

ABSTRACT

Wright, Cody LeForge. Evaluation of Absorption and Post-Absorptive Metabolism of Inorganic and Organic Zinc Sources. (Under the direction of Dr. Jerry W. Spears)

Experiments were conducted to compare the absorption and metabolism of Zn from inorganic or organic sources. In Experiment 1, Holstein bull calves were supplemented with 0 or 20 mg Zn/kg diet as ZnSO₄, Zn proteinate (**ZnProt**) or a mixture of ZnSO₄ and ZnProt (**ZnMix**) for 98 d. From d 98 to 112, supplemental Zn levels for half of the bulls in each treatment were increased to 500 mg Zn/kg. Zinc concentrations in plasma and duodenal, liver, kidney, and muscle samples were greater in bulls supplemented with high levels of ZnProt or ZnMix than ZnSO₄. At high levels, ZnSO₄ increased skin Zn concentrations relative to ZnProt. Skin and hoof wall Zn concentrations were greater in bulls supplemented with low levels of ZnMix compared to ZnSO₄.

Experiments 2 and 3 compared the uptake and transport of Zn from ZnSO₄ and ZnProt or Zn propionate (**ZnProp**) by ruminal and omasal epithelium using parabiotic chambers. Uptake of Zn by omasal epithelium was negligible and Zn transport by ruminal and omasal epithelium was non-detectable in all experiments. Zinc uptake by ruminal epithelium increased as incubation time increased and was greater and tended to be greater from ZnProt and ZnProp, respectively, than from ZnSO₄. In concentration-dependent experiments, Zn uptake was affected by a concentration × source interaction. More Zn was absorbed from ZnProt than from ZnSO₄ when added at 200, but not 10 μM. Concentration-dependent uptake of ZnSO₄ and ZnProp was unaffected by source. Following ruminal digestion, solubility of Zn from ZnSO₄ and ZnProt was influenced and uptake tended to be influenced by a source × concentration interaction with both being

greater from ZnProt than from ZnSO₄. Solubility Zn from ZnSO₄ and ZnProp was influenced by a concentration × source interaction with Zn from ZnSO₄ being more soluble than Zn from ZnProp at 10, but not 200 μM. Zinc uptake from ZnSO₄ and ZnProp following ruminal digestion was affected by a source × concentration × time interaction. At lower Zn levels, uptake was unaffected by source; however, at higher concentrations, uptake increased over time and was greater from ZnSO₄ than ZnProp. Experiments 4 and 5 compared the uptake and transport of Zn from ZnSO₄ and ZnProt or ZnProp using Caco-2 cell monolayers. In time- or concentration-dependent experiments, Zn uptake and transport increased as incubation time and Zn concentration increased; however, neither was affected by Zn source. Addition of inositol hexaphosphate (IP₆) and Ca reduced solubility, uptake and transport of Zn. In the presence of IP₆ and Ca, Zn uptake from ZnSO₄ and ZnProp tended to be affected by a source × concentration interaction with uptake being greater from ZnSO₄ than from ZnProp at 200, but not 10 μM. Following ruminal and intestinal digestions, Zn uptake from ZnProp tended to be greater than from ZnSO₄; whereas, uptake from ZnSO₄ and ZnProt were similar. Following intestinal digestion alone, Zn uptake from ZnSO₄ and ZnProt was affected and uptake from ZnSO₄ or ZnProp tended to be affected by a source × concentration interaction with uptake being greater from ZnProt or ZnProp than from ZnSO₄ when added at 200, but not 10 μM. Zinc solubility following intestinal digestion was affected by a source × concentration interaction. Zinc from ZnSO₄ was more soluble than Zn from ZnProp when added to digestions at 10; however, at 200 μM Zn, Zn from ZnProp was more soluble than from ZnSO₄. Results suggest that at low concentrations or in the absence of antagonists uptake and transport of Zn from inorganic and organic Zn sources

are similar. However, following simulated digestion, uptake of Zn from organic Zn sources appears to be greater than from inorganic source.

**EVALUATION OF ABSORPTION AND POST-ABSORPTIVE METABOLISM OF INORGANIC
AND ORGANIC ZINC SOURCES**

by

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DEDICATION

This dissertation is dedicated...

to my loving wife Stephanie for her unending support, and commitment and devotion to me and my education, without her by my side, this would have been impossible;

to my parents Larry and Jeane Wright, for their compassion and encouragement in all of my endeavors, their love and pride in me means everything;

and to the memory of my beloved grandparents Warren and Eldora Wright, for their love and for instilling in me at a tender age a love and appreciation for agriculture and fine people in agriculture, I miss you.

BIOGRAPHY

Cody LeForge Wright was born in Sioux Falls, South Dakota, on December 21, 1971, to Larry and Jeane Wright. Cody, along with his sister Karli, and twin brothers Casey and Kelly, attended elementary school in Valley Springs, South Dakota and junior and senior high school in Brandon, South Dakota. Upon graduation in 1990, Cody enrolled at South Dakota State University as a Mechanical Engineering major. However, following a humbling semester in the Engineering curriculum, Cody came to his senses and changed his major to Animal Science. In May of 1994, Cody graduated With Honor with a Bachelor of Science degree in Animal Science. Cody and his wife, Stephanie, were married on December 10, 1994. After their marriage, he and Stephanie moved to Manhattan, Kansas, where Cody began pursuit of his Master of Science degree at Kansas State University. In 1996, Cody earned his Master of Science degree in Animal Science under the direction of Dr. Larry Corah. He and Stephanie then moved to Raleigh, North Carolina, where Cody began pursuit of his Doctor of Philosophy degree in Animal Science at North Carolina State University under the direction of Dr. Jerry Spears. While at North Carolina State University, Cody also coached the Livestock Judging Team. Upon completion of his Doctor of Philosophy degree, he and Stephanie will be moving to Brookings, South Dakota, where Cody has accepted an Assistant Professor – Beef Extension Specialist position at South Dakota State University.

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LITERATURE REVIEW

Introduction

Zinc is a group IIb transition metal with an atomic number of 30 and a molecular weight of 65.39. Since Zn possesses a completed *d* subshell, it has only one oxidation state. Zinc is known to be a component of enzymes that catalyze over 50 different reactions and reside in all six major Enzyme Commission classes (Chesters, 1997). Some major Zn-metalloenzymes found in mammalian tissue are alcohol dehydrogenase, alkaline phosphatase, carbonic anhydrase, carboxypeptidases A and B, leucine aminopeptidase, mannosidase, and superoxide dismutase.

Presence of Zn in animal tissues has been known since the end of the nineteenth century; however, clear evidence of the biological function of Zn was not available until 1934 (Chesters, 1997). Todd et al. (1934) first determined that Zn was necessary for growth and health in rats and mice. Shortly thereafter, Tucker and Salmon (1955) discovered that Zn prevented and cured parakeratosis in pigs and Zn deficiency was demonstrated in the chick (O'Dell and Savage, 1957). In ruminants, Zn deficiency is uncommon; however, growth and health responses to supplemental Zn have been observed in cattle and sheep grazing pastures containing 20 mg Zn/kg DM (Underwood and Suttle, 1999).

Zinc Absorption

Zinc absorption occurs primarily from the small intestine in most species (Underwood and Suttle, 1999) and is believed to be facilitated by a carrier-mediated process, which has yet to be clearly defined (Cousins, 1996). In ruminants, Zn is absorbed primarily from the small intestine (Miller and Cragle, 1965; Hampton et al.,

1976); however, Zn absorption from ruminal tissue has been demonstrated. Zinc uptake and apparent absorption from rumen tissue has been clearly demonstrated in vivo using ^{65}Zn dosed lambs (Arora et al., 1969) and wethers fitted with ruminal, abomasal and ileal cannulas (Kennedy and Bunting, 1991, Kirk et al., 1994). Less than 2% of total Zn is absorbed from the large intestine in ruminants (Hampton et al., 1976); however, when intestinal absorption was impaired Zn was absorbed from the cecum and colon in rats (Hara et al., 2000).

Specific Zn absorption mechanisms have yet to be clearly defined; however, absorption kinetics have been examined in several systems. At least two components of Zn uptake have been identified in isolated brush border membrane vesicles of rats (Menard and Cousins, 1983) and pigs (Blakeborough and Salter, 1987). The first component exhibits saturation kinetics with a K_m of 70-350 μM depending on the system used (Chesters, 1997). The second component responded linearly to Zn concentrations up to 2 mM (Lee et al., 1989). Zinc uptake is also known to be greater from the lumen of Zn-deficient rats than from Zn-adequate controls, suggesting that Zn uptake is a major site of homeostatic regulation (Chesters, 1997). Within the mucosa Zn is likely bound by one of several potential intracellular ligands. Hempe and Cousins (1991, 1992) hypothesized that at low Zn concentrations, absorbed Zn is bound by a cysteine-rich intestinal protein (**CRIP**) that is not metallothionein (**MT**). The authors suggested that CRIP may function as an intracellular Zn shuttle to translocate Zn to the basolateral membrane or to one of many potential Zn metalloproteins within the mucosa. In contrast, when Zn was at high concentrations, absorbed Zn induced synthesis of MT (Cousins, 1985) and subsequently a large fraction of the absorbed Zn was bound by MT and sequestered in the mucosa

(Hempe and Cousins, 1992). The authors suggested that, at high Zn concentrations, MT binds Zn to prevent absorption of potentially toxic levels of Zn; whereas, when Zn was at low concentrations, CRIP transferred absorbed Zn to the basolateral membrane for transport into circulation. Researchers have only recently defined zinc transporters. Four distinct Zn transport proteins have been isolated; however only two, ZnT-1 and ZnT-2, appear to function in the small intestine (McMahon and Cousins, 1998). Both of these transport proteins seem to be localized to the basolateral membrane and function to transport Zn out of the enterocyte into circulation (McMahon and Cousins, 1998). Divalent cation transporter 1 (**DCT1**), a transporter of a number of cations, has recently been cloned (Gunshin et al., 1997). Upregulation of DCT1 in response to low dietary iron status but without additional iron in the intestinal lumen may cause more Zn to be absorbed because the affinities for both cations are similar (Cousins and McMahon, 2000).

Peptide and Amino Acid Absorption in Ruminants

Ruminant protein digestion relies on the same complement of pancreatic and intestinal proteases to effect breakdown of protein as do non-ruminants and absorb amino acids and small peptides by similar mechanisms (Merchen, 1988). Amino acid absorption in the small intestine is a Na⁺-dependent, carrier-mediated process, and can be described as a type of secondary active transport (Armstrong and Hutton, 1975). The small intestine has the capacity to transport between 65 and 80% of the total amino acid supply reaching duodenum (Armstrong and Hutton, 1975). The small intestine has been found to be the primary site of amino acid absorption in ruminants. Wilson and Webb (1990) characterized the absorption of lysine and methionine in bovine ileal and jejunal brush

border membrane vesicles. Their observations suggest that amino acid absorption may occur via mediated and non-mediated, and Na⁺-dependent and Na⁺-independent components.

Di- and tri-peptides have also been found to be absorbed in the small intestine via mechanisms driven by H⁺ and(or) Ca gradients (Webb et al., 1993). Evidence is strong for the existence of multiple peptide transport systems, including one type that is electrogenic in nature and cotransports two H⁺ for every peptide transported (Webb et al., 1992). Furthermore, peptides may be absorbed more rapidly than amino acids and subsequently partially hydrolyzed by intracellular peptidases (Webb et al., 1993). Recently, researchers have demonstrated the ability of ovine ruminal and omasal epithelium to absorb and translocate amino acids and di-peptides (Matthews and Webb, 1995). Free and peptide-bound amino acid absorption appeared to be non-saturable and occurred to a greater extent from omasal epithelium than from ruminal epithelium. Furthermore, carnosine was absorbed across both tissues without hydrolysis, whereas methionylglycine was partially hydrolyzed in both tissues and to a greater extent by omasal epithelium (Matthews and Webb, 1995). In sheep and lactating Holstein cows, peptide transporter mRNA has been found in rumen, omasum, duodenum, jejunum, and ileum (Chen et al., 1999). No hybridization was observed with mRNA from abomasum, cecum, colon, liver, kidney, or semitendinosus and longissimus muscles.

Propionate Absorption in Ruminants

Though some volatile fatty acids (VFA) will leave the reticulo-rumen with the digesta, flowing to the lower gastro-intestinal tract, the vast majority of the acids produced by ruminal fermentation are absorbed from the reticulo-rumen (Merchen,

1998). To date there is no evidence for any active transport of VFA from the rumen. Since the pKa of VFA is near 4.8, most VFA are dissociated at normal pH (6-7). Stevens (1970) suggested that free (undissociated) acids may be absorbed via simple diffusion whereas dissociated acids are likely absorbed in exchange for bicarbonate. In vitro experiments support the hypothesis of Stevens. Kramer et al. (1996) used isolated ovine ruminal epithelium in Ussing chambers to evaluate absorption of short-chain fatty acids (SCFA). Their results suggest that SCFA anions either compete with Cl⁻ for binding sites on a common anion exchange mechanism or that SCFA anions are absorbed in exchange with HCO₃⁻.

Bioavailability of Organic Zinc Sources

Feed grade Zn is commercially available as inorganic salts, ZnSO₄ or ZnO. Organic Zn sources have become increasingly more popular in recent years as a result of reported improvements in growth performance, feed efficiency, health, and carcass characteristics in livestock. While the mode of action by which organic trace minerals elicit beneficial responses remains to be elucidated, proposed mechanisms have included enhanced solubility, greater absorption, prevention of antagonistic interactions, and differences in post-absorptive metabolism. Ashmead et al. (1985) suggested that metal ions may be transported in metal:amino acid or metal:peptide complexes. It is possible that these complexes may be transported intact by amino acid and(or) peptide transporters. The following is a brief review of research conducted with various organic Zn sources.

Zinc amino acid chelates. Zinc amino acid chelates are defined as the products resulting from the reaction of a metal ion from a soluble metal salt with a mole ratio of

one mole metal to one to three (preferably two) moles of amino acids to form coordinate covalent bonds (Spears, 1996). The average weight of the hydrolyzed amino acids must be approximately 150 and the resulting molecular weight of the chelate must not exceed 800. Zinc amino acid chelates (**ZnAAC**) have been compared to inorganic Zn sources in chicks, dogs, lambs, and pigs.

Cao et al. (2000) compared numerous organic Zn sources to inorganic ZnSO₄ using several in vitro techniques and broiler chicks and lambs in vivo. Chicks were fed corn-soybean meal diets supplemented with 0, 200, 400, or 600 mg Zn/kg from ZnSO₄ or 200 or 400 mg Zn/kg from ZnAAC or Zn proteinate (**ZnProt**). Feed intake and gain were not different between organic Zn sources; however, both were greater in chicks fed organic Zn relative to those fed inorganic Zn. Relative bioavailability values (%) calculated based on bone Zn concentration were 104 and 116 in week 1, 100 and 135 in week 2, and 83 and 139 in week 3 for ZnAAC and ZnProt, respectively. Lambs were supplemented with 0, 700, 1400, or 2100 mg Zn/kg from ZnSO₄, or 1400 mg Zn/kg from ZnProt, ZnAAC, or ZnMet. Relative bioavailability calculations based on liver Zn and metallothionein, kidney Zn and pancreas Zn concentrations were 130, 110, and 113% for ZnProt, ZnAAC, and ZnMet respectively as compared to ZnSO₄.

In three different experiments with dogs, Lowe et al. (1994a, 1994b, and 1998) observed greater absorption and retention of Zn, and greater hair growth and Zn concentration in dogs fed Zn as ZnAAC than those fed ZnO or Zn polysaccharide. In contrast, Swinkels et al. (1996) fed Zn depleted pigs and pair-fed nondepleted pigs 45 mg Zn/kg diet from ZnSO₄ or ZnAAC for 36 d. Zinc concentrations of serum, liver, pancreas, kidney, brain, and small intestine were not affected by Zn source.

Zinc amino acid complexes. Zinc amino acid complexes are defined as the products resulting from the complexing of a soluble metal salt with an amino acid(s)(Spears, 1996). Generally, Zn amino acid complexes refer to either Zn lysine (**ZnLys**) or Zn methionine (**ZnMet**); however, in some cases multiple amino acids may be complexed with the metal ion.

Malcolm-Callis et al. (2000) compared the effects of 30 mg supplemental Zn/kg diet as ZnSO₄, Zn amino acid complex or Zn polysaccharide on performance, carcass characteristics and serum Zn concentrations of Brangus- and Angus-sired steers. The authors reported no differences in performance or serum Zn concentrations with respect to Zn source. Steers supplemented with organic Zn sources had greater s.c. fat thickness and lower kidney, pelvic, and heart fat percentages than steers supplemented with ZnSO₄.

Individual Zn amino acid complexes, ZnLys and ZnMet, have been researched extensively in several species; however, for sake of brevity literature reviewed here will focus on ruminants. Moreover, many early experiments compared responses of cattle fed ZnMet to those of unsupplemented cattle. The lack of an inorganic Zn source for comparison in these experiments precludes estimation of bioavailability and clouds interpretation of the data. As such, only experiments that included an inorganic Zn treatment will be reviewed.

Effects of ZnMet on growth performance, carcass characteristics, health and immune function have been examined in growing and finishing cattle. Zinc methionine supplementation has improved USDA Quality Grade and marbling score, and increased external and kidney, pelvic, and heart fat in feedlot steers (Greene et al., 1988). Growth performance of growing heifers (Spears, 1989) and feed efficiency of stressed steers

(Spears et al., 1991) tended to be greater in cattle supplemented with ZnMet than in those supplemented with ZnO. Antibody titers to bovine herpes virus-1, but not parainfluenza-3, were greater in stressed steers supplemented with ZnMet than in those supplemented with ZnO (Spears et al., 1991). Lambs born to ewes fed 50 mg Zn per ewe daily were weaned and supplemented with 25 mg Zn/kg diet from ZnMet or ZnO. Blastogenic responses of unstimulated lymphocytes were greater from lambs supplemented with ZnMet than from those supplemented with ZnO. However, when stimulated with phytohemagglutinin, concanavalin A, or pokeweed mitogen, blastogenic responses were unaffected by Zn source. Furthermore, lambs fed ZnMet had smaller welt diameters following intradermal PHA injection than those fed ZnO. In contrast, Engle et al. reported no difference in cell-mediated immune responses between heifers that were repleted with 23 mg Zn/kg as ZnMet or ZnSO₄; however, response values were lower in heifers supplemented with ZnLys. Droke et al. (1998) observed no difference in immune function in lambs supplemented with 25 mg Zn/kg diet from ZnMet or ZnSO₄.

Bioavailabilities of ZnMet and ZnLys have been assessed using apparent absorption and retention, and tissue and plasma Zn and metallothionein concentrations in lambs (Rojas et al., 1995; Spears, 1989) and Holstein heifer calves (Kincaid et al., 1997). Rojas et al. (1995) supplemented lambs with 360 mg Zn/kg diet for 3 wk, then the treatments were withdrawn for 4 wk and subsequently resumed for another week. Zinc accumulation and metallothionein concentrations in kidney, liver, and pancreas in lambs fed ZnLys were greater than values from lambs fed ZnO, ZnSO₄, ZnMet or unsupplemented controls. Tissue Zn concentrations were not different between lambs fed ZnSO₄ and ZnMet. Spears (1989) reported similar apparent absorption of ZnMet and

ZnO in lambs fed a semi-purified diet; however, retention was higher for lambs fed ZnMet than those fed ZnO. Similar responses were observed in Holstein heifer calves. Kincaid et al. (1997) reported elevated serum and liver Zn concentrations in Holstein heifer calves fed 300 mg Zn/kg diet as ZnMet or ZnLys than heifers fed ZnO. Serum Lys and Met concentrations were unaffected by treatment. Collectively, these experiments suggest that Zn from ZnMet or ZnLys is absorbed more effectively than Zn from inorganic Zn sources. Moreover, once absorbed, metabolism of Zn from the organic sources appears to be different than Zn from inorganic sources. The lack of changes in serum Lys and Met suggests that the organic Zn sources may not be absorbed intact. Rather, it is possible that Zn from the organic Zn sources may be transferred more effectively and(or) utilized more effectively by tissues than Zn from inorganic sources. However, specific differences in absorption and post-absorptive utilization of inorganic and organic trace minerals remain to be elucidated.

Zinc polysaccharide complex. Metal polysaccharide complexes are defined as the products resulting from the complexing of a soluble salt with a polysaccharide solution (Spears, 1996). Very little research has been conducted to evaluate the bioavailability of Zn polysaccharide complexes (**ZnPoly**) relative to inorganic Zn sources. Kennedy et al. (1993) examined the distribution of ZnPoly and ZnO in ruminal contents. Six Holstein steers were provided 208, 920, or 896 mg Zn/d from basal, ZnPoly, and ZnO diets, respectively. On the day ruminal contents were sampled, steers were dosed with 800 mg Zn from the appropriate supplement. Zinc concentrations in cell-free fluid, and fluid- and particle-associated microbial and particulate fractions were higher from steers supplemented with ZnPoly than unsupplemented controls or steers supplemented with

ZnO. Zinc from ZnPoly was also more soluble in a buffer solution than was Zn from ZnO.

Malcolm-Callis et al. (2000) compared the effects of 30 mg supplemental Zn/kg diet as ZnSO₄, Zn amino acid complex or ZnPoly on performance, carcass characteristics and serum Zn concentrations of Brangus- and Angus-sired steers. The authors reported no differences in performance or serum Zn concentrations with respect to Zn source. Steers supplemented with organic Zn sources had greater s.c. fat thickness and lower kidney, pelvic, and heart fat percentages than steers supplemented with ZnSO₄.

Zinc propionate. Zinc propionate is a complexed Zn product consisting of one molecule of Zn and two molecules of propionic acid (Kemin Industries, 1995). Research to establish the bioavailability of Zn propionate (**ZnProp**) in ruminants has not been published; however, bioavailability has been examined in chicks and dogs. To investigate the bioavailability of organic and inorganic Zn sources Kemin Industries (1995) fed chicks a corn-soybean meal diet alone or supplemented with 10 or 20 mg Zn/kg diet from ZnMet or ZnProp, or 10, 20, or 30 mg Zn/kg from ZnSO₄ for 21 d. Chicks fed 20 mg Zn/kg from ZnProp and ZnMet gained more weight than chicks fed 20 mg Zn/kg from ZnSO₄; however, feed efficiency was similar between treatments. Plasma Zn was greater in chicks fed 20 mg Zn/kg from ZnProp and ZnMet than those fed ZnSO₄. Tibia ash was not affected by Zn source; however, had the experiment been conducted for a longer time, differences may have become more prominent. Relative bioavailability calculations based on performance measures, and plasma and tibia Zn concentration ranged from 100.3 to 128.2% for ZnProp as compared to ZnSO₄.

Brinkhaus et al. (1998) used 10 adult Beagles to determine the bioavailability of ZnProp relative to ZnO in dogs. Following a 24 h period of feed restriction, dogs were given a gelatin capsule containing 5 mg Zn/kg BW. Plasma Zn concentrations from 0.5 to 6 h after dosing were greater in dogs dosed with ZnProp than in those dosed with ZnO. Wedekind et al. (1998) fed 42 dogs a semi-purified diet for two wk prior to administration of Zn treatments, then fed treatment diets for another three wk. Dogs were supplemented with 40 mg Zn/kg diet as ZnProp or ZnO, in the absence or presence of antagonists (Ca and/or beet pulp). While Zn intake was greater for ZnO, plasma Zn concentrations in the presence of Ca and beet pulp tended to be higher in dogs supplemented with ZnProp. With increasing dietary Ca and beet pulp, Zn concentrations of teeth and testes declined, regardless of Zn source. In the absence of antagonists, calculated bioavailability of ZnProp was 60-80% greater than that of ZnO. In the presence of Ca and beet pulp, bioavailability of both Zn sources declined; however, ZnProp appeared to be more available than ZnO.

