to be a challenge.

Two other factors that can also have an effect on bioavailability estimates include the standard mineral standard source used in the assay, and the choice of response variable. It has been unequivocally demonstrated that copper from copper oxide is poorly available to poultry, pigs, and cattle (Baker *et* al., 1991; Ledoux *et al*., 1991; Kegley and Spears, 1994). Therefore, copper oxide should not be used as a standard for evaluation of bioavailability of organic copper sources. A similar case could be made for zinc oxide, with bioavailability estimates of four feed grade zinc oxide sources ranging from as low as 22% to as high as 93% in one study (Edwards and Baker, 1999). Reports by Schell and Kornegay (1996) and Wedekind *et al*. (1994b) clearly demonstrate the impact of the response variable used on bioavailability estimates. Wedekind *et al*. (1994b) reported bioavailability estimates of 67, 70, and 87% for zinc oxide, 24, 38, and 79% for zinc lysine, and 60, 84, and 95% for zinc methionine, when metacarpal, coccygeal, and plasma zinc, respectively were used as response variables. Schell and Kornegay (1996) used serum, liver, kidney, and bone zinc as response variables and reported bioavailability estimates ranging from 61 to 84% for zinc oxide, 59 to 93% for zinc methionine, and 88 to 94% for zinc lysine. These studies clearly demonstrate that choice or response variable can significantly influence bioavailability estimates.

There are limited data on the mechanisms associated with solubilization, absorption, and transport of organic mineral sources. One hypothesis proposed to explain increased bioavailability of organic sources is that mineral chelates can be absorbed intact. However, results of recent studies suggest that this particular hypothesis may be questionable. Gel filtration chromatography studies of chelated copper (Guo *et al*., 2001) and zinc (Cao *et* al., 2000) sources indicate that very little of the copper and zinc remained in the chelated form at pH 2. This suggests that it is unlikely that chelated mineral sources are absorbed intact, and is consistent with

conclusions reached by researchers in two previous studies investigating ^{65}Zn-methionine absorption (Hill *et al*., 1987; Hempe and Cousins, 1989).

There does appear to be some evidence that organic zinc and copper sources are metabolized differently than inorganic sources. In 1989, Spears reported that zinc from zinc oxide and zinc methionine was absorbed to the same extent by lambs but more zinc was retained from zinc methionine as a result of lower urinary excretion of zinc by lambs. Similar results were reported by Kerley and Ledoux (1992) who also observed similar absorption of zinc from zinc proteinate and zinc oxide, but increased retention of zinc from zinc proteinate as a result of reduced urinary zinc excretion by lambs. More recently, Eckert *et al*. (1999) also reported differences in the way copper from copper proteinate and copper sulfate was metabolized by sheep. Copper from copper proteinate resulted in greater ceruloplasmin activity than copper from copper sulfate. However, ewes fed increasing levels of copper from copper sulfate deposited more copper in the liver compared with ewes fed copper proteinate.

ECONOMIC CONSIDERATIONS

Even if organic mineral sources are more bioavailable than inorganic sources, cost of these sources will be a major factor in the decision on whether or not to use them. One approach to this problem would be to supply only a portion of the supplemental minerals in the organic form. Recent studies suggest that this approach has merit. Veum *et al*. (1995) replaced 15-36% of supplemental inorganic zinc, iron, copper, and manganese with chelated metal proteinates and observed increased gain and feed conversion in nursery pigs compared with those fed only inorganic sources. Uchida *et al.* (2001) replaced a portion of an inorganic mineral supplement with organic sources (zinc, manganese, copper, and cobalt) in a dairy cow diet fed from calving until first breeding service. Cows fed the diet containing the organic mineral sources had fewer days to conception and tended to have fewer days to first service and fewer services per conception. In a similarly designed study, Ballantine *et al.* (2002) also replaced a portion of an inorganic mineral supplement with organic sources (zinc, manganese, copper, and cobalt) but the diet was fed from 21 days prepartum until 250 days postpartum. In cows pregnant at 250 days in milk, those fed organic sources produced more milk, had fewer days open (147 vs 169 days), and tended to have a higher first service conception rate (27 vs 18%). Nocek and Patton (2002) also reported increased milk production and improved reproductive performance in cows fed mineral supplements containing a combination of inorganic and organic mineral sources from 60 days prepartum to 150 days postpartum. These studies suggest that replacing a portion of inorganic mineral supplements with organic sources may be a viable approach and would certainly help address cost concerns.