In vitro, Buetler et al. (1998) compared uptake of Zn from ZnCl₂, ZnMet, and ZnProp using cultured human intestine epithelial cells, monkey kidney fibroblasts, and perfused mouse intestine. Steady-state uptake of Zn by human intestinal epithelial cells and monkey kidney fibroblasts was not affected by Zn source. Furthermore, using gel filtration chromatography, the authors reported that ⁶⁵Zn elution profiles of the three Zn sources were similar.

Zinc proteinate. Metal proteinates are defined as the products resulting from the chelation of a soluble salt with amino acids and/or partially hydrolyzed protein (Spears, 1996). Proteinate compounds are commercially available for copper, cobalt, iron,

manganese, and zinc (Spears, 1996). For sake of brevity, this review will focus exclusively on ZnProt. Zinc proteinate has received minimal attention in published literature to date. Experiments have been conducted to compare the bioavailability and retention of ZnProt relative to inorganic Zn in lambs (Cao et al., 2000; Lardy et al., 1992) and chicks (Cao et al., 2000). The effect of ZnProt supplementation on performance, health, and carcass characteristics of beef steers (Engle et al., 1998; Spears and Kegley, 1994), hoof strength characteristics of feedlot heifers (Reiling et al., 1992), somatic cell counts and incidences of mammary infection in lactating dairy cows (Spain et al., 1993) has been compared to inorganic Zn sources. In vitro experiments have also been conducted to compare the chemical characteristics of several organic Zn sources, including three different ZnProt compounds, to ZnSO₄ (Cao et al., 2000).

Lardy et al. (1992) fed fifteen lambs with either no supplemental Zn or Zn from ZnO or ZnProt to determine Zn retention from each source. Lambs were housed in stainless steel metabolism crates and fed a basal diet composed of 40% soyhulls, 45% cornstarch, 15% casein, and 1% urea. Lambs were allowed to adapt to the treatment diets for 14 d then feces and urine were collected for the next five days. Zinc intake was 18.3, 40.1, and 40.5 mg/d for the control, ZnO and ZnProt treatments, respectively. Apparent Zn digestion was increased by ZnO and ZnProt relative to controls, but was not different between the two Zn sources (16.4, 14.8, and 5.3, respectively). However, Zn retention appeared to be greater from ZnProt than from ZnO or controls (6.4, 0.6, and -4.7 mg/d, respectively).

In three different experiments Cao et al. (2000) utilized broiler chicks and crossbred wether lambs to determine the bioavailability of organic Zn sources compared

to inorganic ZnSO₄. In the first experiment, chicks were fed a corn-soybean meal diet supplemented with 0, 200, 400, or 600 mg Zn/kg DM as reagent grade ZnSO₄ or 200 or 400 mg Zn/kg DM as ZnAAC or ZnProt to determine bioavailability of each of the organic Zn sources relative to ZnSO₄. Diets were fed for three weeks and three chicks from each treatment were sacrificed each week. In a second experiment, broiler chicks were fed a corn-soybean meal diet supplemented with 0, 200, or 400 mg Zn/kg as reagent grade ZnSO₄ or 200 mg Zn/kg as ZnPoly, or two different ZnProt compounds. In both experiments, relative bioavailability was calculated from Zn concentrations in bone, liver and intestine, and liver and intestinal MT concentrations. In a third experiment, 42 crossbred wether lambs were used to compare the bioavailability of ZnProt, ZnAAC, and ZnMet to ZnSO₄. Bioavailability was estimated from the Zn concentrations in liver, kidney and pancreas, and liver MT concentrations. In the first experiment, when ZnSO₄ was assigned a value of 100% as the standard, ZnProt and ZnAAC were estimated to have relative bioavailability values of 139 and 83, respectively. In the second experiment, ZnPoly, and two different ZnProt compounds were found to have relative bioavailability values of 94, 99, and 108, respectively. Finally, using lambs, ZnProt, ZnAAC, and ZnMet were estimated to have relative bioavailability values of 130, 110 and 113 relative to ZnSO₄. Collectively, these experiments suggest that Zn from ZnProt is more available to ruminants and non-ruminants than Zn from ZnSO₄.

Spears and Kegley (1994) used 60 Angus and Angus × Hereford steers to compare the effects of ZnO and two forms of ZnProt on performance and carcass characteristics of growing and finishing steers. Cattle were individually fed a corn silage-based diet in the growing phase and a 90% ground corn diet in the finishing phase. Diets

were supplemented with 25 mg Zn/kg DM regardless of Zn source. In the finishing phase and when the growing and finishing phases were combined, steers supplemented with ZnProt tended to gain faster and more efficiently than steers supplemented with ZnO. Soluble Zn concentrations in rumen fluid were greater in steers supplemented with ZnProt than those fed ZnO. Hot carcass weights and dressing percentage tended to be higher in steers fed ZnProt than those fed ZnO. Quality grades, yield grades, marbling and back fat were increased by Zn supplementation, but was not affected by Zn source. Plasma Zn, and measures of humoral and cell-mediated immunity were not influenced by Zn supplementation (Spears and Kegley, unpublished data).

Reiling et al. (1992) examined the effect of supplementing 180 mg Zn/kg diet as ZnSO₄ or ZnProt on hoof durability in feedlot heifers. Heifers received treatment diets for 45, 60, or 75 d. Hooves from heifers that received ZnProt tended to require greater force for shearing and appeared to be more elastic than hooves from heifers supplemented with ZnSO₄.

Spain et al. (1993) supplemented lactating dairy cows with 800 mg Zn/kg diet as ZnO or a 50:50 mixture of ZnO and ZnProt for 20 weeks. Somatic cell counts and milk production were not affected by Zn source; however, cows supplemented with the 50:50 mixture experienced fewer mammary infections than cows supplemented with ZnO alone. Plasma and tissue Zn values were not reported.

Literature Cited

- Armstrong, D. G., and K. Hutton. 1975. In: I. W. McDonald and A. C. I. Warner (eds.)
Digestion and Metabolism in the Ruminant. pp 432-447. University of New
England Publishing Unit, Armidale, Australia.
- Arora, S. P., E. E. Hatfield, U. S. Garrigus, T. G. Lohman, and B. B. Doane. 1969. Zinc-
65 uptake by rumen tissue. *J. Nutr.* 97:25-28.
- Ashmead, H. D, D. J. Graff, and H. H. Ashmead. 1985. Intestinal absorption of metal
ions and chelates. Charles C. Thomas, Springfield, IL.
- Beutler, K. T., O. Pankewycz, and D. L. Brautigam. 1998. Equivalent uptake of organic
and inorganic zinc by monkey kidney fibroblasts, human intestinal epithelial cells,
and perfused mouse intestine. *Biol. Tr. Elem. Res.* 61:19-31.
- Blakeborough, P., and D. Salter. 1987. The intestinal transport of zinc studied using
brush border membrane vesicles from the piglet. *Br. J. Nutr.* 57:45-55.
- Brinkhaus, F., J. Mann, C. Zorich, and J. Greaves. 1998. Bioavailability of zinc
propionate in dogs. *J. Nutr.* 128:2596S-2597S.
- Cao, J., P. R. Henry, R. Guo, R. A. Holwerda, J. P. Troth, R. C. Littell, R. D. Miles, and
C. B. Ammerman. 2000. Chemical characteristics and relative bioavailability of
supplemental organic zinc sources for poultry and ruminants. *J. Anim. Sci.*
78:2039-2054.
- Chen, H., E. A. Wong, and K. E. Webb, Jr. 1999. Tissue distribution of a peptide
transporter mRNA in sheep, dairy cows, pigs, and chickens. *J. Anim. Sci.*
77:1277-1283.

- Chesters, J. K. 1997. Zinc. In: B. L. O'Dell and R. A. Sunde (eds.) Handbook of Nutritionally Essential Trace Elements. pp 185-230. Marcel Dekker, Inc. New York, NY.
- Cousins, R. J. 1985. Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. *Physiol. Rev.* 65:238-309.
- Cousins, R. J. 1996. Zinc. In: L. J. Filer and E. E. Ziegler (eds.) Present Knowledge in Nutrition, 7th Edition. pp 293-306. International Life Science Institute-Nutrition Foundation, Washington, DC.
- Cousins, R. J., and R. J. McMahon. 2000. Integrative aspects of zinc transporters. *J. Nutr.* 130:1384S-1387S.
- Droke, E. A., G. P. Gengelbach, and J. W. Spears. 1998. Influence of level and source (inorganic vs organic) of zinc supplementation on immune function in growing lambs. *Asian-Austral. J. Anim. Sci.* 11:139-144.
- Greene, L. W., D. K. Lunt, F. M. Byers, N. K. Chirase, C. E. Richmond, R. E. Knutson, and G. T. Schnellling. 1988. Performance and carcass quality of steers supplemented with zinc oxide or zinc methionine. *J. Anim. Sci.* 66:1818-1823.
- Gunshin, H., B. Mackenzie, U. V. Berger, Y. Gunshin, M. F. Romero, W. F. Boron, S. Nussberger, J. L. Gollan, and M. A. Hediger. 1997. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* 388:482-488.
- Hampton, D. L., W. J. Miller, M. W. Neathery, R. L. Kincaid, D. M. Blackmon, and R. P. Gentry. 1976. Absorption of zinc from the small and large intestine of calves. *J. Dairy Sci.* 59:1963-1966.

- Hara, H., A. Konishi, and T. Kasai. 2000. Contribution of the cecum and colon to zinc absorption in the rat. *J. Nutr.* 130:83-89.
- Hempe, J. M., and R. J. Cousins. 1991. Cysteine-rich intestinal protein binds zinc during transmucosal zinc transport. *Proc. Natl. Acad. Sci.* 88:9671-9674.
- Hempe, J. W., and R. J. Cousins. 1992. Cysteine-rich intestinal protein and intestinal metallothionein: an inverse relationship as a conceptual model for zinc absorption in rats. *J. Nutr.* 122:89-95.
- Kemin Industries. 1995. Bioavailability of zinc from organic and inorganic sources using the chick bioassay technique. Tech. Bull. No. 11575. Kemin Industries, Des Moines, IA.
- Kennedy, D. W., and L. D. Bunting. 1991. Alterations in ruminal utilization of magnesium and zinc in lambs fed different ratios of concentrate:forage. *Internat. J. Vit. Nutr. Res.* 61:67-71.
- Kincaid, R. L., B. P. Chew, and J. D. Cronrath. 1997. Zinc oxide and amino acids as sources of dietary zinc for calves: effects on uptake and immunity. *J. Dairy Sci.* 80:1381-1388.
- Kirk, D. J., J. P. Fontenot, and S. Rahnema. 1994. Effects of feeding lasalocid and monensin on digestive tract flow and partial absorption of minerals in sheep. *J. Anim. Sci.* 72:1029-1037.
- Kramer, T., T. Michelberger, H. Gurtler, and G. Gabel. 1996. Absorption of short-chain fatty acids across ruminal epithelium of sheep. *J. Comp. Physiol.* 166:262-269.
- Lardy, G., M. S. Kerley and J. A. Patterson. 1992. Retention of metal proteinates by lambs. *J. Anim. Sci.* 70(suppl. 1):314(abstr.).

- Lee, H. H., A. S. Prasad, G. J. Brewer, and C. Owyang. 1989. Zinc absorption in human small intestine. *Am. J. Physiol.* 256:G87-G91.
- Lowe, J. A., and J. Wiseman. 1998. A comparison of the bioavailability of three dietary zinc sources using four different physiologic parameters in dogs. *J. Nutr.* 128:2809S-2811S.
- Lowe, J. A., J. Wiseman, and D. J. A. Cole. 1994a. Absorption and retention of zinc when administered as an amino acid chelate in the dog. *J. Nutr.* 124:2572S-2574S.
- Lowe, J. A., J. Wiseman, and D. J. A. Cole. 1994b. Zinc source influences zinc retention in hair and hair growth in the dog. *J. Nutr.* 124:2575S-2576S.
- Malcolm-Callis, K. J., G. C. Duff, S. A. Gunter, E. B. Kegley, and D. A. Vermeire. 2000. Effects of supplemental zinc concentration and source on performance, carcass characteristics, and serum values in finishing steers. *J. Anim. Sci.* 78:2801-2808.
- Matthews, J. C., and K. E. Webb, Jr. 1995. Absorption of L-carnosine, L-methionine, and L-methionylglycine by isolated sheep ruminal and omasal epithelium. *J. Anim. Sci.* 73:3464-3475.
- McMahon, R. J., and R. J. Cousins. 1998. Mammalian zinc transporters. *J. Nutr.* 128:667-670.
- Menard, M. P., and R. J. Cousins. 1983. Zinc transport by brush border membrane vesicles from rat intestine. *J. Nutr.* 113:1434-1442.
- Merchen, N. R. 1988. Digestion, absorption, and excretion in ruminants. In: D. C. Church (ed.) *The Ruminant Animal: Digestive Physiology and Nutrition*. pp 172-201. Prentice-Hall, Inc., Englewood Cliffs, NJ.

- Miller, J. K., and R. G. Cragle. 1965. Gastrointestinal sites of absorption and endogenous secretion of zinc in dairy cattle. *J. Dairy Sci.* 48:370-373.
- O'Dell, B. L., and J. E. Savage. 1957. Symptoms of zinc deficiency in the chick. *Fed. Proc.* 16:1394.
- Reiling, B. A., L.L. Berger, G. L. Riskowski and R. E. Rompala. 1992. Effects of zinc proteinate on hoof durability in feedlot heifers. *J. Anim. Sci.* 70(suppl. 1):313(abstr.).
- Rojas, L. X., L. R. McDowell, R. J. Cousins, F. G. Martin, N. S. Wilkinson, A. B. Johnson, and J. B. Velasquez. 1995. Relative bioavailability of two organic and two inorganic zinc sources fed to sheep. *J. Anim. Sci.* 73:1202-1207.
- Spain, J. N., D. Hardin, B. Steevens, and J. Thorne. 1993. Effect of organic zinc supplementation on milk somatic cell count and incidence of mammary gland infections of lactating dairy cows. *J. Dairy Sci.* 76(suppl. 1):354(abstr.).
- Spears, J. W. 1989. Zinc methionine for ruminants: relative bioavailability of zinc in lambs and effects of growth and performance of growing heifers. *J. Anim. Sci.* 67:835-843.
- Spears, J. W. 1996. Organic trace minerals in ruminant nutrition. *Anim. Feed Sci. Technol.* 58:151-163.
- Spears, J. W. and E. B. Kegley. 1994. Influence of zinc proteinate on performance and carcass characteristics of steers. *J. Anim. Sci.* 72(suppl. 1):(abstr.).
- Spears, J. W., R. W. Harvey, and T. T. Brown, Jr. 1991. Effects of zinc methionine and zinc oxide on performance, blood characteristics, and antibody titer response to

- viral vaccination in stressed feeder calves. *J. Am. Vet. Med. Assoc.* 199:1731-1733.
- Stevens, C. E. 1970. In: A. T. Phillipson (ed.) *Physiology of Digestion and Metabolism in the Ruminant*. pp 101-112. Oriol Press Ltd., Newcastle upon Tyne, UK.
- Swinkels, J. W. G. M., E. T. Kornegay, W. Zhou, M. D. Lindemann, K. E. Webb, Jr., and M. W. A. Verstegen. 1996. Effectiveness of a zinc amino acid chelate and zinc sulfate in restoring serum and soft tissue zinc concentrations when fed to zinc-depleted pigs. *J. Anim. Sci.* 74:2420-2430.
- Todd, W. R., C. A. Elvehjem, and E. B. Hart. 1934. Zinc in the nutrition of the rat. *Am. J. Physiol.* 107:146-156.
- Tucker, H. F., and W. D. Salmon. 1955. Parakeratosis or zinc deficiency disease in the pig. *Proc. Soc. Exp. Biol. Med.* 88:613-616.
- Underwood, E. J. and N. F. Suttle. 1999. *The Mineral Nutrition of Livestock*. 3rd ed. CABI Publishing, New York, NY.
- Webb, K. E., Jr., D. B. DiRienzo, and J. C. Matthews. 1993. Recent developments in gastrointestinal absorption and tissue utilization of peptides: a review. 76:351-361.
- Webb, K. E., Jr., J. C. Matthews, and D. B. DiRienzo. 1992. Peptide absorption: a review of current concepts and future perspectives. *J. Anim. Sci.* 70:3248-3257.
- Wedekind, K. J., and S. R. Lowry. 1998. Are organic zinc source efficacious in puppies? *J. Nutr.* 128:2593S-2595S.
- Wilson, J. W., and K. E. Webb, Jr. 1990. Lysine and methionine transport by bovine jejunal and ileal brush border membrane vesicles. *J. Anim. Sci.* 68:504-514.

CHAPTER 1

Effect of zinc source and dietary level on zinc metabolism in Holstein bulls^{1,2}

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ABSTRACT: Forty-eight Holstein bull calves were stratified by origin and weight, and randomly assigned to one of four treatment groups. Dietary treatments were administered in two phases. In Phase 1, treatment groups received no supplemental Zn (**Con**), 20 mg Zn/kg DM as ZnSO₄ (**ZnS**) or ZnProt (**ZnP**) or 20 mg Zn/kg DM with 50% of the Zn supplied from each source (**ZnM**). In Phase 2, calves continued to receive the same Zn source fed in Phase 1; however, half of the calves in each treatment group were randomly selected to receive 500 mg Zn/kg DM (**HiZnS**, **HiZnP**, **HiZnM**). Calf ADFI, ADG and feed efficiency were not affected by treatment in either phase of the experiment. Treatment had no effect on plasma Zn concentration or Alp activity in Phase 1, but liver Zn concentration was greater ($P < 0.05$) in bulls fed ZnS than those fed ZnP. In Phase 2, plasma Zn was greater ($P < 0.01$) in bulls fed HiZnP and HiZnM than in those fed HiZnS. In Phase 2, liver Zn was greater ($P < 0.05$) in bulls fed HiZnP than in those fed HiZnS. Duodenal Zn concentrations were greater ($P < 0.01$) in bulls supplemented with HiZnP and HiZnM than those supplemented with HiZnS. Liver MT was not affected by Zn source. Rumen metallothionein (**MT**) concentrations tended ($P < 0.10$) to be greater in bulls fed ZnP compared to ZnS. Omasal MT tended to be greater ($P < 0.10$) in calves fed ZnM than in those fed ZnS. Duodenal MT concentrations were not affected by Zn source or concentration. Bulls fed HiZnP and HiZnM had higher ($P < 0.05$) kidney Zn concentrations than those fed HiZnS. Muscle Zn concentration tended ($P < 0.10$) to be greater in bulls fed HiZnM than bulls fed HiZnS. Heart, spleen, testicular, and bone Zn concentrations were not affected by Zn source. Bulls fed ZnM or HiZnS had greater ($P < 0.05$) skin Zn concentrations than bulls fed ZnS or HiZnP, respectively. Hoof wall samples contained nearly three-fold greater ($P < 0.01$) Zn concentrations than hoof sole.

Bulls fed ZnS had greater ($P < 0.05$) Zn concentration in hoof wall samples than bulls fed ZnM. Hoof sole Zn concentration was not affected by Zn source or concentration. At low dietary levels, Zn bioavailability from ZnSO₄ and ZnProt was similar; however, at high levels, Zn from ZnSO₄ and ZnProt appeared to be absorbed and metabolized differently.

Key Words : zinc, proteinate, sulfate, bioavailability, cattle

Introduction

Supplementation of ruminant diets with organic trace minerals has become increasingly popular in recent years. While evidence suggests that organic trace mineral sources can, under certain conditions, enhance performance, and improve health and reproduction, specific mechanisms underlying observed responses remain unclear (Spears, 1996).

Previously, Zn proteinate (**ZnProt**) improved performance and carcass characteristics in feedlot steers (Spears and Kegley, 1994), increased force required for shearing of hooves in heifers (Reiling et al., 1992), and increased Zn retention in lambs (Lardy et al., 1992) relative to inorganic Zn sources (ZnSO₄ or ZnO). Observed differences in bioavailability are suggested to result from enhanced absorption of organic relative to inorganic Zn sources. Zinc uptake and apparent absorption from rumen tissue has been clearly demonstrated in vivo using ⁶⁵Zn dosed lambs (Arora et al., 1969) and wethers fitted with ruminal, abomasal and ileal cannulas (Kennedy and Bunting, 1991; Kirk et al., 1994). Absorption of metal-peptide complexes may occur in the rumen and(or) the omasum via peptide transporters described by Matthews and Webb (1995). Absorption from the forestomach could significantly reduce the potential for ionization or digestion of the complex during the digestive process. Metal-peptide complexes may also be transported directly into the intestinal mucosa intact via peptide transport mechanisms (Ashmead et al., 1985).

This experiment was conducted to determine the effect of Zn source and level on plasma Zn concentration and alkaline phosphatase activity (**Alp**), and tissue Zn and metallothionein (**MT**) concentrations in Holstein bull calves.

Materials and Methods

Care and handling of the animals and sampling procedures described herein were approved by the North Carolina State University Animal Care and Use Committee.

Forty-eight Holstein bulls were obtained from research dairies in the North Carolina Department of Agriculture and North Carolina State University systems. Due to age differences, one group of bull calves ($n = 16$; average BW = 183.5 kg) began receiving experimental diets on d 0, while the remaining calves ($n = 32$; average BW = 148.1 kg) began receiving experimental diets 28 d later. Start date did not affect ($P > 0.10$) any measured variables, thus data were pooled for analysis and will be referred to as one experiment.

Bulls were stratified by origin and weight, and randomly assigned to one of four treatment groups. Dietary treatments were administered in two phases. In Phase 1 (d 0 to 98), treatment groups received no supplemental Zn (Con), 20 mg Zn/kg DM as ZnSO₄ (**ZnS**) or ZnProt (**ZnP**; Chelated Minerals Corporation, Salt Lake City, UT) or 20 mg Zn/kg DM with 50% of the Zn supplied from each source (**ZnM**). In Phase 2 (d 99 to 112) cattle continued to receive the same Zn source fed in Phase 1, however half of the calves in each treatment group were randomly selected to receive 500 mg Zn/kg DM (**HiZnS**, **HiZnP**, **HiZnM**). Bulls were fed a corn-soybean meal-cottonseed hull basal diet (28.0 mg Zn/kg DM; Table 1), and were housed two per pen in covered, slotted-floor pens (4 × 4 m). Weights were taken prior to feeding on d -1, 0, 28, 56, 84, 98, 111 and 112. On d 112, all bulls were transported to a commercial abattoir for slaughter.

Blood samples were collected on d 0, 28, 56, 84, 98 and 112 via jugular venipuncture in heparinized-trace mineral free tubes (Vacutainer, Becton Dickinson,

Franklin Lakes, NJ). Whole blood was centrifuged at $1760 \times g$ for 15 min at 4°C , then plasma was aspirated into 5-mL polyethylene tubes (Elkay Products Incorporated, Shrewsbury, MA) and frozen at -20°C until analysis. Plasma Alp activity was determined by kinetic assay (ALP 20, Sigma Diagnostics Incorporated, St. Louis, MO).

Liver biopsies were collected on d 0, 56, and 98. Biopsy sites were clipped of hair, scrubbed three times with betadine (Purdue Frederick, Norwalk, CT) and 70% ethyl alcohol. A small incision was made between the 11th and 12th rib, on a line from the tubercosae to the point of the shoulder. Liver tissue was removed using a Jamshide bone marrow punch (0.4 cm in diameter \times 10 cm in length; Allegiance Healthcare Corp., McGaw Park, IL) while applying suction with a 10 mL syringe (Becton Dickinson, Franklin Lakes, NJ). Liver samples were immediately rinsed with 0.01 mol/L PBS (pH 7.4) and drained to remove contaminating blood. Samples were then transferred to acid-washed 5-mL polyethylene tubes, capped, and placed on ice for transport to the laboratory, where they were stored at -20°C until analysis.

Hair samples were collected by clipping a 4×4 cm area 56 d prior to and the day before slaughter. Samples were stored in plastic bags (Whirl-Pak, Nasco, Fort Atkinson, WI) at room temperature until analysis. Hair samples were washed with 0.1 M SDS solution and rinsed repeatedly with deionized water prior to analysis. Heart, kidney, liver, omasum, rumen, spleen, duodenum samples and the right front leg (below the knee) of each calf were collected at the abattoir, transported on ice to the laboratory where they were stored at -20°C . Duodenal segments (7 to 10 cm in length) were excised from an area approximately 30 to 60 cm from the pylorus, immediately rinsed and flushed with ice-cold 0.85% saline, and placed into ice-cold saline for transport to the laboratory.

Upon arrival, segments were cut longitudinally to expose the mucosa and rinsed again with ice-cold saline to remove remaining digesta. Mucosal cells were then removed by scraping the tissue with a glass microscope slide. Cell scrapings were transferred into pre-weighed 50-mL centrifuge tubes (Sorvall, Kendro Laboratory Products, Newtown, CT), weighed and diluted 1:4 (wt:vol) with glycine buffer containing 0.2 mmol phenylmethylsulfonyl fluoride, 0.6 mg leupeptin, 0.9 mg pepstatin A, and 0.2 sodium azide/L (pH 8.6) to inhibit proteolysis. Samples were then homogenized (Polytron, Brinkmann Instruments, Westbury, NY), and centrifuged at $20,000 \times g$ for 30 min (Sorvall RC5C, Kendro Laboratory Products, Newtown, CT). Supernatant fractions were collected, heated for 2 min at 100°C , and centrifuged again at $20,000 \times g$ for 30 min. Final supernatant fractions were then transferred to 15-mL screw-top tubes (Falcon, Becton Dickinson, Franklin Lakes, NJ) and stored at -20°C .

Tissue MT concentration was determined on liver, rumen, omasum, and small intestinal homogenates using a non-radioactive Ag binding assay procedure (Lee et al., 1989) as modified by Carlson et al. (1999). Briefly, approximately 2 g tissue (liver, and ruminal and omasal epithelium) were diluted 1:4 (wt:vol) with 0.5 mol/L glycine buffer (pH 8.3), homogenized, and heated for 2 min at 100°C . Samples were then centrifuged at $25,000 \times g$ for 2 min. Silver concentrations in each supernatant fraction were determined by flame atomic absorption spectroscopy (AA-6701F, Shimadzu, Kyoto, Japan). The amount of silver present was assumed to be proportional to the MT concentration of the sample.

Bone, hoof, skin, and muscle samples were harvested from the right front leg of each calf. Hoof tissue was collected by first removing a thin layer of tissue to remove

contamination. Then a 0.5 cm slice was cut from each digit parallel to the sole of the hoof. Each hoof sample was washed with 1.0 M SDS, rinsed repeatedly with deionized water, and separated by location on the hoof (wall or sole). Bone (metacarpal), muscle (deep and superficial digital flexors), and skin samples were removed from 1.0 cm slices cut perpendicular to the longitudinal axis of the leg, both in the center of the shaft and approximately 5 cm from the distal end of the metacarpal bone. Hair was shaved from each skin sample and visible connective tissue was removed from both the skin and muscle samples.

Bone samples were processed as described by Armstrong et al. (2000). Briefly, bone cross-sections were weighed and dried for 48 h at 100°C then weighed again to determine dry matter. Samples were then wrapped in filter paper (p8, 09-795D, Fisher Scientific, Pittsburgh, PA) placed in a side-arm Soxhlet extraction apparatus, extracted with petroleum ether for 48 h and allowed to air dry under a hood for 48 h. After lipid extraction, bone sections were dried at 100°C for 18 h and weighed. Percentage of bone ash was calculated after heating the cross-sections of bone in a muffle furnace at 700°C for 48 h. Bone ash was dissolved in 10 mL 6 N HCl and brought to 25 mL with deionized water for Zn analysis.

Feed and tissue samples were dried at 100° C for 48 h, weighed, and wet ashed using a microwave digestion (Model MDS-81D, CEM, Matthews, NC) procedure described by Genglebach et al. (1994). Plasma and tissue homogenates (rumen, omasum, and duodenum) were diluted 1:4 with 5% HNO₃ and centrifuged at 1760 × g for 15 min. Ashed tissue and feed samples and plasma and tissue homogenate supernatant fractions were analyzed for Zn content by flame atomic absorption spectroscopy.

Statistical Analysis. Statistical analysis of liver and plasma Zn concentrations and plasma Alp activity in Phase 1 were analyzed as repeated measures using the Mixed procedure of SAS (Version 6.12, SAS Institute Inc., Cary, NC) as described by Littell et al. (1998). Animal was considered the experimental unit and animal within treatment was used as a random error term. The model included treatment, time and treatment \times time interaction. Single degree of freedom contrasts were used to compare means between unsupplemented (**-Zn**) vs Zn supplemented treatments (**+Zn**), ZnS vs ZnP, and ZnS vs ZnM.

Statistical analysis of performance data from Phase 1 and all Phase 2 data were performed by ANOVA using the General Linear Model procedure of SAS. Pen was considered the experimental unit for ADFI, ADG, and feed efficiency (**G:F**) data. Animal was considered the experimental unit for plasma Zn and ALP, and tissue Zn and MT data. Single degree of freedom contrasts were used to compare means. Comparisons made were: -Zn vs +Zn, high vs low supplemental Zn, ZnS vs ZnP, HiZnS vs HiZnP, ZnS vs ZnM, and HiZnS vs HiZnM. Significance was declared at $P \leq 0.05$ and a trend declared at $P \leq 0.10$.

Results

Phase 1. Average daily feed intake, ADG, and G:F were not affected by dietary treatment (Table 2). Treatment had no effect on plasma Zn concentration or Alp activity. Plasma Zn was higher ($P < 0.08$) on d 84 and d 98 than on d 0 and 28 (Table 3). Plasma Alp activity increased ($P < 0.01$) by d 56 and then increased subsequently on d 84 and 98 (Table 3). Dietary treatment tended ($P < 0.07$) to influence liver Zn concentration. Liver Zn concentration was greater in bulls fed ZnS (156.8 mg/kg) than those fed ZnP (133.3

mg/kg), but was not different between bulls fed ZnS and those fed ZnM (139.7 mg/kg). Zinc supplemented bulls tended ($P < 0.10$) to have greater liver Zn concentration than – Zn bulls.

Phase 2. Average daily feed intake, ADG, and G:F were not affected by dietary treatment. Zinc concentration in duodenal mucosal scrapings from bulls supplemented with HiZnP and HiZnM were greater ($P < 0.01$) than those supplemented with HiZnS (Table 4).

Zinc concentrations were greater in duodenal homogenates from +Zn than in those from –Zn bulls ($P < 0.05$) and in bulls supplemented with high relative to low dietary Zn levels ($P < 0.01$; Table 4). Homogenates of ruminal and omasal epithelium were not affected by Zn source or concentration.

Rumen MT concentration tended to be greater ($P < 0.10$) in rumen epithelial homogenates from bulls fed low supplemental Zn relative to those from bulls fed higher levels (Table 5). Metallothionein concentrations in rumen epithelial homogenates from bulls fed ZnS were 25.3% less ($P < 0.10$) than those from bulls fed ZnP (Table 5). Omasal MT concentrations were greater ($P < 0.10$) from bulls fed ZnM than from those fed ZnS. Metallothionein concentrations in duodenal homogenates were not affected by Zn source or concentration.

Plasma Zn concentrations were greater ($P < 0.01$) in bulls that received HiZnP and HiZnM than in those fed HiZnS and in +Zn relative to –Zn bulls (Table 6). Bulls supplemented with high dietary Zn levels had higher ($P < 0.01$) plasma Zn concentrations than those fed lower Zn levels (Table 6).

Liver Zn was greater ($P < 0.01$) in bulls fed high compared to low dietary Zn and tended ($P < 0.10$) to be greater in +Zn than in -Zn bulls (Table 6). Bulls fed HiZnP had greater ($P < 0.05$) liver Zn concentrations than bulls fed HiZnS (Table 6). Liver MT concentrations were increased ($P < 0.01$) by Zn supplementation and were nearly 50% greater ($P < 0.01$) in bulls fed higher dietary Zn levels relative to those fed lower levels (Table 6).

Bulls supplemented with HiZnP and HiZnM had higher ($P < 0.01$ and $P < 0.05$, respectively) kidney Zn concentrations than those fed HiZnS, and bulls fed HiZnM tended ($P < 0.10$) to have greater muscle Zn concentrations than bulls fed HiZnS (Table 7). Zinc supplemented bulls had greater kidney ($P < 0.01$) and muscle ($P < 0.05$) Zn concentrations, but tended to have lower spleen ($P < 0.10$) Zn concentrations than unsupplemented controls (Table 7). Bulls fed higher dietary Zn levels had greater ($P < 0.01$) kidney and muscle Zn concentrations than those supplemented with low Zn. Heart and testicular Zn concentrations were not affected by Zn source or concentration. Bone Zn concentration varied by location on the bone shaft. Zinc concentrations in bone slices harvested from the distal endplate region of the bone were 84.3% greater ($P < 0.01$) than slices harvested from the center of the shaft. Bone shaft slices from bulls supplemented with high dietary Zn had higher ($P < 0.05$) Zn concentrations than bone from bulls supplemented at lower levels; however, bone Zn was not affected by Zn source.

At low Zn concentration, bulls fed ZnM had greater ($P < 0.05$) skin Zn concentrations than bulls fed ZnS (Table 8). When fed high dietary Zn, bulls fed HiZnS had greater ($P < 0.05$) skin Zn concentration than bulls fed HiZnP (Table 8). As with bone Zn, hoof Zn concentration varied by location. Samples collected from the hoof wall

contained nearly three-fold greater ($P < 0.01$) Zn concentrations than samples taken from sole of the hoof (Table 8). Bulls supplemented with ZnS had greater ($P < 0.05$) Zn concentration in hoof wall samples than bulls supplemented with ZnM. Zinc concentration of hoof sole tissue was not affected by Zn source or concentration.

Discussion

In the current experiment, performance was not different between cattle supplemented with ZnSO₄ and ZnProt. In contrast, steers fed a finishing diet supplemented 25 mg/kg Zn with ZnProt tended to gain faster and more efficiently than steers supplemented with ZnO or unsupplemented controls (Spears and Kegley, 1994). The effect of Zn source on performance has also been examined using Zn amino acid complexes, Zn polysaccharide complexes and Zn methionine. Malcolm-Callis et al. (2000) observed differences in performance of feedlot steers supplemented with 30 mg Zn/kg as ZnSO₄, Zn amino acid complex, or Zn polysaccharide complex; however responses were inconsistent between Zn sources. Supplementation of growing cattle with Zn methionine has improved (Spears, 1989) or had no effect on ADG and G:F (Greene et al., 1988) relative to controls supplemented with inorganic Zn.

Doudenal homogenate Zn concentrations were greater in intestinal segments from bulls supplemented with HiZnP and HiZnM than in samples from bulls supplemented with HiZnS treatment; however, duodenal MT concentrations were unaffected by Zn source. These observations suggest that, when supplemented at high levels, Zn from ZnProt accumulates in duodenal mucosa to a greater extent than Zn from ZnSO₄, but does not induce MT synthesis. It is possible that Zn from ZnProt was more soluble in the duodenal lumen than Zn from ZnSO₄ and hence was taken up more effectively. The lack

of difference in MT concentration suggests that Zn from ZnProt was either undissociated or was associated with another intracellular ligand preventing the induction of mucosal MT. All or part of the supplemental ZnProt may have remained intact through the digestion process and been absorbed as a chelate, preventing interaction with dietary antagonists. Ashmead et al. (1985) suggested that metal ions may be absorbed as part of a metal:peptide complex, thereby facilitating absorption of Zn via intestinal transport mechanisms distinct from inorganic Zn.

Rumen and omasal homogenate Zn concentrations were not influenced by Zn source or concentration; however, MT concentrations tended to be greater in ruminal epithelium of bulls supplemented with ZnP compared to ZnS, and in omasal epithelium of bulls supplemented with ZnM compared to ZnS. The ability of rumen tissue to take up Zn has been demonstrated in vivo using ^{65}Zn dosed lambs (Arora et al., 1969) and apparent absorption of Zn from the reticulo-rumen has been observed in wethers fitted with ruminal, abomasal and ileal cannulas (Kennedy and Bunting, 1991; Kirk et al., 1994). Previously, when animals were supplemented with high levels of Zn, excess Zn accumulated with MT in various tissues. Whanger et al. (1981) observed Zn accumulation with MT in liver, kidney, pancreas, and small and large intestine, but not with MT in heart, testes, rumen papillae, abomasal mucosa or choroid plexus when cattle and sheep were fed 2000 mg Zn/kg. In the present experiment, high dietary Zn supplementation resulted in higher MT concentrations in liver but not in small intestine. Hempe and Cousins (1991) observed that more Zn was associated with MT in the cytosol of intestinal tissues from rats fed 180 μg Zn/g diet than those fed 1 μg Zn/g diet. It is possible that MT may have been induced by one of any number of dietary or

physiological stimuli (Cousins, 1985); however, supplemental Zn levels were not high enough to cause Zn accumulation with MT. Current theories of intestinal Zn absorption state that when dietary Zn is low, a large portion of the absorbed Zn is bound by intracellular species that are not MT. Intestinal mucosa has been found to contain a cysteine-rich intestinal protein (**CRIP**) that is not MT (Hempe and Cousins, 1991). Specific interrelationships that exist between CRIP and MT remain to be elucidated. However, it has been hypothesized that when low Zn levels are fed, Zn that enters the enterocyte is bound by CRIP and is subsequently shuttled to the basolateral membrane where it is transferred into the plasma (Hempe and Cousins, 1992). When high Zn levels are fed, MT binds Zn, perhaps to prevent the absorption of toxic levels of the metal; whereas, only a small portion of the absorbed Zn is bound by CRIP (Hempe and Cousins, 1992). The reason for the tendency toward lower MT concentrations in ruminal epithelium from bulls fed high supplemental Zn is unclear.

Plasma Zn and Alp were not affected by Zn source in Phase 1; however, in Phase 2, bulls supplemented with HiZnP and HiZnM had greater plasma Zn concentrations than bulls supplemented with HiZnS. As expected, plasma Zn was greater in bulls supplemented with high relative to low dietary Zn levels. The lack of responses with respect to Zn source in Phase 1 is consistent with previous research with growing and finishing steers supplemented with 25 mg Zn/kg as ZnProt or ZnSO₄. (Spears and Kegley, 1994). The effect of ZnProt supplemented at high levels on plasma Zn and(or) Alp has not been previously evaluated. However, observations in the current experiment are consistent with those of Kincaid et al. (1997) who reported greater plasma Zn

concentrations in Holstein heifer calves supplemented with 300 mg Zn/kg as Zn methionine or Zn lysine than in those receiving ZnO.

In Phase 1, liver Zn was greater in bulls fed ZnS than in those fed ZnP; however, in Phase 2, bulls fed HiZnP had higher liver Zn concentrations than those fed HiZnS. This response correlates well with the observed increase in plasma Zn in bulls fed HiZnP or HiZnM and suggests that liver Zn may have increased in response to higher plasma Zn concentrations in the bulls supplemented with ZnProt. Alternatively, increased liver Zn may indicate that Zn from ZnProt is retained more effectively than Zn from ZnSO₄. Greater retention of Zn from organic Zn has been previously demonstrated in sheep supplemented with ZnProt (Lardy et al., 1992) and Zn methionine (Spears, 1989). Liver MT was not affected by Zn source. Recently, Cao et al. (2000) reported higher Zn concentrations in liver, kidney and pancreas and higher liver MT concentrations in lambs supplemented with 700, 1400, or 2100 mg Zn/mg for 21 d as ZnProt than in those fed ZnSO₄. In Phase 1, dietary Zn levels may have been insufficient to allow delineation of differences between Zn sources; however, liver Zn concentrations in Phase 2 agree strongly with the findings of Cao et al. (2000). Increased liver Zn in the absence of an increase in liver MT suggests that Zn from ZnProt may be metabolized differently by hepatocytes than Zn from inorganic Zn. It is possible that Zn from the complex is bound to intracellular ligands other than MT. Alternatively, if intestinal transport indeed occurred via paracellular transport or simple diffusion, it is possible that the chelate was delivered to the liver intact and was metabolized and stored as such. Spears (1989) observed similar absorption of ZnSO₄ and Zn methionine; however, based on greater Zn

retention in lambs fed Zn methionine, the author suggested that post-absorptive differences in metabolism might exist.

Zinc concentrations in other organs varied by tissue analyzed. Kidney Zn concentrations were greater in bulls fed HiZnP and HiZnM than in bulls fed HiZnS. Since it has been established that kidney tissue contains MT induced by high circulating Zn concentrations (Cousins, 1985), kidney Zn concentrations likely increase in response to high dietary Zn. Bone and muscle are the primary Zn storage organs, and bone degeneration and(or) deformation is a clinical sign of Zn deficiency (Underwood and Suttle, 1999). Muscle Zn concentration was greater in bulls fed HiZnM than in those fed HiZnS, while HiZnP was intermediate. These data parallel liver Zn concentration, further supporting the hypothesis that, at high dietary levels, Zn from ZnProt is absorbed and(or) retained more effectively than Zn from inorganic sources. Furthermore, this observation lends credence to the suggestion that ZnProt may be absorbed and metabolized differently by tissues than inorganic Zn. Bone Zn concentrations were unaffected by source, however bulls that received high dietary Zn had greater bone Zn concentrations than those that were fed lower levels. Diets containing higher Zn levels were only fed for 14 d in the current experiment. Supplementing the bulls for a longer time period may have allowed for greater differences to become apparent.

Zinc is essential for proper keratinization of epithelial tissue (Mills et al., 1967). As such, hair and wool Zn concentrations reflect dietary intakes in all species studied, but individual variability is high and there is significant variation with age, breed, sampling site, and seasonal conditions (Underwood and Suttle, 1999). However, since Zn is an integral component of several metalloproteins and functions in gene expression, it is

reasonable to assume that it would be essential for rapidly growing epithelial tissues such as skin, hair, and hoof. In the current experiment, skin samples from bulls fed HiZnS contained more Zn than samples from bulls fed HiZnP. Furthermore, bulls fed ZnM had greater skin Zn and lower hoof wall Zn concentrations than bulls fed ZnS.

Supplementing growing and finishing cattle with 620 mg Zn/kg as ZnO increased hair Zn concentration relative to cattle supplemented at lower levels (Beeson et al., 1977). Supplementation of Zn amino acid chelate has increased hair growth rate and hair Zn concentration relative to ZnO or Zn polysaccharide complex in dogs (Lowe et al., 1994). Greater hoof elasticity and required shear force were observed in heifers supplemented with 180 mg Zn/hd/d as Zn proteinate compared to heifers supplemented with ZnSO₄ (Reiling et al., 1992). Hoof strength characteristics were not evaluated in the current experiment, however several observations were made with respect to Zn concentration. Interestingly, Zn concentration in hoof wall tissue was three-fold greater than in samples from the sole of the hoof. Since the hoof wall is a significantly harder tissue, more Zn may be required to facilitate keratinization. Also, more Zn accumulated in the hoof wall of heifers fed ZnSO₄ relative to heifers fed Zn proteinate. Smith et al. (1999) observed no difference in hoof Zn concentration in lactating Holstein cows supplemented with ZnSO₄ and Zn methionine

In summary, plasma and tissue Zn concentrations were generally not affected by Zn source when Zn was supplemented at 20 mg Zn/kg to a basal diet that contained 28 mg Zn/kg. However, when supplemented at 500 mg Zn/kg, Zn from ZnProt appears to be absorbed and(or) retained more effectively than Zn from ZnSO₄.

Implications

While chronic Zn deficiency is not a common occurrence in the beef industry, certain management practices or nutritional interactions may cause an acute Zn deficiency to develop. These data suggest that the Zn status of growing bull calves receiving a basal diet supplemented with low levels of Zn is not affected by Zn source. However, short-term supplementation of ZnProt at high levels may more effectively elevate Zn status than ZnSO₄. Given the large disparity in Zn concentrations and limited supplementation time used in this experiment, more research is warranted to evaluate ZnSO₄ and ZnProt supplementation at various concentrations and for various lengths of time.

References Cited

- Armstrong, T. A., J. W. Spears, T. D. Crenshaw, and F. H. Nielsen. 2000. Boron supplementation of a semipurified diet for weanling pigs improves feed efficiency and bone strength characteristics and alters plasma lipid metabolites. *J. Nutr.* 139:2575-2581.
- Arora, S. P., E. E. Hatfield, U. S. Garrigus, T. G. Lohman, and B. B. Doane. 1969. Zinc-65 uptake by rumen tissue. *J. Nutr.* 97:25-28.
- Ashmead, H. D., D. J. Graff, and H. H. Ashmead. 1985. Intestinal absorption of metal ions and chelates. Charles C. Thomas, Springfield, IL.
- Beeson, W. M., T. W. Perry, and T. D. Zurcher. 1977. Effect of supplemental zinc on growth and on hair and blood serum levels of beef cattle. *J. Anim. Sci.* 45:160-165.
- Cao, J., P. R. Henry, R. Guo, R. A. Holwerda, J. P. Troth, R. C. Littell, R. D. Miles, and C. B. Ammerman. 2000. Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. *J. Anim. Sci.* 78:2039-2054.
- Carlson, M. S., G. M. Hill, and J. E. Link. 1999. Early- and traditionally-weaned nursery pigs benefit from phase-feeding pharmacological concentrations of zinc oxide: effect on metallothionein and mineral concentrations. *J. Anim. Sci.* 77:1199-1207.
- Cousins, R. J. 1985. Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. *Physiol. Rev.* 65:238-309.

- Eckert, G. E., L. W. Greene, G. E. Carstens, and W. S. Ramsey. 1999. Copper status of ewes fed increasing amounts of copper from copper sulfate or copper proteinate. *J. Anim. Sci.* 77:244-249.
- Greene, L. W., D. K. Lunt, F. M. Byers, N. K. Chirase, C. E. Richmond, R. E. Knutson, and G. T. Schnellling. 1988. Performance and carcass quality of steers supplemented with zinc oxide or zinc methionine. *J. Anim. Sci.* 66:1818-1823.
- Hempe, J. M., and R. J. Cousins. 1991. Cysteine-rich intestinal protein binds zinc during transmucosal zinc transport. *Proc. Natl. Acad. Sci.* 88:9671-9674.
- Hempe, J. M., and R. J. Cousins. 1992. Cysteine-rich intestinal protein and intestinal metallothionein: an inverse relationship as a conceptual model for zinc absorption in rats. *J. Nutr.* 122:89-95.
- Kennedy, D. W., and L. D. Bunting. 1991. Alterations in ruminal utilization of magnesium and zinc in lambs fed different ratios of concentrate:forage. *Internat. J. Vit. Nutr. Res.* 61:67-71.
- Kincaid, R. L., B. P. Chew, and J. D. Cronrath. 1997. Zinc oxide and amino acids as sources of dietary zinc for calves: effects on uptake and immunity. *J. Dairy Sci.* 80:1381-1388.
- Kirk, D. J., J. P. Fontenot, and S. Rahnama. 1994. Effects of feeding lasalocid and monensin on digestive tract flow and partial absorption of minerals in sheep. *J. Anim. Sci.* 72:1029-1037.
- Lardy, G., M. S. Kerley and J. A. Patterson. 1992. Retention of metal proteinates by lambs. *J. Anim. Sci.* 70(suppl. 1):314 (abstr.).