ENVIRONMENTAL APPLICATIONS

Another potential application for organic mineral sources, that is currently receiving a lot of attention, is the use of organic sources of copper and zinc as a replacement for pharmacological concentrations of zinc oxide and copper sulfate

currently being used as growth promotants. The rationale for this approach is that if organic sources are more available, then the beneficial effects of high levels of zinc oxide and copper sulfate should be achieved with much lower concentrations of organic sources. The use of lower zinc and copper concentrations would address current environmental concerns with respect to high fecal excretion of both zinc and copper. To date, results of studies have been mixed. Case and Carlson (2002) conducted 3 experiments comparing the growth promoting ability of a zinc amino acid complex (500 mg/kg) and a zinc polysaccharide complex (500 mg/kg) to that of zinc oxide (3000 mg/kg). In two of the three experiments, body weight gain of pigs fed the zinc polysaccharide complex was similar to that of pigs fed zinc oxide. Pigs fed zinc polysaccharide excreted 4 fold less zinc than pigs fed zinc oxide in experiment 3. In a second study, Carlson *et al.* (2004) evaluated various inclusion rates of organic zinc either as zinc polysaccharide (0 – 500 mg/kg) or proteinate (0 – 800 mg/kg) compared with zinc oxide (2000 mg.kg) on growth performance, plasma, and excretion of nursery pigs. Organic zinc either as a polysaccharide or proteinate had no effect on growth performance; however, feeding the lower concentrations of organic zinc greatly decreased zinc excretion. Veum et al. (2004) reported that replacing 250 mg Cu/kg as copper sulfate in practical phase 1 and phase 2 nursery diets with 50 or 100 mg/kg copper as copper proteinate increased growth performance of 6-kg pigs. A growth response to copper source was not observed in a balance study, the two copper proteinate diets (50 and 100 mg/kg) did however reduce copper excretion by 77 and 61%, respectively, compared with 250 mg Cu/kg as copper sulfate. Results of these studies suggest that there is the potential for using organic sources of copper and zinc to reduce the negative environmental impact of pharmacological concentrations of zinc and copper.

MINERAL INTERACTIONS

O'Dell (1997) defines mineral interactions as ''interrelationships among mineral elements as revealed by physiological or biochemical responses''. O'Dell (1997) divided interactions into two major classes, positive (commonly synergistic) and negative (antagonistic). A high concentration of an antagonist element decreases the biologic effectiveness of its target element. Antagonistic interactions are often expressed as a mutual inhibition of absorption from the intestinal tract but can also occur at the cellular level (Henry and Miles, 2000). Interactions can occur between two minerals (e.g. calcium and phosphorus), or multiple minerals (e.g. copper, molybdenum, and sulfur). The current presentation will be limited to a discussion of mineral interactions that can have practical implications for ruminant animals.

Copper-Molybdenum-Sulfur Interaction

One interaction of practical importance in ruminant nutrition, recognized since the 1950s, is the copper-molybdenum-sulfur (Cu-Mo-S) interrelationship (Dick, 1953). In the reducing environment of the rumen, dietary sulfur is reduced to sulfide, which then interacts with molybdenum to form thiomolybdates (Mason, 1986). Thiomolybdates have been shown to affect copper metabolism in two ways. In the gastrointestinal tract,

some thiomolybdates have been shown to bind copper preventing its absorption (Allen and Gawthome, 1987). Thiomolybdates that are absorbed have also been shown to cause systemic effects on copper metabolism including: 1) increased biliary excretion of copper from liver stores; 2) reduced transport of available copper for biochemical processes; and 3) removal of copper from metalloenzymes (Spears, 2003). The formation of thiomolybdates appears to depend on total dietary sulfur concentration. Spears (2003) cited data to show that at low ruminal sulfide concentrations, molybdenum may have little effect on copper availability, whereas at higher ruminal sulfide concentrations copper availability was significantly decreased.