- Lee, D-Y., G. J. Brewer, and Y. Wang. 1989. Treatment of Wilson's disease with zinc. VII. Protection of the liver from copper toxicity by zinc-induced metallothionein in a rat model. *J. Lab. Clin. Med.* 114:639-646.
- Littel, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. *J. Anim. Sci.* 76:1216-1231.
- Lowe, J. A., J. Wiseman, and D. J. A. Cole. 1994. Zinc source influences zinc retention in hair and hair growth in the dog. *J. Nutr.* 124:2575S-2576S.
- Matthews, J. C., and K. E. Webb, Jr. 1991. Absorption of L-carnosine, L-methionine, and L-methionylglycine by isolated sheep ruminal and omasal epithelium. *J. Anim. Sci.* 73:3464-3475.
- Malcolm-Callis, K. J., G. C. Duff, S. A. Gunter, E. B. Kegley, and D. A. Vermeire. 2000. Effects of supplemental zinc concentration and source on performance, carcass characteristics, and serum values in finishing steers. *J. Anim. Sci.* 78:2801-2808.
- Mills, C. F., A. C. Dalgarno, R. B. Williams, and J. Quarterman. 1967. Zinc deficiency and the zinc requirements of calves and lambs. *Br. J. Nutr.* 21:751.
- Reiling, B. A., L.L. Berger, G. L. Riskowski and R. E. Rompala. 1992. Effects of zinc proteinate on hoof durability in feedlot heifers. *J. Anim. Sci.* 70(suppl. 1):313 (abstr.).
- Richards, M .P. 1989. Recent developments in trace element metabolism and function: role of metallothionein in copper and zinc metabolism. *J. Nutr.* 119:1062-1070.
- Smith, M. B., H. E. Amos, and M. A. Froetschel. 1999. Influence of ruminally undegraded protein and zinc methionine on milk production, hoof growth and composition, and selected plasma metabolites. *Prof. Anim. Sci.* 15:268-277.

- Spears, J. W. 1989. Zinc methionine for ruminants: relative bioavailability of zinc in lambs and effects of growth and performance of growing heifers. *J. Anim. Sci.* 67:835-843.
- Spears, J. W. 1996. Organic trace minerals in ruminant nutrition. *Anim. Feed Sci. Technol.* 58:151-163.
- Spears, J. W. and E. B. Kegley. 1994. Influence of zinc proteinate on performance and carcass characteristics of steers. *J. Anim. Sci.* (suppl. 1):(abstr.).
- Underwood, E. J. and N. F. Suttle. 1999. *The Mineral Nutrition of Livestock*. 3rd ed. CABI Publishing, New York, NY.
- Ward, J. D., J. W. Spears, and E. B. Kegley. 1996. Bioavailability of copper proteinate and copper carbonate relative to copper sulfate in cattle. *J. Dairy Sci.* 79:127-132.
- Whanger, P. D., S. OH, and J. T. Deagen. 1981. Ovine and bovine metallothioneins: accumulation and depletion of zinc in various tissues. *J. Nutr.* 111:1196-1206.

Table 1. Diet composition.

Ingredient	% of diet DM
Cottonseed hulls	40.00
Cracked corn	45.10
Soybean meal	11.18
CaCO ₃	1.40
Urea	1.00
CaSO ₄	0.60
Dicalcium phosphate	0.50
NaCl	0.20
Vitamin premix ^a	0.02
Mineral premix ^b	+

^a Vitamin premix contained: 6,600,000 IU vitamin A, 4,400,000 IU vitamin D, and 2,200 IU vitamin E/kg.

^b Trace minerals provided in mg/kg of diet: 25 mg Fe as FeSO₄, 20 mg Mn as MnSO₄, 10 mg Cu as CuSO₄, 0.5 mg I as Ca(IO₃)₂·H₂O, 0.1 mg Co as CoCO₃, and 0.1 mg Se as NaSeO₃.

Table 2. Effect of Zn source and level on performance.

Item	Treatment ^a							SEM	Contrasts ^b
	Con	ZnS	ZnP	ZnM	HiZnS	HiZnP	HiZnM		
----- kg -----									
Phase 1									
ADFI	7.4	7.0	7.1	7.0	---	---	---	0.4	NS
ADG	1.4	1.3	1.3	1.3	---	---	---	0.1	NS
G:F ^c	0.19	0.19	0.18	0.18	---	---	---	0.01	NS
Phase 2									
ADFI	9.2	7.0	8.7	7.8	8.8	8.1	8.9	0.7	NS
ADG	1.5	0.9	1.0	1.3	1.5	1.2	1.5	0.3	NS
G:F	0.16	0.13	0.15	0.12	0.18	0.19	0.13	0.02	NS
Total									
ADFI	7.6	7.1	7.6	6.9	7.0	7.0	7.4	0.5	NS
ADG	1.4	1.3	1.2	1.3	1.3	1.3	1.2	0.1	NS
G:F	0.18	0.18	0.17	0.18	0.19	0.18	0.17	0.01	NS

^a Treatments consisted of 0 (Con), 20 or 500 mg supplemental Zn/kg DM as ZnSO₄ (ZnS and HiZnS), Zn proteinate (ZnP and HiZnP) or a mixture supplying 50% of supplemental Zn from each source (ZnM and HiZnM).

^b NS = not significant.

^c Gain:feed.

Table 3. Effect of time on plasma Zn concentration and alkaline phosphatase (Alp) activity (Phase 1).

Item	d 0	d 28	d 56	d 84	d 98	SEM
Plasma Zn (mg/L) ^a	1.04	1.02	1.05	1.14	1.12	0.04
Plasma Alp (U/L) ^b	84.8	89.2	98.1	109.0	118.9	3.62

^a Time effect ($P < 0.08$).

^b Time effect ($P < 0.01$).

Table 4. Effect of Zn source and level tissue homogenate Zn concentration.

Item	Treatment ^a							SEM	Contrasts ^{b,c}
	Con	ZnS	ZnP	ZnM	HiZnS	HiZnP	HiZnM		
	----- mg/L -----								
Duodenum	0.8	1.0	0.7	0.9	0.7	2.6	2.0	0.3	A*, B**, C**, D**
Rumen	8.8	8.2	10.3	9.1	10.3	9.1	9.0	0.9	NS
Omasum	10.7	10.5	10.5	10.3	10.5	11.8	10.5	0.9	NS

^a Treatments consisted of 0 (Con), 20 or 500 mg supplemental Zn/kg DM as ZnSO₄ (ZnS and HiZnS), Zn proteinate (ZnP and HiZnP) or a mixture supplying 50% of supplemental Zn from each source (ZnM and HiZnM).

^b Single degree of freedom contrasts: A = no Zn vs Zn, B = low Zn vs high Zn, C = HiZnS vs HiZnP, D = HiZnS vs HiZnM.

^c NS = not significant.

* $P < 0.05$.

** $P < 0.01$.

Table 5. Effect of Zn source and level tissue metallothionein (MT) concentration.

Item	Treatment ^a							SEM	Contrasts ^{b,c}
	Con	ZnS	ZnP	ZnM	HiZnS	HiZnP	HiZnM		
	----- µg MT/g wet tissue -----								
Duodenum	106.8	100.6	111.8	88.7	106.9	113.0	118.9	14.3	NS
Rumen	188.8	186.7	233.9	180.3	185.8	168.2	168.4	18.5	A [†] , B [†]
Omasum	26.7	23.4	26.4	27.2	27.8	27.1	26.2	1.4	C [†]

^a Treatments consisted of 0 (Con), 20 or 500 mg supplemental Zn/kg DM as ZnSO₄ (ZnS and HiZnS), Zn proteinate (ZnP and HiZnP) or a mixture supplying 50% of supplemental Zn from each source (ZnM and HiZnM).

^b Single degree of freedom contrasts: A = low Zn vs high Zn, B = ZnS vs ZnP, C = ZnS vs ZnM

^c NS = not significant.

[†] $P < 0.10$.

Table 6. Effect of Zn source and level on plasma Zn concentrations and alkaline phosphatase (Alp) activity (Phase 2).

Item	Treatment ^a							SEM	Contrasts ^{b,c}
	Con	ZnS	ZnP	ZnM	HiZnS	HiZnP	HiZnM		
Plasma Zn (mg/L)	1.1	1.2	1.2	1.1	1.8	2.3	2.3	0.1	A ^{**} , B ^{**} , C ^{**} , D ^{**}
Liver Zn (mg/kg)	158.2	138.0	152.8	163.9	237.1	378.0	293.3	42.9	A [†] , B ^{**} , C [*]
Liver MT (µg/g tissue)	334.8	425.4	418.8	377.1	616.1	683.6	668.8	56.3	A ^{**} B ^{**}

^a Treatments consisted of 0 (Con), 20 or 500 mg supplemental Zn/kg DM as ZnSO₄ (ZnS and HiZnS), Zn proteinate (ZnP and HiZnP) or a mixture supplying 50% of supplemental Zn from each source (ZnM and HiZnM).

^b Single degree of freedom contrasts: A = no Zn vs Zn, B = low Zn vs high Zn, C = HiZnS vs HiZnP, D = HiZnS vs HiZnM.

^c NS = not significant.

[†] $P < 0.10$.

^{*} $P < 0.05$.

^{**} $P < 0.01$.

Table 7. Effect of Zn source and level on final tissue Zn concentrations (Phase 2).

Item	Treatment ^a							SEM	Contrasts ^{b,c}
	Con	ZnS	ZnP	ZnM	HiZnS	HiZnP	HiZnM		
	----- mg/kg -----								
Bone ^d									
Shaft	49.7	45.6	44.6	49.5	55.2	53.8	59.5	4.7	B*
Endplate	84.1	86.6	87.1	109.7	85.9	89.2	116.8	21.0	NS
Heart	102.8	80.1	85.3	89.6	90.2	90.7	102.1	12.1	NS
Kidney	95.8	97.2	89.7	114.3	183.1	356.7	299.5	33.5	A**, B**, C**, D*
Muscle	13.3	16.2	11.0	12.0	18.1	21.5	24.2	2.2	A*, B**, D†
Spleen	112.5	97.6	97.6	92.8	105.2	99.3	99.3	9.0	A†
Testicle	98.3	83.7	77.5	69.4	84.1	83.2	104.9	11.4	NS

^a Treatments consisted of 0 (Con), 20 or 500 mg supplemental Zn/kg DM as ZnSO₄ (ZnS and HiZnS), Zn proteinate (ZnP and HiZnP) or a mixture supplying 50% of supplemental Zn from each source (ZnM and HiZnM).

^b Single degree of freedom contrasts: A = no Zn vs Zn, B = low Zn vs high Zn, C = HiZnS vs HiZnP, D = HiZnS vs HiZnM.

^c NS = not significant.

^d Location effect ($P < 0.01$).

† $P < 0.10$.

* $P < 0.05$.

** $P < 0.01$.

Table 8. Effect of Zn source and level on hair, hoof, and skin Zn concentrations (Phase 2).

Item	Treatment ^a							SEM	Contrasts ^{b,c}
	Con	ZnS	ZnP	ZnM	HiZnS	HiZnP	HiZnM		
	----- mg/kg -----								
Hair	155.9	156.6	174.4	157.1	172.0	172.7	160.9	7.3	NS
Hoof ^d									
Sole	61.6	58.7	66.7	54.3	51.5	54.9	51.3	5.5	NS
Wall	159.7	175.6	185.0	149.5	157.5	174.4	171.7	9.0	B*
Skin	18.5	14.9	14.2	21.1	23.8	15.3	20.6	2.2	A*, B*

^a Treatments consisted of 0 (Con), 20 or 500 mg supplemental Zn/kg DM as ZnSO₄ (ZnS and HiZnS), Zn proteinate (ZnP and HiZnP) or a mixture supplying 50% of supplemental Zn from each source (ZnM and HiZnM).

^b Single degree of freedom contrasts: A = HiZnS vs HiZnP, B = ZnS vs ZnM.

^c NS = not significant.

^d Location effect ($P < 0.01$).

* $P < 0.05$

CHAPTER 2

Uptake and transport of zinc from zinc sulfate and zinc proteinate by ruminal and omasal epithelium¹

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ABSTRACT: Uptake and transport of Zn from ZnSO₄ and Zn proteinate (**ZnProt**) by ruminal and omasal epithelium were examined using a parabiotic chamber system and ⁶⁵Zn-labelled ZnSO₄ and ZnProt. Time-dependent experiments were conducted for 4 h with mucosal buffer (pH 6.0, Krebs-Ringer phosphate) containing 20 μM Zn as ZnSO₄ or ZnProt. Concentration-dependent experiments were conducted for 4 hr with 10 or 200 μM Zn as ZnSO₄ or ZnProt in the mucosal buffer. Zinc uptake and transport were also evaluated following simulated ruminal digestion. Digestion solutions contained substrate and 10 or 200 μM added Zn as ZnSO₄ or ZnProt. Zinc uptake and transport by omasal tissue were negligible, thus remaining experiments were conducted with rumen epithelium only. In time-dependent experiments, Zn uptake increased ($P < 0.01$) as incubation time increased, but was unaffected by Zn source. Zinc uptake in concentration-dependent experiments, was affected by a source × concentration interaction ($P < 0.02$). At 10 μM Zn, uptake was not influenced by Zn source, whereas when 200 μM Zn was added, Zn uptake from ZnProt was greater than from ZnSO₄. Zinc uptake in concentration-dependent experiments was also affected by a concentration × time interaction ($P < 0.01$). Increasing incubation time increased Zn uptake at 200 μM Zn; however at 10 μM, Zn uptake increased to 30 min then remained constant over time. Following simulated ruminal digestion, Zn solubility was influenced by a source × concentration interaction ($P < 0.03$). When digestion solutions contained 10 μM added Zn, solubility was not different between sources; however when 200 μM Zn was added, Zn from ZnProt was more soluble than from ZnSO₄ and tended to be taken up by rumen epithelium to a greater extent. Zinc uptake from digestions containing 200 μM added Zn was greater ($P < 0.01$) than from those containing 10 μM added Zn. Zinc transport was

non-detectable in every experiment. Tissue Zn concentration was not affected by Zn source or incubation time in any experiment; however, in concentration-dependent experiments and following ruminal digestion, tissue Zn concentration was greater at high than at low added Zn concentrations. In the absence of dietary inhibitors and with 200 μM Zn in the uptake buffer, Zn uptake was greater from ZnProt than from ZnSO₄. With dietary antagonists present, solubility of Zn at the high Zn concentration was greater from ZnProt than from ZnSO₄, and uptake was numerically greater from ZnProt than from ZnSO₄.

Key Words: rumen, omasum, parabiotic, proteinate, zinc

Introduction

Zinc bioavailability from a variety of inorganic and organic sources has been evaluated in numerous *in vivo* experiments (Spears, 1996). However, little research to date has compared zinc proteinate (**ZnProt**) to inorganic Zn sources. Previously, ZnProt has improved performance and carcass characteristics in feedlot steers (Spears and Kegley, 1994), increased the force required to shear hoof tissue in heifers (Reiling et al., 1992), and increased Zn retention in lambs (Lardy et al., 1992) relative to inorganic Zn sources (ZnSO₄ or ZnO). While the mechanism(s) responsible for these observed differences remains unclear, it has been hypothesized that organic Zn is more soluble and(or) more bioavailable for absorption.

Zinc uptake and apparent absorption from rumen tissue has been demonstrated *in vivo* using ⁶⁵Zn-dosed lambs (Arora et al., 1969) and apparent absorption of Zn from the reticulo-rumen has been observed in wethers fitted with ruminal, abomasal and ileal cannulas (Kennedy and Bunting, 1991; Kirk et al., 1994). Ashmead et al. (1985) suggested that metal-peptide complexes might be transported directly into the intestinal mucosa intact via peptide transport mechanisms. Amino acid- or peptide-bound Zn may be absorbed from the rumen or omasum via peptide transport mechanisms described by Matthews and Webb (1995). Absorption from the forestomach could significantly reduce the potential for ionization or digestion of the complex during the digestive process.

A series of experiments was conducted to determine the effects of time, Zn concentration, and simulated ruminal digestion on uptake and transport of Zn from ⁶⁵Zn-labelled ZnSO₄ and ZnProt by ruminal epithelium.

Materials and Methods

Parabiotic chamber experiments. Uptake and transport of Zn by ovine ruminal and omasal epithelium were examined in a parabiotic chamber system described by Matthews and Webb (1995). Serosal (pH 7.4; Krebs-Ringer phosphate + 10 mM glucose) and mucosal (pH 6.0; Krebs-Ringer phosphate + 10 mM mannitol and 500 μ M phenol red) buffers and 0.85% saline were prepared the day prior to each experiment, and warmed overnight in a 39°C water bath. On the day of each experiment, serosal and mucosal buffers were oxygenated by bubbling an O₂:CO₂ (95:5) gas mixture through each solution for one hour. Treatment solutions were prepared by adding the appropriate amount of Zn (10, 20 or 200 μ M) from ZnSO₄ or Zn proteinate (**ZnProt**; Chelated Minerals Corporation, Salt Lake City, UT) and 7.4 kBq ⁶⁵Zn/mL to a volume of mucosal buffer. One Dorset or Katahdin wether was stunned by captive bolt and killed by exsanguination. The entire rumen and omasum were excised, digesta was removed, and tissues were rinsed with warm tap water and pre-warmed (39°C) 0.85% saline. Tissues were transported to the laboratory in pre-warmed (39°C), oxygenated serosal buffer. At the laboratory, eighteen parabiotic chambers were prepared as described by Matthews and Webb (1995). Two lambs were used to provide eight replicate chambers per treatment in time- and concentration-dependent experiments. One lamb was used to provide four replicate chambers per treatment in experiments following simulated ruminal digestion.

Preliminary experiments determined that Zn uptake from omasal tissue was negligible, thus all experiments were subsequently conducted solely with ruminal epithelium. Furthermore, Zn transport was not influenced by time, Zn source or

concentration, or the presence of 2.5 mg/mL BSA in the serosal buffer. As such, only Zn uptake, as calculated by decreasing specific radioactivity of mucosal buffer, is reported herein. Initial time-dependent experiments conducted for 6 h determined that minimal Zn uptake occurred beyond 4 h, thus all experiments were conducted during 4 h incubations at 39°C. Concentration-dependent transport was assessed with either 10 or 200 µM Zn as ZnSO₄ or ZnProt in the mucosal buffer. Zinc uptake by ruminal epithelium was also determined following a simulated ruminal digestion (described below). Aqueous fractions from each digestion were pooled prior to being placed into mucosal side of each chamber. In all experiments, aliquots from each test solution were withdrawn from both the serosal and mucosal sides of each chamber at 0, 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 h after inoculation and placed in microcentrifuge tubes for storage. Aliquots (0.2 mL) from each sample were transferred into 5-mL polyethylene tubes for gamma counting (Cobra II, Packard Instrument Company, Meriden, CT). At the conclusion of the experiments, excess buffer was discarded and tissue exposed to buffer was excised, washed 3 times in ice-cold 5 mM EDTA solution, dried and weighed. Tissue samples were transferred to 5-mL polyethylene tubes for gamma counting.

Simulated ruminal digestion. Buffered mineral medium (McDougall, 1948) was prepared fresh and warmed (39°C) prior to each experiment. Rumen fluid was collected from fistulated steers and strained through 8 layers of cheesecloth into a pre-warmed (39°C), insulated container for transport to the laboratory. Upon arrival, rumen inoculum and mineral medium were combined in a 2:1 ratio and anaerobic conditions were maintained by bubbling CO₂ through the mixture. Urea was added to provide a final concentration of 0.05% and the solution was mixed well under CO₂. Aliquots (10 mL)

were transferred into 50 mL centrifuge tubes containing 0.118 g substrate (74% corn, 11% soybean meal, 10% oat straw, and 5% CaCl₂·2H₂O). Zinc sulfate or ZnProt and ⁶⁵Zn-labelled ZnSO₄ and ZnProt were combined prior to the experiment as described above and added to provide either 10 or 200 μM Zn and 18.5 kBq ⁶⁵Zn/mL of final digestion solution. Four replicate digestions were maintained for each source × concentration combination. Tubes were mixed gently, blanketed in CO₂, and stoppers with one-way valves were installed to maintain an anaerobic environment. Tubes were then incubated in a waterbath for 18 h at 39°C, and were mixed gently periodically during the incubation. After incubation, 1-mL aliquots of the complete digestion solution in each digestion tube were transferred into 5-mL polyethylene tubes for gamma counting. Digestion tubes were then centrifuged at 25,000 × g for 30 min. Following centrifugation, a 0.2-mL aliquot from the aqueous fraction of each digestion solution was transferred into a 5-mL polyethylene tube for gamma counting. The remaining aqueous fractions from the four replicates of each Zn source × concentration combination were pooled prior to being added directly to the mucosal side of each parabiotic chamber. Solubility of each Zn source was calculated from the specific activity of the digestion solution before centrifugation and the aqueous fraction of each digestion solution after centrifugation.

Statistical analysis. Analysis of repeated measures data was conducted by ANOVA using the Mixed procedure of SAS (Version 6.12, SAS Institute Inc., Cary, NC) as described by Littell et al. (1998). Chamber was considered the experimental unit and chamber within source was included as a random error term. Eight replicate chambers were included in analysis of time- and concentration-dependent experiments. Four

replicate chambers were included in analysis of data from experiments following simulated digestion. The models included concentration (in concentration-dependent experiments), source, time, and all appropriate interactions. Analysis of final tissue Zn concentration was conducted by ANOVA using the General Linear Model procedure of SAS. Chamber was considered the experimental unit, and the models included source, concentration and concentration \times source (in concentration-dependent experiments).

Results

In time-dependent experiments, Zn uptake increased ($P < 0.01$) at a decreasing rate as incubation time increased; however, uptake was not affected by Zn source (Figure 1).

In concentration-dependent experiments, Zn uptake was affected by a source \times concentration interaction ($P < 0.02$; Figure 2). When 10 μM Zn was added to the mucosal buffer, uptake was not influenced by Zn source, whereas when 200 μM Zn was added, Zn uptake from ZnProt was greater than from ZnSO₄. Zinc uptake in concentration-dependent experiments was also affected by a concentration \times time interaction ($P < 0.01$). Increasing incubation time resulted in increased Zn uptake when mucosal buffer contained 200 μM Zn; however, when mucosal buffer contained 10 μM , Zn uptake did not change after 30 min.

Following simulated ruminal digestion, Zn solubility was influenced by a source \times concentration interaction ($P < 0.03$). When digestion solutions contained 10 μM added Zn, solubility was not different between ZnSO₄ and ZnProt (39.7 and 14.7%, respectively). However, when 200 μM Zn was added, Zn from ZnProt was more soluble than from ZnSO₄ (41.5 and 8.8%, respectively). Zinc uptake from aqueous fractions of

simulated rumen digestions containing 200 μM added Zn was greater ($P < 0.01$) than from those containing 10 μM added Zn (Figure 3). Uptake of Zn from ZnProt tended to be greater than ZnSO_4 for ruminal digestions containing 200 μM added Zn, but not those containing 10 μM of Zn.

Final tissue specific activity in time-dependent experiments was not affected by Zn source or incubation time (Table 1). Tissue specific activities in concentration-dependent experiments and experiments following simulated ruminal digestion were greater when 200 μM Zn was added compared to 10 μM added Zn (Table 1). Tissue specific activity was unaffected by Zn source in any experiment.