Copper-Sulfur Interaction

Independent of its role in the Cu-Mo-S interaction, sulfur has also been shown to reduce copper bioavailability. Increasing dietary sulfur (inorganic or organic) reduced copper bioavailability by 30-56% in hypocupremic ewes fed low molybdenum diets (Suttle, 1974). Sulfur reduced to sulfide in the rumen is believed to combine with copper to form insoluble copper sulfide a form of copper that is unavailable to ruminants. Evidence for this hypothesis was provided by Bird (1970) who reported that increasing dietary sulfur from 0.8 to 2.5 g sulfur/kg diet reduced omasal flow of soluble copper by approximately 50% in sheep.

A recent report on the effects of a molasses-based mineral supplement on trace mineral status of beef cattle is a prime example of a mineral interaction that can occur under practical conditions. In contrast to a corn-based mineral supplement, a molasses-based supplement resulted in lower liver copper concentrations of beef heifers (Arthington and Pate, 2002). The authors attributed the lower liver copper to Cu-Mo-S and Cu-S interactions as a consequence of the higher sulfur and molybdenum content in molasses compared to that of corn. Addition of sulfur to the corn-based mineral supplement resulted in liver copper values that were intermediate between the corn-based and molasses-based mineral supplements (Arthington and Pate, 2002).

In a recent review of copper antagonists in cattle nutrition, Arthington (2003) described other sources of sulfur that could also contribute to both the Cu-Mo-S and Cu-S interactions. Sources included fertilizers, high sulfur water, and sulfur containing supplements. Forages fertilized with ammonium sulfate may have a much higher concentration of sulfur compared with forages fertilized with ammonium nitrate. Bahiagrass pastures fertilized with ammonium sulfate contained 0.50% sulfur compared with 0.22% for pastures fertilized with ammonium nitrate (Arthington *et al*., 2002). Cows grazing these pastures had lower liver copper concentrations (72 ppm) compared with cows on pastures receiving no fertilizer (204 ppm) or fertilized with ammonium nitrate (137 ppm; Arthington *et al*., 2002). The authors concluded that application of sulfur-containing fertilizers may affect the copper status of grazing cattle, and choice of fertilizer source may be critical in areas where grazing cattle may be prone to copper deficiency.

Water containing high concentrations of sulfur can also significantly contribute to total sulfur intake. Weeth and Hunter (1971) reported that beef heifers consuming water containing 5000 ppm sulfur had reduced performance. Paterson *et al.* (1999) reported that sulfate content of water samples from 12 Montana ranches ranged from a low of 100 ppm to a high of 1300 ppm. These data suggest that the sulfur contribution from water could be substantial depending on water consumption by animals on these ranches. Arthington (2003) suggested that sulfate in water may be more available to interact with Mo and Cu compared with sulfur from dietary sources, and is a greater management challenge for livestock producers compared with sulfate fertilizers.

A final source of sulfur that is sometimes overlooked is sulfur present in energy, protein, and mineral supplements. The use of molasses in mineral supplements to increase palatability and hence intake, and the consequences of the sulfur and molybdenum content of molasses has already been discussed. High protein supplements will generally contain higher concentrations of sulfur due to the contribution of sulfur amino acids, and the amount of sulfur could be further increased with addition of molasses to these supplements.

Copper-Iron Interaction

Ruminants consuming forage based diets can be exposed to high levels of iron. Grazing animals can consume high levels of iron as a consequence of soil consumption during grazing and through consumption of soil contaminated forages. Suttle (1975) estimated that during winter, soil ingestion can exceed 10% of the dry matter intake of grazing sheep and cattle. This level of soil ingestion markedly reduced copper absorption (Suttle, 1975). High dietary iron has been shown to reduce copper status in cattle (Standish *et al*., 1971; Campbell *et al*., 1974; Humphries *et al*., 1983; Phillipo *et* al., 1987; Mullis *et al*., 2003) and sheep (Prabowo *et al*., 1988; Grace and Lee, 1990). It is believed that sulfide in the rumen combines with iron to form ferrous sulfide complexes that dissociate in the low pH abomasum where the sulfide then forms insoluble complexes with copper (Gengelbach *et al*., 1994).

Selenium-Sulfur Interaction

Spears (2003) cited studies to suggest that there may be an antagonistic interaction between selenium and sulfur. Sulfur addition to pregnant ewe diets low in selenium increased the incidence of white muscle disease in their lambs. Increasing dietary sulfur from 2.1 to 7 g/kg diet resulted in a linear decrease in plasma selenium and absorption of selenium in lactating dairy cows. In sheep, concentrations of selenium were reduced in liver and rumen microbes when dietary sulfur was increased from 2.2 to 4 g/kg diet. The mechanism of the interaction has not been determined but selenium and sulfur have similar physical and chemical properties, and elements with similar chemical properties are likely to interact competitively (O'Dell, 1997).