Discussion

At 10 and 20 μM Zn concentrations uptake by ruminal epithelial cells was not affected by Zn source. However at 200 μM Zn, Zn uptake by ruminal epithelial tissue was greater from ZnProt than from ZnSO_4 . These data suggest that, in the absence of dietary antagonists, uptake of Zn from ZnSO_4 and ZnProt may be absorbed via different mechanisms depending on concentration. Inorganic ZnSO_4 is known to dissociate in solution, whereas the dissociation of chelated Zn depends heavily upon the specific chelation chemistry of the compound. Using various in vitro techniques, Cao et al. (2000) investigated the chemical characteristics and bioavailability of several organic Zn sources relative to ZnSO_4 . In gel filtration experiments Zn from all of the organic Zn products eluted in the same range of fractions as Zn from ZnSO_4 when examined at pH 2 and pH 5. However, in deionized H_2O , small peaks appeared for each organic Zn source before that of ZnSO_4 indicating that a small percentage (ranging from 2.2 to 11.7%) of the organic products remained chelated or complexed. Values for three different ZnProt

compounds examined ranged from 10.2 to 11.7% and were three of the four highest values suggesting that ZnProt compounds remain intact, at least to some extent, in solution. Given the observations of Cao et al. (2000), it is reasonable to assume that uptake of Zn from the dissociated fractions would be similar between inorganic and organic Zn sources. However, if approximately 10% of the Zn from ZnProt remained chelated, it is possible that ZnProt may have been transported into the rumen epithelium intact via amino acid and(or) peptide transport mechanisms. Ashmead et al. (1985) suggested that it may be possible for metal ions to be transported into the intestinal mucosa as part of metal:peptide complexes via mechanisms distinct from ionic Zn. Furthermore, researchers recently demonstrated the ability of ruminal and omasal tissue to effectively absorb and translocate methionine and the dipeptides carnosine and methionylglycine (Matthews and Webb, 1995). Alternatively, Zn from ZnSO₄ may have interacted with phosphates contained in the KRP buffer used for time- and concentration-dependent experiments. This suggestion is also consistent that a fraction of the added ZnProt remained intact in solution, thereby preventing the antagonism.

Following simulated ruminal digestion, Zn uptake was not significantly influenced by Zn source; however, when 200 µM Zn was added to digestion solutions Zn uptake was numerically greater from ZnProt (10.3 nmol Zn/mg dry tissue) than from ZnSO₄ (7.9 nmol Zn/mg dry tissue). Enhanced uptake following simulated ruminal digestion may at least partially be explained by greater Zn solubility from ZnProt than from ZnSO₄ at high Zn concentrations. Greater solubility and uptake of ZnProt at high concentration supports that hypothesis that a fraction of ZnProt remains chelated in solution preventing potential interactions with dietary antagonists. Ionic Zn from ZnSO₄

or ZnProt was likely bound by one of many potential dietary ligands, many of which may negatively impact solubility and uptake. In contrast, intact ZnProt would remain soluble and available for uptake by reducing or eliminating interactions with dietary antagonists.

Matthews and Webb (1995) clearly demonstrated the uptake and transport of amino acids and dipeptides across ruminal and omasal epithelium. Thus, the lack of Zn uptake by omasal tissue and the lack of Zn transport in both tissues is puzzling. Amino acid and peptide transporters found in ruminal and omasal tissue may differ in such a way as to allow uptake of amino acid- and(or) peptide-bound Zn in the rumen, and preclude such uptake in the omasum. Binding of Zn by intracellular ligands such as metallothionein (MT) may also partially explain the lack of Zn transport. In vivo, excess Zn accumulated with MT in liver, kidney, pancreas, and small and large intestine, but not with MT in heart, testes, rumen papillae, abomasal mucosa or choroid plexus in cattle and sheep fed 2000 mg Zn/kg (Whanger et al., 1981). Hempe and Cousins (1991) observed that more Zn was associated with MT in the cytosol of intestinal tissues from rats fed 180 μg Zn/g diet than those fed 1 μg Zn/g diet. While MT was not quantified in the current experiments, in vivo rumen MT concentration was not affected by Zn source or concentration (Wright and Spears, unpublished data). Zinc may have entered the epithelium and been bound to MT that was already present. While ZnProt may have been taken up intact as suggested above, cytosolic MT may have bound Zn from both Zn sources. Metallothionein is known to have a high affinity for Zn (Cousins, 1985), thus MT may have removed Zn from the ZnProt chelate.

Alternatively, presence of cytosolic peptidases in rumen tissue may also be responsible for hydrolysis of ZnProt compounds and subsequent release of bound Zn.

Though cytosolic peptidases in rumen tissue have not been isolated experimentally, presence of peptides in rumen fluid and peptide transporters in ruminal and epithelial tissue supports their existence. Partial hydrolysis of methionylglycine but not carnosine by rumen tissue (Matthews and Webb, 1995) suggests that peptidases are specific and as such hydrolysis of ZnProt would depend on the specific amino acid composition of the compound.

The lack of Zn transport into the basolateral buffer may have several potential explanations. Addition of 5 mM EDTA to the wash solution may have removed loosely bound Zn. Since EDTA is known to be a strong Zn chelator, Zn may have been removed from the epithelial tissue potentially eliminating any measurable differences in Zn that may have occurred. Alternatively, translocation of Zn from the apical to basolateral buffers may require more time than the 4 h allowed in the current experiment. Finally, transport of Zn across the basolateral membrane may require some factor(s) found in the plasma that was not present in the basolateral buffer used in vitro.

Results of the current experiments suggest that in the absence of dietary inhibitors, Zn uptake by ruminal tissue is greater from ZnProt than from ZnSO₄. In the presence of dietary antagonists, Zn solubility was greater and Zn uptake tended to be greater from aqueous fractions of simulated ruminal digestions containing 200 μM added Zn as ZnProt than from ZnSO₄. When 10 μM Zn was added to simulated ruminal digestions, Zn solubility was greater from ZnSO₄ than from ZnProt; however, uptake of Zn was unaffected by Zn source.

Implications

Numerous organic Zn sources are available commercially and while they have been touted as being more available or more effectively utilized by livestock, specific mechanisms remain to be elucidated. These data suggest that uptake of Zn from ZnProt and ZnSO₄ may occur via distinct mechanisms and that Zn from ZnProt appears to be more available for uptake by ruminal epithelium than Zn from ZnSO₄. More research is required to further define differences in absorption between inorganic and organic Zn sources.

Literature Cited

- Arora, S. P., E. E. Hatfield, U. S. Garrigus, T. G. Lohman, and B. B. Doane. 1969. Zinc-65 uptake by rumen tissue. *J. Nutr.* 97:25-28.
- Ashmead, H. D., D. J. Graff, and H. H. Ashmead. 1985. Intestinal absorption of metal ions and chelates. Charles C. Thomas, Springfield, IL.
- Cao, J., P. R. Henry, R. Guo, R. A. Holwerda, J. P. Toth, R. C. Littell, R. D. Miles, and C. B. Ammerman. 2000. Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. *J. Anim. Sci.* 78:2039-2054.
- Cousins, R. J. 1985. Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. *Physiol. Rev.* 65:238-309.
- Hempe, J. W., and R. J. Cousins. 1992. Cysteine-rich intestinal protein and intestinal metallothionein: an inverse relationship as a conceptual model for zinc absorption in rats. *J. Nutr.* 122:89-95.
- Lardy, G., M. S. Kerley and J. A. Patterson. 1992. Retention of metal proteinates by lambs. *J. Anim. Sci.* 70(suppl. 1):314 (abstr.).
- Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. *J. Anim. Sci.* 76:1216-1231.
- Kennedy, D. W., and L. D. Bunting. 1991. Alterations in ruminal utilization of magnesium and zinc in lambs fed different ratios of concentrate:forage. *Internat. J. Vit. Nutr. Res.* 61:67-71.

- Kirk, D. J., J. P. Fontenot, and S. Rahnema. 1994. Effects of feeding lasalocid and monensin on digestive tract flow and partial absorption of minerals in sheep. *J. Anim. Sci.* 72:1029-1037.
- Matthews, J. C., and K. E. Webb, Jr. 1995. Absorption of L-carnosine, L-methionine, and L-methionylglycine by isolated sheep ruminal and omasal epithelium. *J. Anim. Sci.* 73:3464-3475.
- McDougall, E. I. 1948. Studies on ruminant saliva. I. The composition and output of sheep saliva. *Biochem. J.* 3:15-18.
- Reiling, B. A., L.L. Berger, G. L. Riskowski and R. E. Rompala. 1992. Effects of zinc proteinate on hoof durability in feedlot heifers. *J. Anim. Sci.* 70(suppl. 1):313 (abstr.).
- Spears, J. W. 1996. Organic trace minerals in ruminant nutrition. *Anim. Feed Sci. Technol.* 58:151-163.
- Spears, J. W. and E. B. Kegley. 1994. Influence of zinc proteinate on performance and carcass characteristics of steers. *J. Anim. Sci.* 72(suppl. 1):(abstr.).
- Whanger, P. D., S. OH, and J. T. Deagen. 1981. Ovine and bovine metallothioneins: accumulation and depletion of zinc in various tissues. *J. Nutr.* 111:1196-1206.

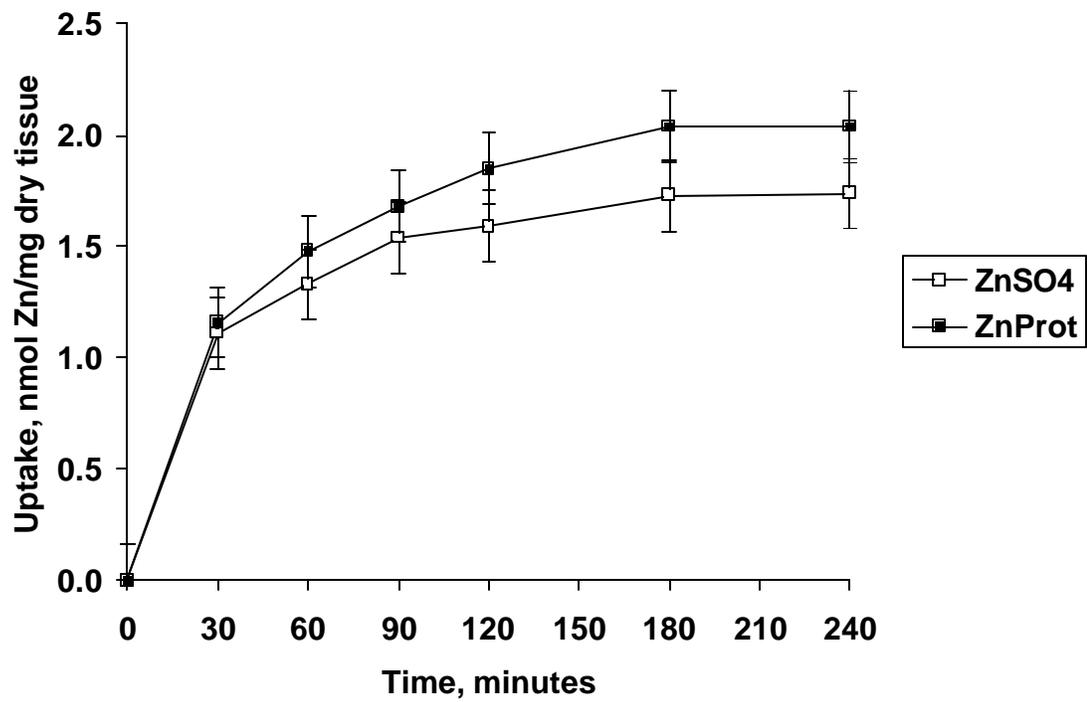


Figure 1. Uptake of Zn by rumen epithelium from mucosal buffer containing 20 μ M Zn as ZnSO₄ or ZnProt during a 240 min incubation at 39°C. Time effect ($P < 0.01$).

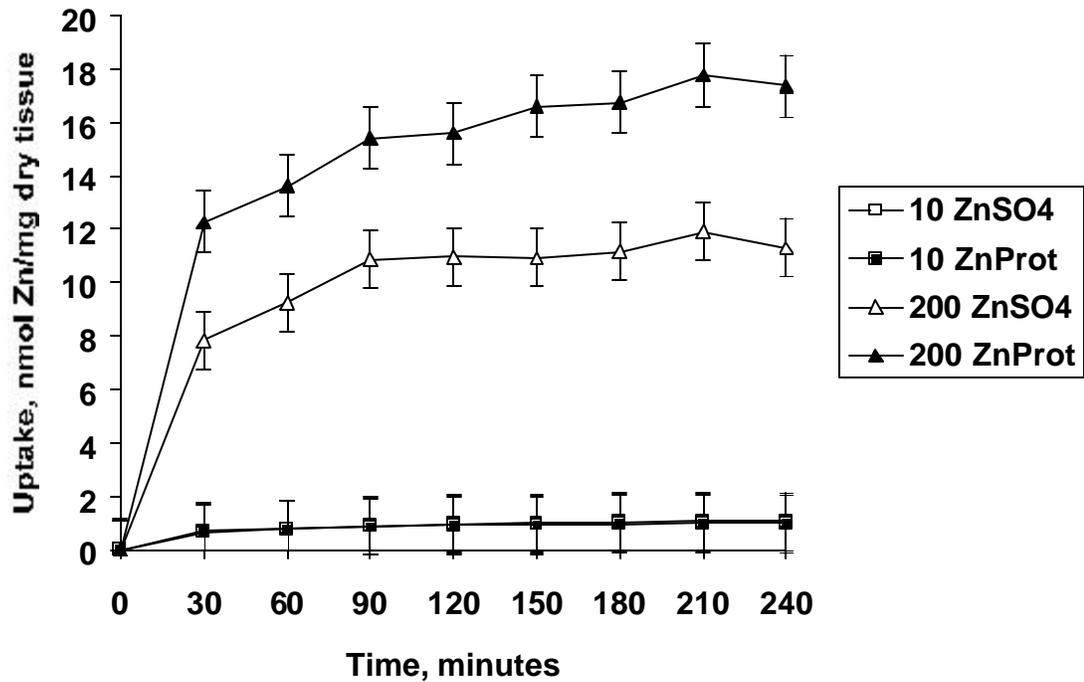


Figure 2. Uptake of Zn by rumen epithelium from mucosal buffer containing 10 or 200 μM Zn as ZnSO_4 or ZnProt during a 240 min incubation at 39°C . Concentration effect ($P < 0.01$), Source effect ($P < 0.01$), Time effect ($P < 0.01$), Concentration \times source interaction ($P < 0.03$), Concentration \times time interaction ($P < 0.01$). Data lines for ZnSO_4 and ZnProt at 10 μM are superimposed upon each other.

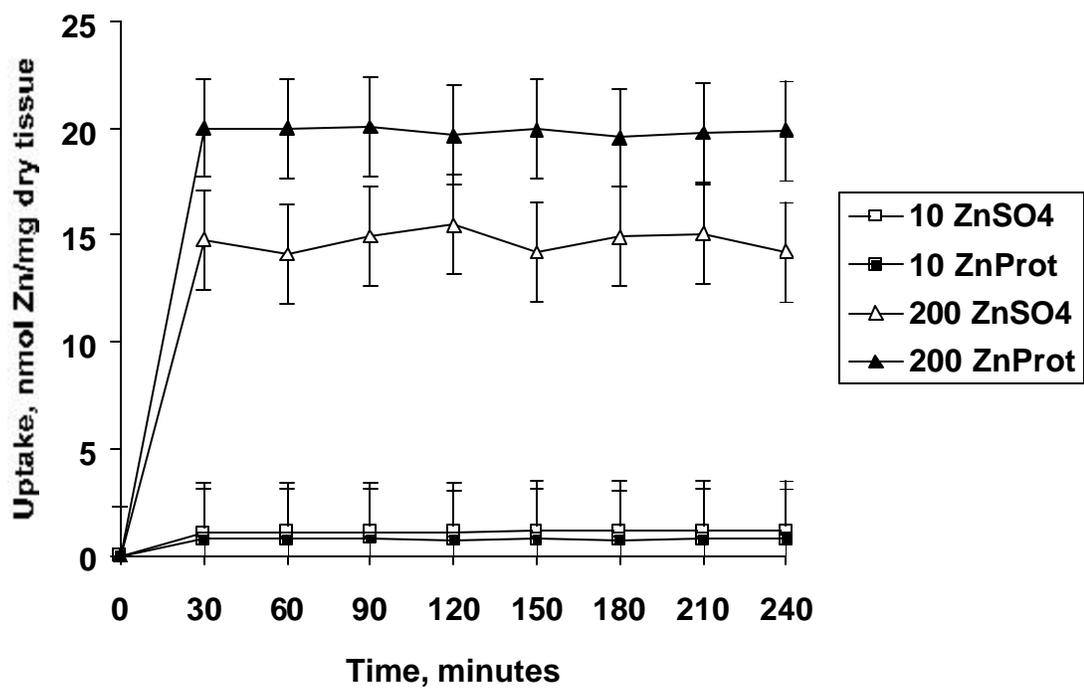


Figure 3. Uptake of Zn by rumen epithelium from aqueous fractions of simulated rumen digestions containing 10 or 200 μM added Zn as ZnSO_4 or ZnProt during a 240 min incubation at 39°C . Concentration effect ($P < 0.01$).

Table 1. Final tissue Zn uptake.

Experiment	Mucosal buffer Zn concentration			SEM
	10	20	200	
----- nmol Zn/mg dry tissue -----				
Time-dependent uptake				
ZnSO ₄	---	1.81	---	0.33
ZnProt	---	2.07	---	0.33
Concentration-dependent uptake ^a				
ZnSO ₄	1.00	---	7.09	0.59
ZnProt	1.01	---	8.93	0.60
Uptake following simulated ruminal digestion ^a				
ZnSO ₄	0.06	---	0.82	0.16
ZnProt	0.04	---	0.34	0.16

^a Concentration effect ($P < 0.01$).

CHAPTER 3

Uptake and transport of zinc from zinc sulfate and zinc propionate by ruminal and omasal epithelium¹

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ABSTRACT: Uptake and transport of Zn from ZnSO₄ and Zn propionate (**ZnProp**) by ruminal and omasal epithelium was examined using a parabiotic chamber system and ⁶⁵Zn-labelled ZnSO₄ and ZnProp. Time-dependent experiments were conducted for 4 h with mucosal buffer (pH 6.0, Krebs-Ringer phosphate) containing 20 μM Zn as ZnSO₄ or ZnProp. Concentration-dependent experiments were conducted for 4 h with 10 or 200 μM Zn as ZnSO₄ or ZnProp in the mucosal buffer. The effect of dietary antagonists was evaluated following simulated ruminal digestion. Digestion solutions contained substrate and 10 or 200 μM added Zn as ZnSO₄ or ZnProp. Zinc uptake and transport by omasal tissue was negligible, and Zn transport was non-detectable in both ruminal and omasal tissue. In time-dependent experiments, Zn uptake increased ($P < 0.01$) with increasing incubation time and tended to be greater ($P < 0.10$) from ZnProp than from ZnSO₄. In concentration-dependent experiments Zn uptake was affected by a concentration × time interaction ($P < 0.01$). With 10 μM Zn in the mucosal buffer, Zn uptake did not increase over time, whereas with 200 μM Zn in the mucosal buffer, Zn uptake increased as incubation time increased. Zinc uptake was not affected by Zn source in concentration-dependent experiments. Following ruminal digestion, Zn uptake was affected by a source × concentration × time interaction ($P < 0.03$). Zinc uptake from digestion solutions containing 10 μM added Zn was not affected by Zn source or increasing incubation time. However, when digestion solutions contained 200 μM added Zn, uptake increased over time and was greater from digestions containing ZnSO₄ than those containing ZnProp. Zinc solubility after digestion was affected by a concentration × source interaction ($P < 0.01$). At low concentrations of added Zn, Zn from ZnSO₄ was more soluble than from ZnProp; however, at high concentrations, solubility was not affected by source. Final

tissue specific activity in time-dependent experiments was not affected by Zn source or incubation time. Tissue specific activity in concentration-dependent experiments and following simulated ruminal digestion increased ($P < 0.01$) as Zn concentration in the mucosal buffer increased from 10 to 200 μM Zn, but was unaffected by Zn source. In the absence of dietary antagonists, Zn uptake was similar between Zn sources. However, following simulated ruminal digestion Zn uptake appeared to be greater from ZnSO_4 than from ZnProp.

Key Words: zinc, propionate, rumen, omasum, parabiotic

Introduction

Bioavailability of Zn from organic and inorganic sources has been the subject of numerous in vivo experiments (Spears, 1996). However, minimal research to date has specifically addressed absorption characteristics of organic and inorganic Zn sources.

Relatively little research has been conducted to evaluate the bioavailability of Zn propionate (**ZnProp**) relative to inorganic Zn. To date, ZnProp has been demonstrated to be more bioavailable in dogs (Brinkhaus et al., 1998; Wedekind and Lowry, 1998), and chicks (Kemin Industries, 1995). In contrast, Zn uptake by kidney fibroblasts (CV-1) and epithelial cells (T-84) in vitro was not different between three different Zn sources (ZnCl₂, ZnProp, and Zn methionine; Buetler et al., 1998). Observed differences in bioavailability have been hypothesized to be the result of enhanced absorption and(or) solubility of organic relative to inorganic sources. Zinc uptake by rumen tissue has been clearly demonstrated in vivo using ⁶⁵Zn-dosed lambs (Arora et al., 1969). Apparent absorption of Zn from the rumen has also been observed in experiments using indigestible markers and wethers fitted with ruminal, abomasal and ileal cannulas (Kennedy and Bunting, 1991; Kirk et al., 1994). Since propionate is readily absorbed from the rumen (Merchen, 1988), it is possible that ZnProp may be absorbed from the rumen intact.

A series of experiments was conducted to examine the effects of incubation time, Zn concentration and simulated ruminal digestion on the uptake and transport of Zn from ZnSO₄ and ZnProp by ruminal and omasal epithelium.

Materials and Methods

Parabiotic chamber experiments. Uptake and transport of Zn by ovine ruminal and omasal epithelium were examined in a parabiotic chamber system described by Matthews and Webb (1995). Serosal (pH 7.4; Krebs-Ringer phosphate + 10 mM glucose) and mucosal (pH 6.0; Krebs-Ringer phosphate + 10 mM mannitol and 500 μ M phenol red) buffers and 0.85% saline were prepared the day prior to each experiment, and warmed overnight in a 39°C water bath. On the day of each experiment, serosal and mucosal buffers were oxygenated by bubbling an O₂:CO₂ (95:5) gas mixture through each solution for one hour. Treatment solutions were prepared by adding the appropriate amount of Zn (10, 20 or 200 μ M) from ZnSO₄ or ZnProp (Kemin Industries, Des Moines, IA) and 7.4 kBq ⁶⁵Zn/mL to a volume of mucosal buffer. One Dorset or Katahdin wether was stunned by captive bolt and killed by exsanguination. The entire rumen and omasum were excised, digesta was removed, and tissues were rinsed with warm tap water and pre-warmed (39°C) 0.85% saline. Tissues were transported to the laboratory in pre-warmed (39°C), oxygenated serosal buffer. At the laboratory, eighteen parabiotic chambers were prepared as described by Matthews and Webb (1995). Two lambs were used to provide eight replicate chambers per treatment in time- and concentration-dependent experiments. One lamb was used to provide four replicate chambers per treatment in experiments following simulated ruminal digestion.