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Table 1. Bioavailability of organic copper sources

MINERAL SOURCE	BIOAVAILABILITY ESTIMATE	REFERENCE
Cattle		
Copper lysine	=	Ward *et. al*., 1993
Copper lysine	100	Kegley and Spears, 1994
Copper proteinate	147	Kincaid *et al*., 1986
Copper proteinate	102	Wittenberg *et al*., 1990
Sheep		
Copper glycine	96	MacPherson and Hemingway, 1968
Copper amino acid chelate	>	Ryan *et al*., 2002
Poultry		
Copper-lysine	99	Pott *et al.,* 1994
Copper-lysine	116	Baker *et al*., 1991
Copper-lysine	120	Aoyagi and Baker, 1993a
Copper-lysine	122, 111	Guo *et al*., 2001
Copper-methionine	88, 96	Aoyagi and Baker, 1993b
Copper-amino acid chelate	122	Guo *et al*., 2001
Copper-proteinate A	109	Guo *et al*., 2001
Copper-proteinate B	105	Guo *et al*., 2001
Copper-proteinate C	111	Guo *et al*., 2001
Pigs		
Copper-methionine	107	Bunch *et al*., 1965
Copper chelate	=	Stansbury *et al*., 1990
Copper-lysine	=	Apgar and Kornegay, 1996

Adapted from Ammerman *et al*., 1995.

Table 2. Bioavailability of organic zinc sources

MINERAL SOURCE	BIOAVAILABILITY ESTIMATE	REFERENCE
Cattle		
Zinc-methionine	103, 133	Kincaid *et al.,* 1984
Zinc-methionine	99	Greene et al., 1988
Zinc-methionine	106	Spears, 1989
Zinc-methionine	105	Spears and Kegley, 1991
Zinc-polysaccharide complex	173	Kennedy *et al*., 1993
Sheep		
Zinc chelated	91, 125	Ho and Hiridoglou, 1977
Zinc, sequestered	108, 103	Ho and Hiridoglou, 1977
Zinc-methionine	95, 103	Spears, 1989
Zinc-methionine	107 – 120	Cao *et al*., 2000
Zinc proteinate	128 –131	Cao *et al*., 2000
Zinc amino acid chelate	103 –114	Cao *et al*., 2000
Zinc amino acid chelate	>	Ryan *et al*., 2002
Zinc-lysine	>	Rojas *et al*., 1995
Zinc-methionine	=	Rojas *et al*., 1995
Poultry		
Zinc-methionine	124, 176	Wedekind *et al*., 1992
Zinc-methionine	100	Pimental *et al*., 1991
Zinc proteinate	100	Pensack *et al*., 1958
Zinc amino acid chelate	76 – 83	Cao *et al*., 2000
Zinc proteinate A	133 – 139	Cao *et al*., 2000
Zinc proteinate B	99	Cao *et al*., 2000
Zinc proteinate C	108	Cao *et al*., 2000
Zinc polysaccaride	94	Cao *et al.,* 2000
Pigs		
Zinc-lysine	100	Hahn and Baker, 1993
Zinc-lysine	38 - 79	Wedekind *et al*., 1994b
Zinc-lysine	88 – 99	Schell and Kornegay, 1996
Zinc-lysine	69 – 88	Schell and Kornegay, 1996
Zinc-lysine	=	Cheng *et al*., 1998
Zinc-methionine	>100	Hahn and Baker, 1993
Zinc-methionine	60 - 95	Wedekind *et al*., 1994b
Zinc-methionine	59 – 93	Schell and Kornegay, 1996
Zinc-methionine	80 – 99	Schell and Kornegay, 1996
Zinc-methionine	=	Hill *et al*., 1986
Zinc-methionine	=	Revy *et al*., 2002
Zinc amino acid chelate	=	Swinkels *et al.,* 1996
Zinc amino acid chelate	109 – 127	Swiatkiewicz *et al*., 2001

Adapted from Ammerman *et al*., 1995.