Preliminary experiments determined that Zn uptake from omasal tissue was negligible, thus all experiments were subsequently conducted solely with ruminal epithelium. Furthermore, Zn transport was not influenced by time, Zn source or concentration, or the presence of 2.5 mg/mL BSA in the serosal buffer. As such, only Zn

uptake, as calculated by decreasing specific radioactivity of mucosal buffer, is reported herein. Initial time-dependent experiments conducted for 6 h determined that minimal Zn uptake occurred beyond 4 h, thus all experiments were conducted during 4 h incubations at 39°C. Concentration-dependent transport was assessed with either 10 or 200 µM Zn as ZnSO₄ or ZnProp in the mucosal buffer. Zinc uptake by ruminal epithelium was also determined following a simulated ruminal digestion (described below). Aqueous fractions from each digestion were pooled prior to being placed into mucosal side of each chamber. In all experiments, aliquots from each test solution were withdrawn from both the serosal and mucosal sides of each chamber at 0, 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 h after inoculation and placed in microcentrifuge tubes for storage. Aliquots (0.2 mL) from each sample were transferred into 5-mL polyethylene tubes for gamma counting (Cobra II, Packard Instrument Company, Meriden, CT). At the conclusion of the experiments, excess buffer was discarded and tissue exposed to buffer was excised, washed 3 times in ice-cold 5 mM EDTA solution, dried and weighed. Tissue samples were transferred to 5-mL polyethylene tubes for gamma counting.

Simulated ruminal digestion. Buffered mineral medium (McDougall, 1948) was prepared fresh and warmed (39°C) prior to each experiment. Rumen fluid was collected from fistulated steers and strained through 8 layers of cheesecloth into a pre-warmed (39°C), insulated container for transport to the laboratory. Upon arrival, rumen inoculum and mineral medium were combined in a 2:1 ratio and anaerobic conditions were maintained by bubbling CO₂ through the mixture. Urea was added to provide a final concentration of 0.05% and the solution was mixed well under CO₂. Aliquots (10 mL) were transferred into 50 mL centrifuge tubes containing 0.118 g substrate (74% corn,

11% soybean meal, 10% oat straw, and 5% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$). Zinc sulfate or ZnProp and ^{65}Zn -labelled ZnSO_4 and ZnProp were combined prior to the experiment as described above and added to provide either 10 or 200 μM Zn and 18.5 kBq/mL of final digestion solution. Four replicate digestions were maintained for each source \times concentration combination. Tubes were mixed gently, blanketed in CO_2 , and stoppers with one-way valves were installed to maintain an anaerobic environment. Tubes were then incubated in a waterbath for 18 h at 39°C , and were mixed gently periodically during the incubation. After incubation, 1-mL aliquots of the complete digestion solution in each digestion tube were transferred into 5-mL polyethylene tubes for gamma counting. Digestion tubes were then centrifuged at $25,000 \times g$ for 30 min. Following centrifugation, a 0.2-mL aliquot from the aqueous fraction of each digestion solution was transferred into a 5-mL polyethylene tube for gamma counting. The remaining aqueous fractions from the four replicates of each Zn source \times concentration combination were pooled prior to being added directly to the mucosal side of each parabiotic chamber. Solubility of each Zn source was calculated from the specific activity of the digestion solution before centrifugation and the aqueous fraction of each digestion solution after centrifugation.

Statistical analysis. Analysis of repeated measures data was conducted by ANOVA using the Mixed procedure of SAS (Version 6.12, SAS Institute Inc., Cary, NC) as described by Littell et al. (1998). Chamber was considered the experimental unit and chamber within source was included as a random error term. Eight replicate chambers were included in analysis of time- and concentration-dependent experiments. Four replicate chambers were included in analysis of data from experiments following simulated digestion. The models included concentration (in concentration-dependent

experiments), source, time, and all appropriate interactions. Analysis of final tissue Zn concentration was conducted by ANOVA using the General Linear Model procedure of SAS. Chamber was considered the experimental unit, and the models included source, concentration and concentration \times source (in concentration-dependent experiments).

Results

In time-dependent experiments, Zn uptake increased ($P < 0.01$) as incubation time increased from 0 to 240 min, and tended to be 26.3% greater ($P < 0.10$) from ZnProp than from ZnSO₄ (Figure 1). Final tissue specific activity in time-dependent experiments was not affected by Zn source or incubation time (Table 1).

Concentration-dependent experiments were affected by a concentration \times time interaction ($P < 0.01$; Figure 2). With 10 μ M Zn in the mucosal buffer, Zn uptake did not increase over time, whereas with 200 μ M Zn in the mucosal buffer, Zn uptake increased as incubation time increased from 0 to 240 min. Zinc uptake was not affected by Zn source in concentration-dependent experiments. Tissue specific activity in concentration-dependent experiments increased as Zn concentration in the mucosal buffer increased from 10 to 200 μ M Zn, but was unaffected by Zn source.

Zinc solubility following simulated ruminal digestion was affected by a concentration \times source interaction ($P < 0.01$). When added to digestion solutions at low concentration, Zn from ZnSO₄ was more soluble than Zn from ZnProp (5.3 and 2.4%, respectively); however, when added at high concentration, Zn solubility was not different between ZnSO₄ and ZnProp (2.3 and 1.7%, respectively). Uptake of Zn from aqueous fractions of simulated ruminal digestions was affected by a source \times concentration \times time interaction ($P < 0.03$; Figure 3). Zinc uptake from aqueous fractions of digestion

solutions containing 10 μM added Zn was not affected by Zn source or increasing incubation time. However, when digestion solutions contained 200 μM added Zn, uptake increased over time and was greater from aqueous fractions of digestion solutions containing ZnSO_4 than those containing ZnProp. Final tissue Zn specific activity following simulated ruminal digestion was greater ($P < 0.01$) when exposed to aqueous fractions of digestions containing 200 μM added Zn than those containing 10 μM .

Discussion

In time-dependent experiments, Zn uptake increased at a decreasing rate as incubation time increased, and tended to be greater from ZnProp than from ZnSO_4 . Zinc uptake in concentration-dependent experiments increased when Zn concentration was increased from 10 to 200 μM , but was not affected by Zn source. These observations are consistent with those of Buetler et al. (1998), who observed no difference in uptake of ^{65}Zn by cultured monkey kidney fibroblasts (CV-1) or intestinal epithelial cells (T-84) when supplied as ZnProp, ZnCl_2 , or Zn methionine. The reason for inconsistent Zn uptake with respect to Zn source between time- and concentration-dependent experiments is unclear. Inorganic ZnSO_4 is known to completely dissociate in solution, whereas the dissociation of complexed Zn depends heavily upon the specific chelation chemistry of the compound. Using gel filtration chromatography, Buetler et al. (1998) reported similar elution ^{65}Zn peaks for ZnCl_2 , and ZnProp, suggesting that if indeed complexes were formed, they readily dissociate in solution. However, if a small fraction of the Zn from ZnProp remained complexed, it is possible that ZnProp may have been transported into the rumen epithelium intact via a different mechanism than ZnSO_4 .

Research suggests that propionic acid/propionate is absorbed from the rumen as an undissociated acid via passive diffusion or as an anion by a short-chain fatty acid anion/HCO₃⁻ exchanger (Merchen, 1988; Kramer et al., 1996). The curvilinear response in Zn uptake with low concentration in the uptake buffer suggests that Zn uptake is either saturable or Zn contained in the mucosal buffer was completely taken up by rumen tissue. In most chambers, regardless of Zn source, the total amount of Zn removed from the mucosal buffer was at or near the amount of Zn added. When Zn was added at high concentration Zn uptake was also curvilinear; however, total Zn uptake was only 50-60% of the Zn present in the mucosal buffer. The curvilinear response suggests the presence of two different uptake mechanisms for Zn. One that rapidly takes up Zn, and another that takes up Zn more slowly and is saturable at high Zn concentration.

The complete lack of Zn transport was unexpected. Binding of Zn by intracellular ligands such as metallothionein (MT) may partially explain this observation. In vivo, excess Zn accumulates with MT in liver, kidney, pancreas, and small and large intestine, but not with MT in heart, testes, rumen papillae, abomasal mucosa or choroid plexus in cattle and sheep fed 2000 mg Zn/kg (Whanger et al., 1981). In the current experiments rumen MT concentrations, measured in companion experiments (data not shown), were not affected by Zn source or concentration; however, Zn may have entered the epithelium and been bound to MT that was already present. Alternatively, translocation of Zn from the apical to basolateral buffers may require more time than the 4 h allowed in the current experiment. Transport of Zn across the basolateral membrane may also require some factor(s) found in the plasma that was not present in the basolateral buffer used in vitro.

In vivo, ZnProp has been reported to be more bioavailable than ZnO in dogs (Brinkhaus et al., 1998; Wedekind and Lowry, 1998) and ZnSO₄ in chicks (Kemin Industries, 1995); however, numerous physiological and dietary factors may influence Zn absorption in vivo. As components of the diet are degraded, Zn is presented to enterocytes as smaller Zn-binding ligands, primarily peptides, amino acids, and nucleotides, and perhaps as free Zn (Cousins, 1996). Depending on their chemical nature and complexation constants, organic chelators may affect bioavailability either positively or negatively (Baker and Ammerman, 1997). The effect of other dietary ligands, such as phytate, citrate, cysteine, EDTA, histidine, and picolinate on Zn absorption has depended on experimental conditions (Wapnir, 1989). In the current experiment, Zn uptake from aqueous fractions of simulated ruminal digestions containing 200 μM ZnSO₄ was greater than from those containing ZnProp. Zinc solubility following ruminal digestion was greater from ZnSO₄ than from ZnProp when added at 10 μM. When added at 200 μM, Zn solubility was not statistically different between Zn sources; however, numerically Zn from ZnSO₄ was more soluble than Zn from ZnProp. Greater solubility of Zn from ZnSO₄ may explain the greater uptake by rumen epithelial tissue. Lower solubility of Zn from ZnProp than from ZnSO₄ was also unexpected. Perhaps Zn from ZnProp exchanged with dietary ligands more readily or at a faster rate than Zn from ZnSO₄. As discussed above, a number of potential ligands may depress Zn solubility and(or) Zn availability.

Tissue Zn accumulation increased as Zn concentration increased; however, the specific activity of tissue samples could not account for all of the Zn removed from the mucosal buffer. It is possible that lack of differences in tissue specific activity may result from the addition of 5 mM EDTA to the wash solution. Since EDTA is known to be a

strong Zn chelator, loosely bound Zn may have been removed from the epithelial tissue, potentially eliminating any measurable differences in Zn that may have occurred, whereas tightly bound Zn remained in the tissue.

Results of the current experiments suggest that, in the absence of dietary inhibitors, Zn from ZnProp is taken up more readily by ruminal epithelial tissue than Zn from ZnSO₄ when at 20 μM but not at 10 or 200 μM added Zn. However, following simulated ruminal digestion, Zn from ZnSO₄ appears to be more soluble and taken up to a greater extent than Zn from ZnProp. Furthermore, in the current experimental conditions, Zn was not taken up by omasal epithelium and was not transported into the serosal buffer by either ruminal or omasal epithelium.

Implications

Numerous organic Zn sources, available commercially have been touted as being more available or more effectively utilized by livestock. Specific mechanisms responsible for observed effects of organic Zn sources on performance, reproduction, and health remain to be elucidated. Data from the current experiment suggest that in the absence of dietary antagonists, uptake of Zn from ZnProp appears to be greater than from ZnSO₄. In the presence of potential dietary antagonists, Zn from ZnSO₄ appears to be more soluble and be taken up more readily than Zn from ZnProp. Given the myriad of dietary and physiological factors that may influence Zn absorption in vivo, more research is required to clearly define differences in absorption between inorganic and organic Zn sources in various systems.

Literature Cited

- Arora, S. P., E. E. Hatfield, U. S. Garrigus, T. G. Lohman, and B. B. Doane. 1969. Zinc-65 uptake by rumen tissue. *J. Nutr.* 97:25-28.
- Baker, D. H., and C. B. Ammerman. 1995. Zinc bioavailability. In: *Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins*. pp 367-398.
- Beutler, K. T., O. Pankewycz, and D. L. Brautigam. 1998. Equivalent uptake of organic and inorganic zinc by monkey kidney fibroblasts, human intestinal epithelial cells, and perfused mouse intestine. *Biol. Tr. Elem. Res.* 61:19-31.
- Brinkhaus, F., J. Mann, C. Zorich, and J. Greaves. 1998. Bioavailability of zinc propionate in dogs. *J. Nutr.* 128:2596S-2597S.
- Cousins, R. J. 1996. Zinc. In: L. J. Filer and E. E. Ziegler (eds.) *Present Knowledge in Nutrition*, 7th Edition. pp 293-306. International Life Science Institute-Nutrition Foundation, Washington, DC.
- Kennedy, D. W., and L. D. Bunting. 1991. Alterations in ruminal utilization of magnesium and zinc in lambs fed different ratios of concentrate:forage. *Internat. J. Vit. Nutr. Res.* 61:67-71.
- Kirk, D. J., J. P. Fontenot, and S. Rahnema. 1994. Effects of feeding lasalocid and monensin on digestive tract flow and partial absorption of minerals in sheep. *J. Anim. Sci.* 72:1029-1037.
- Kemin Industries. 1995. Bioavailability of zinc from organic and inorganic sources using the chick bioassay technique. *Tech. Bull. No. 11575*. Kemin Industries, Des Moines, IA.

- Kramer, T., T. Michelberger, H. Gurtler, and G. Gabel. 1996. Absorption of short-chain fatty acids across ruminal epithelium of sheep. *J. Comp. Physiol.* 166:262-269.
- Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. *J. Anim. Sci.* 76:1216-1231.
- Matthews, J. C., and K. E. Webb, Jr. 1995. Absorption of L-carnosine, L-methionine, and L-methionylglycine by isolated sheep ruminal and omasal epithelium. *J. Anim. Sci.* 73:3464-3475.
- McDougall, E. I. 1948. Studies on ruminant saliva. I. The composition and output of sheep saliva. *Biochem. J.* 3:15-18.
- Merchen, N. R. 1988. Digestion, absorption, and excretion in ruminants. In: D. C. Church (ed.) *The Ruminant Animal: Digestive Physiology and Nutrition.* p 172. Prentice-Hall, Inc., Englewood Cliffs, NJ.
- Spears, J. W. 1996. Organic trace minerals in ruminant nutrition. *Anim. Feed Sci. Technol.* 58:151-163.
- Wedekind, K. J., and S. R. Lowry. 1998. Are organic zinc source efficacious in puppies? *J. Nutr.* 128:2593S-2595S.
- Whanger, P. D., S. OH, and J. T. Deagen. 1981. Ovine and bovine metallothioneins: accumulation and depletion of zinc in various tissues. *J. Nutr.* 111:1196-1206.

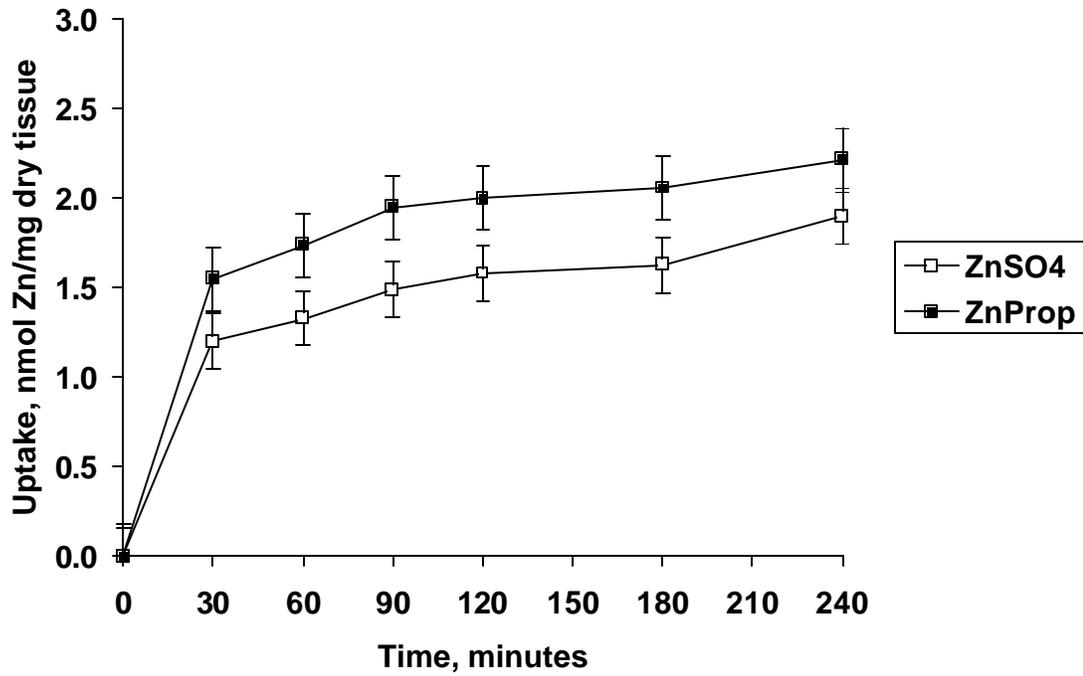


Figure 1. Uptake of Zn by rumen epithelium from mucosal buffer containing 20 μ M Zn as ZnSO₄ or ZnProp during a 240 min incubation at 39°C. Time effect ($P < 0.01$), Source effect ($P < 0.10$).

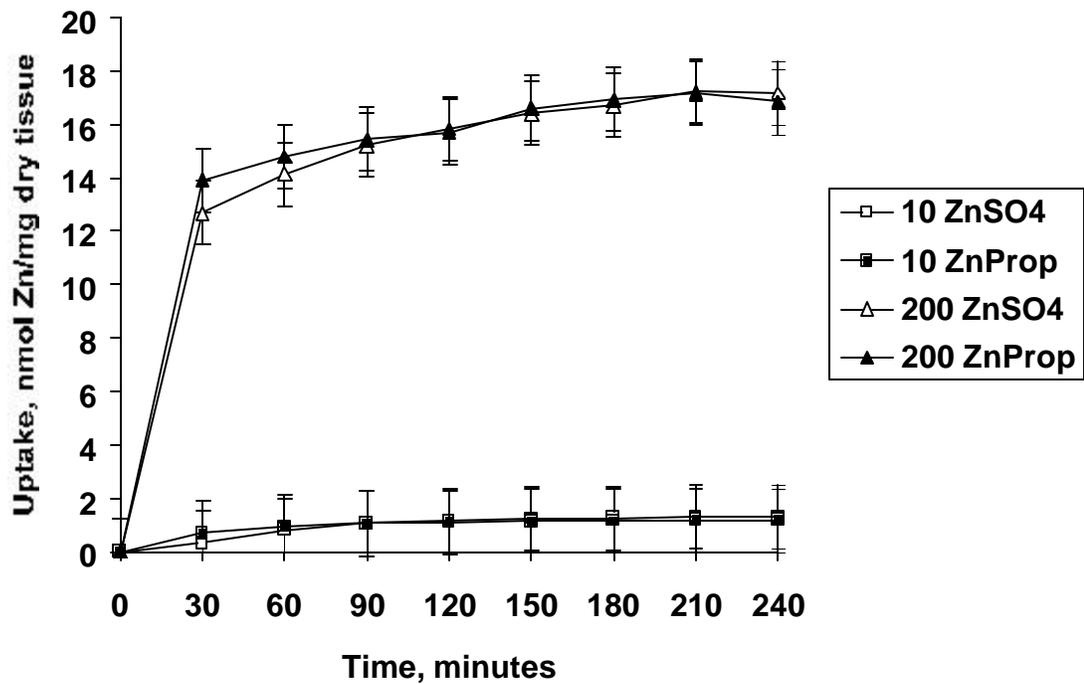


Figure 2. Uptake of Zn by rumen epithelium from mucosal buffer containing 10 or 200 μM Zn as ZnSO_4 or ZnProp during a 240 min incubation at 39°C . Concentration effect ($P < 0.01$), Time effect ($P < 0.01$), Concentration \times time ($P < 0.01$).

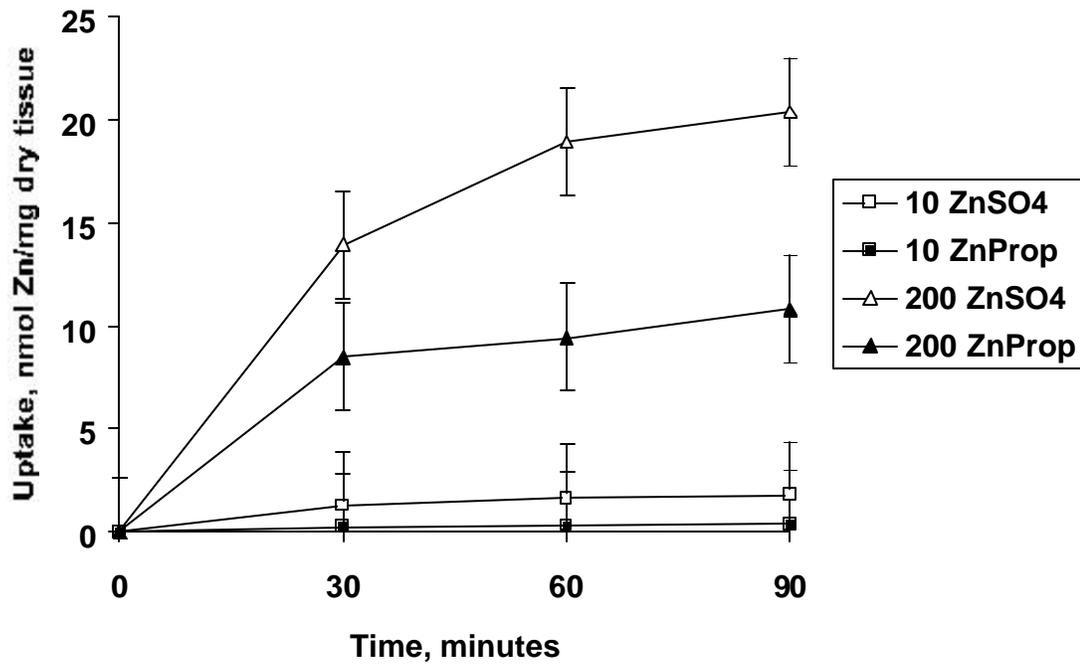


Figure 3. Uptake of Zn by rumen epithelium from aqueous fractions of simulated rumen digestions containing 10 or 200 μM Zn as ZnSO_4 or ZnProp during a 240 min incubation at 39°C . Uptake of Zn beyond 90 min remained constant, thus only values for 0, 30, 60, and 90 min are presented. Concentration effect ($P < 0.01$), Source effect ($P < 0.08$), Time effect ($P < 0.01$), Concentration \times time interaction ($P < 0.01$), Source \times time interaction ($P < 0.01$), Concentration \times source \times time interaction ($P < 0.03$).

Table 1. Final tissue zinc uptake.

	Added Zn concentration			SEM
	10	20	200	
	----- nmol Zn/mg dry tissue -----			
Time-dependent uptake				
ZnSO ₄	---	3.84	---	0.35
ZnProp	---	3.89	---	0.40
Concentration-dependent uptake ^a				
ZnSO ₄	1.58	---	7.56	0.54
ZnProp	1.30	---	8.16	0.54
Uptake following simulated digestion ^a				
ZnSO ₄	0.29	---	2.82	0.64
ZnProp	0.17	---	2.41	0.64

^a Concentration effect ($P < 0.01$).

CHAPTER 4

Uptake and transport of zinc from zinc sulfate and zinc proteinate by Caco-2 cells^{1,2}

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ABSTRACT: Experiments were conducted to evaluate effects of time, Zn concentration, inositol hexaphosphate (**IP₆**) addition, and simulated ruminal and intestinal digestion on the uptake and transport of Zn from ZnSO₄ and Zn proteinate (**ZnProt**) by Caco-2 cells. Experiments were conducted with cells grown on plastic (uptake only) or on membrane inserts (uptake and transport). Reported data refer to both systems unless stated otherwise. Increasing incubation time up to 120 min and Zn concentration up to 200 μM increased ($P < 0.01$) Zn uptake and transport. In the absence of antagonists, Zn uptake and transport were not affected by Zn source. Addition of 200 μM IP₆ and 200 μM Ca, to uptake buffer resulted in a concentration × IP₆ interaction ($P < 0.05$) for Zn uptake and transport by cells. In the absence of antagonists, uptake and transport increased with increasing Zn concentration; however, when antagonists were added, uptake increased up to 20 μM added Zn then remained constant and Zn transport was lower at 200 μM added Zn than at 10 μM. Zinc solubility in the presence of IP₆ and Ca was affected by a concentration × IP₆ interaction ($P < 0.01$). In the absence of IP₆ and Ca, Zn solubility from both Zn sources was nearly 100%; however, when the antagonists were added, solubility declined as Zn concentration increased. Zinc source did not affect Zn solubility in the presence of IP₆ and Ca. Following ruminal and intestinal digestions, Zn uptake and transport were greater from digestions containing 200 μM added Zn than from those containing 10 μM. Following intestinal digestion alone, uptake by monolayers grown on plastic was affected by a concentration × source interaction ($P < 0.04$). Uptake from digestions containing 10 μM added Zn was unaffected by Zn source; however, uptake from digestions containing 200 μM added Zn was greater from ZnProt than from ZnSO₄. Zinc solubility following intestinal digestion alone was greater ($P < 0.05$) when

digestions contained 200 μM relative to 10 μM added Zn, but was not affected by Zn source. Uptake and transport of Zn by cells grown on inserts was greater ($P < 0.01$) from intestinal digestions containing 200 μM relative to 10 μM added Zn, but neither was influenced by Zn source. In the absence of antagonists, uptake and transport of Zn from ZnSO_4 and ZnProt was similar. However, following simulated digestion, uptake of Zn from ZnProt was greater than from ZnSO_4 by Caco-2 cells when added at 200 μM .

Key Words: zinc, proteinate, Caco-2, uptake, transport

Introduction

Bioavailability of Zn from organic and inorganic sources has been the subject of numerous in vivo experiments (Spears, 1996). Zinc proteinate (**ZnProt**) is an organic Zn compound formed from the chelation of Zn from a soluble Zn salt with amino acids and(or) partially hydrolyzed protein (Spears, 1996). Previously, ZnProt has improved performance in feedlot steers (Spears and Kegley, 1994), increased Zn retention in lambs (Lardy et al., 1992), and increased the force required to shear hoof tissue in heifers (Reiling et al., 1992) relative to inorganic Zn sources (ZnSO_4 or ZnO). Observed differences in bioavailability are hypothesized to be the result of enhanced absorption of organic relative to inorganic Zn sources. Minimal research to date has specifically compared absorption characteristics of organic and inorganic Zn sources. Metal-peptide complexes may be transported directly into the intestinal mucosa intact via peptide transport mechanisms (Ashmead et al., 1985). However, if complexes are degraded or ionized during the digestive process, metal uptake may be similar to that found with inorganic salts.

Caco-2, a transformed human colonic cell line, has been used as an in vitro model to evaluate nutrient and drug metabolism by intestinal absorptive epithelium (Han et al., 1994). Monolayers of Caco-2 cells spontaneously differentiate into highly polarized cells that possess many of the properties of mature intestinal absorptive epithelial cells (Hidalgo et al., 1989). Characteristics of Ca (Giuliano and Wood, 1991), Cu (Reeves et al., 1996), Fe (Alvarez-Hernandez et al., 1991; Halleux and Schneider, 1991), Mn (Finley and Monroe, 1997) and Zn (Raffaniello and Wapnir, 1991; Raffaniello et al., 1992) transport have been evaluated using Caco-2 cells.

A series of experiments was conducted to determine the effects of time, Zn concentration, inositol hexaphosphate (**IP₆**) and simulated digestion on uptake and transport of Zn from ZnSO₄ and ZnProt by Caco-2 cells.

Materials and Methods

Materials. Dulbecco's modified Eagle medium (**DMEM**), fetal calf serum (**FCS**), medium supplements except antibiotics and test reagents were purchased from Sigma Chemical Company (St. Louis, MO). Gentamicin and fungizone were purchased from GIBCO (Grand Island, NY). ⁶⁵Zinc (185 GBq/g) was obtained from New England Nuclear (Boston, MA). All other supplies were purchased from Fisher Scientific Company (Pittsburgh, PA) unless specifically stated otherwise.

Caco-2 cells. The TC-7 clone of the Caco-2 human colonic adenocarcinoma cell line (HTB-37) was generously donated by Dr. Monique Rousset (INSERM, Cedex, France). Cells were grown in 75-cm² plastic flasks (Falcon, Becton Dickinson & Company, Franklin Lakes, NJ) in high glucose complete Dulbecco's modified minimal essential medium (**cdMEM**; pH 7.0) containing DMEM (D7777), heat inactivated fetal calf serum (**FCS**; 10%, v/v), NaHCO₃ (44 mM), HEPES (15 mM), non-essential amino acids (1%), L-glutamine (4 mM), and antibiotics (50 mg gentamicin and 0.5 mg fungizone/L) in an atmosphere of air:CO₂ (95:5) at 37°C and 90% humidity. Stock cultures were seeded at a density of 5333 cells/cm², and harvested by treatment with Hank's balanced salt solution without Ca²⁺ or Mg²⁺ and containing trypsin (0.25%) and EDTA (2 mM) for re-seeding when the monolayer was 70-80% confluent. Suspended cells were transferred either to 12-well cluster dishes (Falcon, Becton Dickinson & Company, Franklin Lakes, NJ) to assess uptake alone or track-etched polyethylene

terephthalate membrane filters (0.4 μm pore size, Falcon, Becton Dickinson & Company, Franklin Lakes, NJ) in 6-well cluster dishes (Falcon, Becton Dickinson & Company, Lincoln Park, NJ) to examine Zn uptake and transport together. Medium was changed every second day and the day before cultures were used for experiments. Differentiated cultures were used 10 to 14 d after reaching confluency between passages 79 and 91 (Ellwood et al., 1993).

Uptake experiments. Preliminary experiments were conducted to determine solubility of the Zn sources in the uptake buffer and to determine the optimum pH for uptake and transport. Results indicated no difference in solubility between the Zn sources and that Zn uptake was maximal at pH 6.0 and declined steadily as pH was either reduced to 5.0 or increased to 7.5. Test solutions were prepared and utilized in an identical manner for uptake experiments alone and uptake and transport experiments together. Solutions were prepared fresh prior to the experiment by adding an aliquot of ZnSO_4 or ZnProt (SoluKey, Chelated Minerals Corporation, Salt Lake City, UT) stock solution and ^{65}Zn to a volume of uptake buffer (pH 6.0; 130 mM NaCl, 10 mM KCl, 10 mM 2-[N-morpholino]ethanesulfonic acid, 5 mM glucose, and 1 mM MgSO_4) to provide the appropriate Zn concentration(s) and 9.25 kBq $^{65}\text{Zn}/\text{mL}$. To determine the effect of dietary inhibitors on Zn uptake, 200 μM IP_6 and 200 μM Ca were added to uptake solutions. Stock solutions of IP_6 (1000 μM) and Ca (1000 μM Ca as $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) were prepared in uptake buffer, then appropriate aliquots were added to test solutions following addition of Zn treatments and a 15 min incubation period. Final test solutions were allowed to equilibrate for 15 min at 37°C prior to the experiment. Spent medium was aspirated and each well was washed twice with 1 mL uptake buffer at 37°C. Test solutions (1 mL) were

added to each well and cultures were incubated at 37°C for 5, 10, 15, 30, 60 or 120 min in time-dependent uptake experiments or 60 min in other uptake experiments. After incubation, test solutions were removed and each well was washed three times with ice-cold wash solution (pH 7.0; 150 mM NaCl, 10 mM HEPES, and 5 mM tetrasodium EDTA). Preliminary experiments comparing the effectiveness of multiple washes on removal of non-specifically bound ^{65}Zn determined that more than 3 washes resulted in no further reduction in the ^{65}Zn content of cell suspensions. Cellular material was collected by adding 0.5 mL harvest solution (pH 7.0; 1.7 mM SDS and 1.0 mM tetrasodium EDTA) to each well, scraping the surface with a rubber policeman, and transferring the material to microfuge tubes for solication and storage. The process was then repeated. Cellular material was homogenized by sonication (50 Sonic Dismembrator, Fisher Scientific, Pittsburgh, PA) for 2 to 3 sec. Aliquots of each cell suspension (0.4 mL) and test solution (0.1 mL) were transferred to 5 mL-polypropylene tubes (Sarstedt, Newton, NC) for gamma counting (Cobra II, Packard Instrument Company, Meriden, CT). The quantity of Zn taken up by the cells was calculated from the specific radioactivity of each test solution. Total protein content of each cell suspension was determined by colorimetric assay (Bio-Rad Laboratories Incorporated, Hercules, CA) using a plate reader (PowerWave_x, Bio-Tek Instruments, Winooski, VT). Cell suspensions were diluted 1:5 with harvest solution and aliquots (0.01 mL) of each dilution and BSA standard were pipetted into 96-well dishes (Costar, Corning Incorporated, Corning, NY) and combined with assay reagent. Data were expressed as nmol Zn/mg protein to normalize data between samples.

Transport experiments. Test solutions were prepared as described above, however phenol red (500 μ M) was added to the uptake buffer to facilitate a measure of monolayer integrity. Phenol red is not metabolized or transported by Caco-2 cells, thus appearance of phenol red in the basolateral buffer would be indicative of either paracellular transport or compromised monolayer integrity. A preliminary experiment determined that basal Dulbecco's modified minimal essential medium (**bDMEM**; pH 7.0, containing 44 mM NaHCO₃ and 15 mM HEPES) and cDMEM maintained monolayer integrity more effectively than uptake buffer during a 120 min incubation at 37°C. Since cDMEM contains several potential Zn ligands (FCS and glutamine), bDMEM was selected as the basolateral media and BSA (2.5 mg/mL) was added to provide a single ligand for Zn transported across the basolateral membrane. Previous research has demonstrated that apical-to-basolateral Zn transport was markedly increased by the addition of BSA to the basolateral media up to 2.5 mg/mL (Fleet et al., 1993). Spent medium was aspirated and each insert and well was washed twice with uptake buffer (1 or 2 mL, respectively) at 37°C. Test solution was added to each insert (1 mL) and bDMEM + BSA was added to each well (2 mL). After incubation, inserts were removed and placed into new 6-well cluster dishes containing 2 mL ice-cold wash solution per well. Aliquots of each basolateral buffer were transferred to 5-mL polypropylene tubes for gamma counting (1 mL) and to 96-well dishes for determination of phenol red content (150 μ L). Phenol red content of basolateral buffers was determined colorimetrically after the addition of 20 μ L 1 M NaOH. Collection and gamma counting of cellular material and determination of total protein were determined as described above.

Solubility experiments. Test solutions were prepared fresh prior to each experiment by adding an aliquot of ZnSO₄ or ZnProt stock solution and ⁶⁵Zn to a volume of uptake buffer to provide appropriate Zn concentrations and 9.25 kBq ⁶⁵Zn/mL. To determine the effect of dietary inhibitors on Zn solubility, stock solutions of IP₆ and Ca were added to the test solutions to provide 200 μM IP₆ and 200 μM Ca and test solutions were allowed to equilibrate for 15 min. Test solutions were then vortexed and an aliquot was transferred to 5-mL polyethylene tubes for gamma counting. Test solutions were then allowed to equilibrate at room temperature for 1 hr and centrifuged at 8,160 × g for 5 min (5415C, Eppendorf Scientific Incorporated, Westbury, NY). Following centrifugation, a second aliquot was transferred to 5-mL polyethylene tubes for gamma counting. Solubility was determined from the specific radioactivity of aliquots collected before and after centrifugation.

Simulated ruminal digestion. Buffered mineral medium (McDougall, 1948) was prepared fresh prior to the experiment. Rumen fluid was collected from fistulated steers and strained through 8 layers of cheesecloth into a pre-warmed (39°C), insulated container for transport to the laboratory. Upon arrival, pH was measured and rumen fluid was strained again through 8 layers of cheesecloth. Rumen inoculum and mineral medium were combined in a 2:1 ratio and anaerobic conditions were maintained by bubbling CO₂ through the mixture. Urea was added to provide a final concentration of 0.05% and the solution was mixed well under CO₂. Aliquots (10 mL) were transferred into 50 mL centrifuge tubes containing 0.118 g substrate (74% corn, 11% soybean meal, 10% oat straw, and 5% CaCl₂·2H₂O). Zinc sulfate or ZnProt and ⁶⁵Zn were combined prior to the experiment as described above and added to provide either 10 or 200 μM Zn

and 18.5 kBq/mL in the final intestinal digestion (only 8 mL from each ruminal digestion solution was required for each intestinal digestion solution). Tubes were mixed gently, blanketed in CO₂, and stoppers with one-way valves were installed to preserve an anaerobic environment. Tubes were then incubated in a waterbath for 18 h at 39°C, and were mixed gently periodically during the incubation. After incubation, digestion mixtures were immediately transferred to 15-mL tubes (Sarstedt, Newton, NC) and used in a simulated intestinal digestion procedure described below.

Simulated intestinal digestion. Two grams of substrate (85% corn, 10% soybean meal, and 5% CaCl₂·2H₂O) were diluted in 160 mL of saline (120 mM NaCl and 150 μM butylated hydroxytoluene) and then homogenized for 3 min at 80 rpm (Tissumizer, Tekmar Company, Cincinnati, OH). The pH of the solution was lowered to 2.1 using 1 M HCl, and porcine pepsin was added to provide a final concentration of 2 mg/mL. The solution was homogenized for 3 min at 80 rpm and aliquots (8 mL) were transferred into 15 mL-tubes (Sarstedt, Newton, NC). Zinc sulfate or ZnProt and ⁶⁵Zn were combined prior to the experiment as described above and added to provide either 10 or 200 μM Zn and 18.5 kBq/mL in the final digestion volume (10 mL). Digestion solutions from simulated ruminal digestions entered the intestinal phase of the digestion process with the addition of porcine pepsin and reduction in pH. From that point all tubes, regardless of origin (ruminal or intestinal) were treated in an identical manner. Three replicate digestion tubes were maintained for each treatment combination. Solubility of endogenous Zn and the contribution of added ZnSO₄ and ZnProt to soluble Zn concentration were determined in parallel digestion tubes. Endogenous Zn was determined in triplicate digestion tubes containing the meal alone with no added Zn. The

contribution of added Zn was determined by adding either 10 or 200 μM Zn from each source to triplicate digestion tubes without ^{65}Zn . Tubes were wrapped in laboratory film (Parafilm, American National Can, Chicago, IL) and incubated in a shaking water bath (95 rpm; YB-531, American Scientific) for 60 min at 37°C. After incubation, the pH was raised to 6.0 with 1 M NaHCO_3 , and bile extract, pancreatin and pancreatic lipase (all of porcine origin) were added to each tube to provide final concentrations of 2.4, 0.4, and 0.2 mg/mL, respectively. The pH was then adjusted to 6.9 with 1 M NaHCO_3 and the final volume was brought to 10 mL with saline. Tubes were blanketed with nitrogen gas, wrapped in laboratory film and incubated in a shaking water bath (95 rpm) for 120 min at 37°C.

Following the final incubation, aliquots (4.5 mL each) from each digestion were transferred into ultracentrifuge tubes (Beckman Quick-Seal, Beckman Coulter Incorporated, Fullerton, CA). Tubes were then capped and centrifuged at $142,646 \times g$ for 40 min at 4°C (Beckman L7-65, 50.3 Ti rotor, Beckman Coulter Incorporated, Fullerton, CA). Aqueous fractions were collected by puncturing the side of the tubes with 18 ga needles (Precision Glide, Becton Dickinson and Company, Franklin Lakes, NJ) attached to 10 mL syringes (Becton Dickinson and Company, Franklin Lakes, NJ) and filtered through syringe filters (0.2 μm pores, polysulfone membrane, Acrodisc, Pall Gelman Laboratory, Ann Arbor, MI) into 5-mL polypropylene tubes. Aliquots (0.1 mL) of each test solution prior to and after ultracentrifugation were transferred into 5-mL polypropylene tubes for quantification of ^{65}Zn . Solubility of Zn in samples containing ^{65}Zn was determined from the specific radioactivity of each sample prior to and after ultracentrifugation. Solubility of endogenous Zn and of Zn contributed by ZnSO_4 and

ZnProt was assessed by determining the Zn concentrations in non-radioactive digestion solutions prior to and after ultracentrifugation. Zinc concentrations were determined by flame atomic absorption spectrophotometry (AA-6701F, Shimadzu, Kyoto, Japan). Remaining aqueous fractions were pooled among replicate digestions from each concentration \times source combination. Pooled aqueous fractions were used as is for each experiment, and were placed directly onto cell monolayers.

Statistical analysis. All variables were tested in at least triplicate and each experiment was repeated twice. Results are presented as pooled means between replicates. Time-dependent data were analyzed by ANOVA using the Mixed procedure of SAS (Version 6.12, SAS Institute Inc., Cary, NC) as described by Littell et al. (1998). The model for time-dependent data included time, Zn source and time \times Zn source interaction. Remaining data were analyzed by ANOVA using the General Linear Models procedure of SAS. The models included Zn source, Zn concentration, and Zn source \times Zn concentration interaction. All data are presented as means \pm SE, and significance was declared at $P < 0.05$.

Results

Uptake Experiments. The effect of time on zinc uptake was examined by incubating cultures with uptake buffer containing 20 μ M Zn from each source while increasing incubation times from 5 to 120 min. Incremental increases in incubation time resulted in corresponding increases ($P < 0.01$) in Zn uptake (Figure 1). To determine the effect of Zn concentration on Zn uptake, cultures were incubated for 60 min at 37°C with uptake buffer containing incremental increases in Zn concentrations from 10 to 200 μ M Zn from each source. Zinc uptake increased ($P < 0.01$) accordingly as Zn concentration

increased from 10 to 150 μM Zn; however, increasing the Zn concentration to 200 μM failed to further increase uptake (Figure 2). Time- and concentration-dependent uptake were unaffected by Zn source.

Since interactions with IP_6 and Ca are known to decrease Zn bioavailability, solubility and uptake of Zn from both sources at concentrations ranging from 10 to 200 μM Zn were examined in the presence of 200 μM IP_6 and 200 μM Ca. Cultures were incubated for 60 min at 37°C. Uptake was affected by a concentration \times IP_6 interaction ($P < 0.01$), but was not affected by Zn source (Figure 3). In the absence of IP_6 and Ca, Zn uptake increased with increasing Zn concentration. However, when IP_6 and Ca were present, uptake increased with increasing Zn concentration up to 20 μM , but remained constant as Zn concentration increased further. Zinc solubility was affected by a concentration \times IP_6 interaction ($P < 0.01$; Figure 4). In the absence of antagonists, Zn solubility was nearly 100% from both Zn sources; however, when antagonists were added, Zn solubility declined as Zn concentration increased.

Complete diets contain several ligands that are potentially beneficial or detrimental to Zn absorption. Solubility and uptake of Zn from each source were evaluated following simulated ruminal and intestinal digestion processes in series. Digestion solutions contained either 10 or 200 μM Zn. Cultures were incubated with the aqueous fractions of each digestion solution for 60 min at 37°C. Zinc uptake was 11-fold greater ($P < 0.01$) from supernatant fractions digested with 200 μM Zn relative to 10 μM Zn, but was unaffected by Zn source (Figure 5). Zinc solubility following simulated ruminal and intestinal digestion was not affected by Zn source or concentration.

Zinc uptake following intestinal digestion alone was affected by a source \times concentration interaction ($P < 0.04$; Figure 6). Uptake from aqueous fractions of digestions containing 10 μM Zn was not affected by source; however, uptake was greater from aqueous fractions from digestions containing 200 μM ZnProt than those containing 200 μM ZnSO₄. Solubility of Zn from both sources declined ($P < 0.05$) from 44.0 to 22.7% as the concentration of Zn added to digestion solutions increased from 10 to 200 μM .

Transport experiments. Preliminary experiments determined that minimal transport of Zn into the basolateral buffer occurred prior to 30 min. Thus, the effect of increasing incubation times from 30 to 120 min was investigated with 20 μM Zn in the uptake buffer. Uptake and transport of Zn increased with increasing incubation time, but were not influenced by Zn source. Uptake of Zn increased nearly two-fold between 30 to 60 min and again between 90 and 120 min; however, Zn uptake did not increase between 60 and 90 min of incubation (Figure 7). Increasing incubation times from 30 to 60 min and from 60 to 90 min resulted in two-fold increases in Zinc transport. Further increasing incubation time to 120 min resulted in a three-fold increase in Zn transport over that at 90 min.

Effects of increasing Zn concentration in the uptake buffer from 10 to 200 μM Zn on uptake into and transport across cell monolayers were evaluated by incubating cultures for 120 min at 37°C. Zinc uptake and transport were greater ($P < 0.01$) when cultures were incubated with 200 μM Zn than when incubated with 10 μM Zn (Figure 8), however, uptake and transport were not influenced by Zn source.

Zinc bioavailability in the presence of dietary inhibitors was examined by adding 200 μM IP₆ and Ca to the uptake buffer and measuring uptake into and transport of Zn across monolayers following incubation at 37°C for 120 min. In the presence of IP₆ and Ca, Zn uptake was affected by a concentration \times IP₆ interaction ($P < 0.05$; Figure 9). In the absence of IP₆ and Ca, Zn uptake increased as Zn concentration of the uptake buffer increased; however, following addition of the inhibitors, Zn uptake was greater with 10 μM Zn in the uptake buffer than with 200 μM . Zinc transport was also influenced by a concentration \times IP₆ interaction ($P < 0.01$; Figure 10). In the absence of inhibitors, Zn transport at 10 μM Zn was 41.6% of transport at 200 μM Zn. The amount of IP₆ and Ca added nearly completely attenuated Zn transport at both concentrations. Neither uptake nor transport of Zn in the presence of IP₆ and Ca were affected by Zn source.

Bioavailability of Zn was also evaluated following simulated ruminal and intestinal digestions. Uptake of Zn from supernatant fractions following ruminal and intestinal digestion was affected by a source \times concentration interaction ($P < 0.03$; Figure 11). When 10 μM Zn was added to the digestion, subsequent uptake was not different between Zn sources; however, when 200 μM Zn was added, Zn uptake from ZnProt was 65% greater than from ZnSO₄. Zinc transport was 25-fold greater ($P < 0.05$) from digestions containing 200 μM added Zn compared to 10 μM , but was not influenced by Zn source during the 120 min incubation.

Zinc uptake and transport were greater ($P < 0.01$; 16-fold and 12-fold, respectively) from aqueous fractions of digestions containing 200 μM added Zn relative to those containing 10 μM (Figure 12). However, neither uptake nor transport was

affected by Zn source. Uptake of Zn from the aqueous fractions of digestions containing 200 μM added Zn was numerically greater ($P < 0.18$) from ZnProt than from ZnSO_4 .

Discussion

Uptake and transport of Zn by Caco-2 cell monolayers increased as incubation time and Zn concentration in the uptake buffer increased. These observations agree with those of Raffaniello et al. (1992) and Finley et al. (1995) who observed linear increases in Zn uptake during incubations of 50 min and 25 h, respectively. The lack of a linear response in Zn uptake from 60 to 90 min by monolayers grown on inserts and subsequent increases in both Zn uptake and transport may indicate that transport of Zn from the cytosol to the basolateral buffer may be necessary to allow for continued Zn uptake from the apical compartment. Zinc transport responded to increasing incubation time in a similar manner to that reported by Raffaniello et al. (1992). Transport increased at an increasing rate as incubation times increased, suggesting a lag in Zn transport following initial Zn uptake into the cell. Binding of Zn to one of several potential intracellular ligands likely accounts for the lag in Zn transport. Previously, Fleet et al. (1993) observed a curvilinear response in Zn transport as Zn concentration of the uptake buffer increased from 10 to 1000 μM , suggesting the presence of both saturable and non-saturable transport mechanisms. The saturable component was calculated to have a K_t of 226 μM . Two distinct components of Zn absorption have also been identified in rat brush border membrane vesicles (**BBMV**) and isolated doubly perfused rat intestine. One component exhibits saturation kinetics with a K_m of 70-350 μM in BBMV and 30-55 μM in perfused rat intestine, while the second responds linearly to Zn concentrations up to 2 mM Zn (Chesters, 1999). Only two Zn concentrations were used in the current transport

experiments, 10 and 200 μM , precluding examination of transport kinetics; however, based on the data of Fleet et al. (1993) saturation of any potential transport mechanism was unlikely at the highest concentration used in the present study.

The lack of differences in Zn uptake and transport in time- and concentration-dependent experiments with respect to Zn source is inconsistent with the observations of Matsui and Yano (1998). Using Caco-2 cells as a model, Matsui and Yano (1998) found that uptake of Zn from inorganic sources was greater than from a Zn amino acid chelate; however, Zn transport into basolateral buffers was greater from Zn amino acid chelate than from inorganic Zn sources. The authors suggested that MT was sequestering Zn from inorganic sources in the enterocyte; whereas, Zn from the Zn chelate was protected from interacting with MT allowing it to traverse the enterocyte more rapidly. Caco-2 cells have been reported to possess MT (Raffaniello and Wapnir, 1991), and MT has been suggested to interfere with intracellular Zn transport (Hempe and Cousins, 1992). Data from the current experiments suggest that, in the absence of dietary inhibitors, Zn from ZnSO_4 and ZnProt are absorbed at a similar rate possibly via similar mechanisms.

In solution, inorganic Zn sources dissociate, thus the potential exists for Zn to be absorbed as is or to bind to one of several potential ligands in the intestinal lumen. Similar absorption characteristics of the Zn sources used herein may be partially explained by dissociation of both Zn sources. Cao et al. (2000) recently used gel filtration chromatography to evaluate the chelation effectiveness of several feed grade sources of Zn, including three different ZnProt compounds. Soluble fractions from each source in three different media (deionized water, and pH 2 and 5 buffers) were analyzed. In pH 2 and 5 buffers, all Zn from organic products eluted in the same range of fractions as Zn

from ZnSO₄, suggesting that none of the material remained chelated in the given conditions. However, when soluble fractions of the Zn sources in deionized water were analyzed, the percent of total Zn that remained chelated ranged from 2.2% to 12.2%. Percentages for three ZnProt compounds ranged from 10.2% to 11.7%. The current experiments were conducted at pH 6.0, thus it is possible that a fraction of the ZnProt was dissociated and as such utilized a similar absorption mechanism as Zn from ZnSO₄. The undissociated fraction of ZnProt may have been absorbed as well, albeit via a distinct mechanism from the ionic Zn mentioned above.

Zinc proteinate may be absorbed intact via peptide transporters. Caco-2 cells have been shown to contain two distinct types of peptide transport proteins that are highly homologous and similar to those found in human and rat small intestine (Erickson et al., 1995). Ashmead et al. (1985) suggested that complexing metal ions with di- and tri-peptides may facilitate uptake of the metal ions into intestinal mucosal cells via peptide transporters. This hypothesis has yet to be examined or proven conclusively. Using pig isolated BBMV, Tacnet et al. (1990) determined that Zn uptake could be facilitated by a Zn receptor (transporter), chelated Zn, or endocytotic co-transport with an anion. Subsequent experiments provided experimental evidence against anion co-transport; however, active transport as a peptide complex using a peptide carrier system remains a possibility (Tacnet et al., 1993). Zinc may also be transported by multi-element transport mechanisms; however, these have not been well defined experimentally (Gunshin et al., 1997; McMahon and Cousins, 1998).

Phytate is known to reduce absorption and utilization of Zn in several monogastric species (Baker and Ammerman, 1995), thus prevention of antagonistic

interactions with phytate would clearly be beneficial. Chelation of Zn with an organic ligand has been hypothesized to reduce or prevent interactions with dietary antagonists. In the current experiment, solubility, uptake and transport of Zn were reduced by addition of 200 μM IP_6 and 200 μM Ca; but were not affected by Zn source. Reduced Zn solubility in the presence of IP_6 and Ca observed in the current experiment agrees with those of Han et al. (1994). Zinc uptake by Caco-2 cells from a buffer containing 10 μM Zn as a Zn:citrate complex (1:2) was 96% attenuated by the addition of 100 μM IP_6 (Han et al., 1994). Reduced Zn bioavailability in the presence of phytate and Ca is likely a consequence of reduced Zn solubility. In animals, molar phytate:Zn ratios above 12 to 15 have been found to be detrimental to Zn status when dietary Zn was near the required level (Baker and Ammerman, 1997). The current experiment included 200 μM IP_6 and 200 μM Ca in uptake buffer containing Zn concentrations ranging from 10 to 200 μM , representing IP_6 :Zn molar ratios ranging from 20:1 to 1:1. Monolayers used by Han et al. (1994) were exposed to uptake buffer containing a 10:1 IP_6 :Zn ratio. Han et al. (1994) reported a 92% reduction in Zn solubility following the addition of a 10-fold molar excess of IP_6 to a Ca-free uptake buffer. In the current experiment, Zn solubility in the presence of both IP_6 and Ca declined from 90% to 10% as Zn concentration in the uptake buffer increased from 10 to 200 μM .

Reduced Zn solubility in the presence of IP_6 and Ca and increasing Zn concentration seems counterintuitive given the depressed Zn bioavailability associated with high phytate:Zn ratios in vivo. Lonnerdahl et al. (1984) suggested that Ca might block the phytate binding sites, leaving Zn available for absorption. Given this suggestion and the 1:1 phytate:Ca molar ratio used in the current experiment, it is reasonable to

assume that competition for binding sites prevents Zn from associating with phytate at low Zn concentrations, allowing greater Zn solubility. As Zn concentration increases, Zn would become increasingly competitive for binding sites, thereby reducing Zn solubility. Lack of differences in Zn solubility, between sources suggests that either both sources dissociated in the given conditions or that the amount of IP₆ and Ca added was simply too overwhelming for the concentration of Zn present, resulting in a significant reduction in Zn solubility from both Zn sources. Reduced uptake and transport of Zn are likely a result of depressed Zn solubility. In contrast to the current data, Hansen et al. (1996) observed a beneficial response in Zn binding and uptake by Caco-2 cells following the addition of casein phosphopeptides. The authors reported a 79% reduction in Zn binding and uptake by Caco-2 cells from solutions containing 4.6 μM Zn and 415 μM phytate. Addition of 14 μM casein phosphopeptides increased uptake to 94%, whereas 36 and 72 μM casein phosphopeptides depressed zinc binding and uptake (75 and 39%, respectively). It is possible that small peptides function as ligands that bind Zn and aid in facilitation of the absorption process when antagonists are present.

As components of the diet are degraded, Zn is presented to enterocytes as smaller Zn-binding ligands, primarily peptides, amino acids, and nucleotides, and perhaps as free Zn (Cousins, 1996). Depending on their chemical nature and complexation constants, organic chelators may affect bioavailability either positively or negatively (Baker and Ammerman, 1997). The effect of dietary ligands, such as citrate, cysteine, EDTA, histidine, and picolinate on Zn absorption has varied depending on experimental conditions (Wapnir, 1989). When 10 μM Zn was added to simulated ruminal and(or) intestinal digestions, Zn uptake and transport were not affected by Zn source; however,

when 200 μM Zn was added, uptake of Zn from ZnProt was greater than from ZnSO_4 . Solubility of added Zn following ruminal and(or) intestinal digestions was not affected by source. These observations suggest that at high concentrations, and in the presence of dietary antagonists, Zn from organic Zn sources may be taken up more effectively than Zn from inorganic sources. This suggestion is consistent with increased plasma and tissue Zn concentrations observed in growing Holstein bull calves supplemented with 500 mg Zn/kg diet as ZnProt relative to those supplemented with 500 mg Zn/kg diet as ZnSO_4 (Wright and Spears, unpublished data). Since solubility was not different between Zn sources, uptake of all or part of the Zn from ZnProt may have occurred via a mechanism distinct from that used for uptake of inorganic Zn.

The current series of experiments suggest that, in the absence of dietary inhibitors, uptake and transport of Zn from ZnSO_4 and ZnProt by Caco-2 cells were not different. Zinc uptake and transport by Caco-2 cell monolayers increased with increasing incubation time and Zn concentration in the uptake buffer. In the presence of IP_6 and Ca, uptake, transport, and solubility of Zn were reduced, but were unaffected by Zn source. When supplemental Zn was added to ruminal and(or) intestinal digestions at 10 μM , uptake, transport, and solubility of Zn were not affected by Zn source. However, when 200 μM Zn was added to digestion solutions, uptake and transport tended to be greater from ZnProt than from ZnSO_4 . Furthermore, these data suggest that solubility of Zn from ZnSO_4 and ZnProt is similar in the digestion conditions used.

Implications

Commercially available organic Zn sources have been touted as being more bioavailable than inorganic sources. Enhanced absorption has been proposed to be a

primary mechanism by which organic Zn sources elicit responses in growth, reproduction, and health in various species. Results of the current experiment suggest that, following simulated ruminal and(or) intestinal digestion processes, Zn from ZnProt may be more soluble and may be taken up by intestinal mucosa cells to a greater extent than Zn from ZnSO₄. Potential differences in post-absorptive metabolism certainly warrant further investigation in an attempt to explain responses to organic Zn supplementation observed in vivo.

Literature Cited

- Alvarez-Hernandez, X., G. M. Nichols, and J. Glass. 1991. Caco-2 cell line: a system for studying intestinal iron transport across epithelial cell monolayers. *Biochim. Biophys. Acta* 1070:205-208.
- Ammerman, C. B., D. H. Baker, and A. J. Lewis. 1995. Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins. Academic Press, San Diego, CA.
- Ashmead, H. D., D. J. Graff and H. H. Ashmead. 1985. Intestinal absorption of metal ions and chelates. Charles C. Thomas, Springfield, IL.
- Baker, D. H., and C. B. Ammerman. 1995. Zinc bioavailability. In: Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins. pp 367-398. Academic Press, San Diego, CA.
- Cao, J., P. R. Henry, R. Guo, R. A. Holwerda, J. P. Toth, R. C. Littell, R. D. Miles, and C. B. Ammerman. 2000. Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. *J. Anim. Sci.* 78:2039-2054.
- Chesters, J. K. 1997. Zinc. In: B. L. O'Dell and R. A. Sunde (eds.) *Handbook of Nutritionally Essential Trace Elements*. pp 185-230. Marcel Dekker, Inc. New York, NY.
- Cousins, R. J. 1996. Zinc. In: L. J. Filer and E. E. Ziegler (eds.) *Present Knowledge in Nutrition*, 7th Edition. pp 293-306. International Life Science Institute-Nutrition Foundation, Washington, DC.
- Ellwood, K. C., C. Chatzidakis, and M. L. Failla. 1993. Fructose utilization by the human intestinal epithelial cell line, Caco-2. *Proc. Soc. Exp. Biol. Med.* 202:440-446.

- Erickson, R. H., J. R. Gum, Jr., M. M. Lindstrom, D. McKean, and Y. S. Kim. 1995. Regional expression and dietary regulation of rat small intestinal peptide and amino acid transporter mRNAs. *Biochim. Biophys. Res. Comm.* 216:249-257.
- Finley, J. W., M. Briske-Anderson, P. G. Reeves and L. K. Johnson. 1995. Zinc uptake and transcellular movement by Caco-2 cells: studies with media containing fetal bovine serum. *J. Nutr. Biochem.* 6:137-144.
- Finley, J. W., and P. Monroe. 1997. Mn absorption: the use of Caco-2 cells as a model of the intestinal epithelia. *Nutr. Biochem.* 8:92-101.
- Fleet, J. C., A. J. Turnbull, M. Bourcier, and R. J. Wood. 1993. Vitamin D-sensitive and quinacrine-sensitive zinc transport in human intestinal cell line Caco-2. *Am. J. Physiol.* 264:G1037-G1045.
- Giuliano, A. R., R. T. Franceschi, and R. J. Wood. 1991. Characterization of the vitamin D receptor from the Caco-2 human colon carcinoma cell line: effect of cellular differentiation. *Arch. Biochem. Biophys.* 285:261-269.
- Giuliano, A. R., and R. J. Wood. 1991. Vitamin D-regulated calcium transport in Caco-2 cells: unique in vitro model. *Am. J. Physiol.* 260:G207-G212.
- Gunshin, H., B. Mackenzie, U. V. Berger, Y. Gunshin, M. F. Romero, W. F. Boron, S. Nussberger, J. L. Gollan, and M. A. Hediger. 1997. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* 388:482-488.
- Halleux, C., and Y. Schneider. 1991. Iron absorption by intestinal epithelial cells. 1. Caco-2 cultivated in serum-free medium, in polyethyleneterephthalate microporous membrane, as an in vitro model. *In Vitro Cell. & Dev. Biol.* 27:293-302.

- Han, O., M. L. Failla, A. D. Hill, E. R. Morris, and J. C. Smith. 1994. Inositol phosphates inhibit uptake and transport of iron and zinc by a human intestinal cell line. *J. Nutr.* 124:580-587.
- Hansen, M., B. Sandstrom, and B. Lonnerdal. 1996. The effect of casein phosphopeptides on zinc and calcium absorption from high phytate infant diets assessed in rat pups and Caco-2 cells. *Ped. Res.* 40:547-552.
- Hempe, J. W., and R. J. Cousins. 1992. Cysteine-rich intestinal protein and intestinal metallothionein: an inverse relationship as a conceptual model for zinc absorption in rats. *J. Nutr.* 122:89-95.
- Hidalgo, I. J., T. J. Raub, and R. T. Borchart. 1989. Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. *Gastroenterology* 96:736-749.
- Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. *J. Anim. Sci.* 76:1216-1231.
- Lonnerdahl, B., A. Cederblad, L. Davidson, and B. Sandstrom. 1984. The effect of individual components of soy formulas and cow's milk formula on zinc bioavailability. *Am. J. Clin. Nutr.* 40:1064-1070.
- Matsui, T., and H. Yano. 1998. Formulation of low pollution feeds for animal pollution. In: *Proceedings of the 8th World Conference on Animal Production: Symposium Series 1, Seoul, Korea.* pp 110-119.
- McDougall, E. I. 1948. Studies on ruminant saliva. I. The composition and output of sheep saliva. *Biochem. J.* 3:15-18.

- McMahon, R. J., and R. J. Cousins. 1998. Mammalian zinc transporters. *J. Nutr.* 128:667-670.
- Raffaniello, R. D., and R. A. Wapnir. 1991. Zinc-induced metallothionein synthesis by Caco-2 cells. *Biochem. Med. Metab. Biol.* 45:101-107.
- Raffaniello, R. D., S. Lee, S. Teichberg, and R. A. Wapnir. 1992. Distinct mechanisms of zinc uptake at the apical and basolateral membranes of Caco-2 cells. *J. Cell. Physiol.* 152:356-361.
- Reeves, P. G., M. Briske-Anderson, and S. M. Newman, Jr. 1996. High zinc concentrations in culture media affect copper uptake and transport in differentiated human colon adenocarcinoma cells. *J. Nutr.* 126:1701-1712.
- Spears, J. W. 1996. Organic trace minerals in ruminant nutrition. *Anim. Feed Sci. Technol.* 58:151-163.
- Tacnet, F., D. W. Watkins, and P. Ripoche. 1990. Studies of Zn transport into brush-border membrane vesicles isolated from pig small intestine. *Biochim. Biophys. Acta* 1024:323-330.
- Tacnet, F., F. Lauthier, and P. Ripoche. 1993. Mechanisms of zinc transport into pig small intestine brush-border membrane vesicles. *J. Physiol.* 465:57-72.

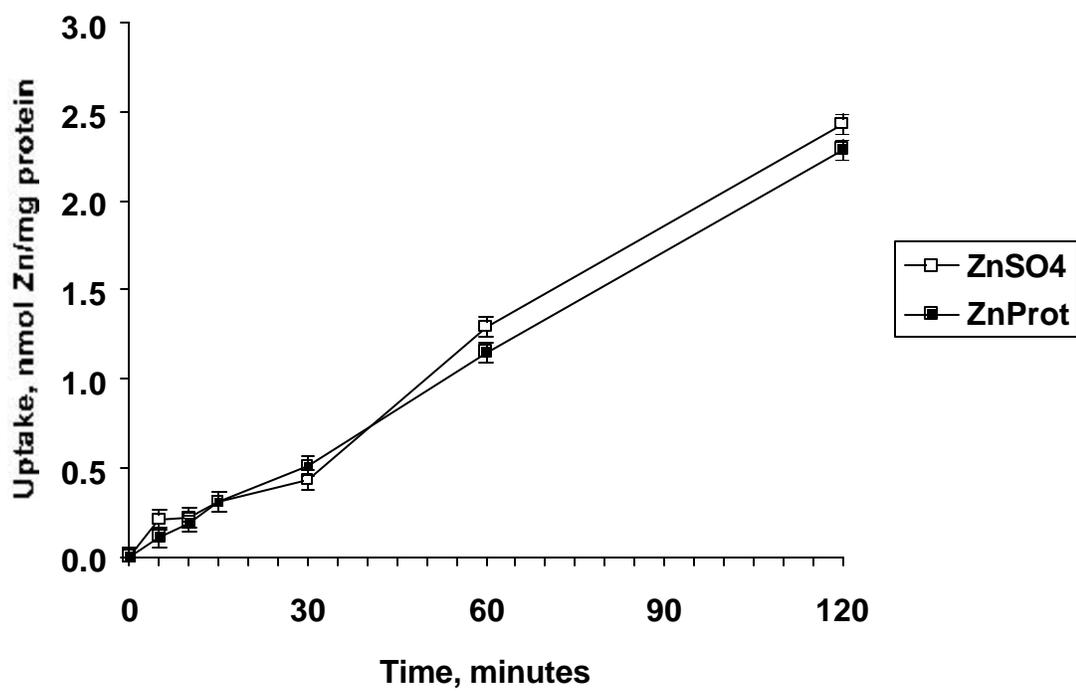


Figure 1. Uptake of Zn from buffer containing 20 μ M Zn as ZnSO₄ or ZnProt incubated for increasing times at 37°C. Time effect ($P < 0.01$).

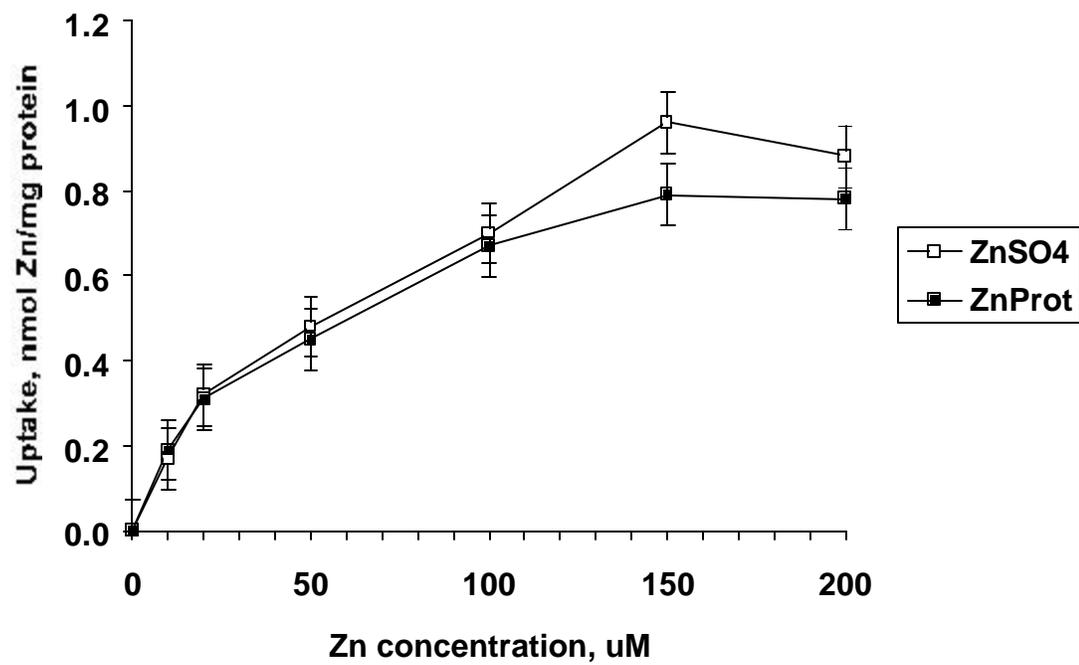


Figure 2. Uptake of Zn from buffer containing increasing Zn concentrations as ZnSO₄ or ZnProt, during a 60 min incubation at 37°C. Concentration effect ($P < 0.01$).

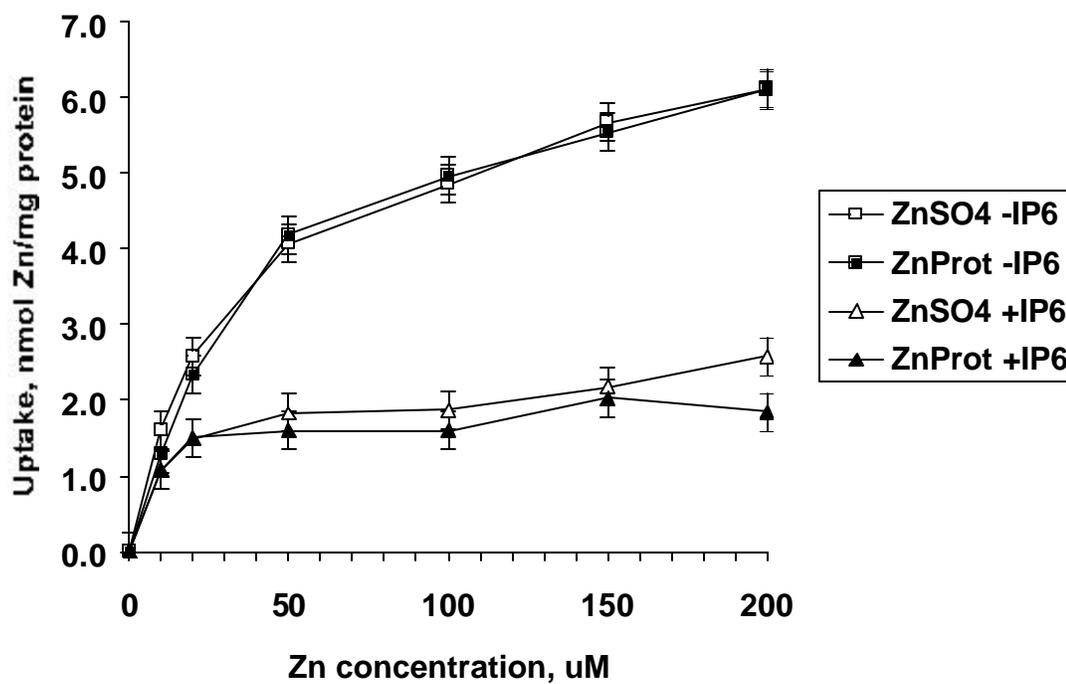


Figure 3. Uptake of Zn from buffers containing increasing concentrations of Zn as ZnSO₄ or ZnProt, in the presence or absence of 200 μM IP₆ and μM Ca. Cells were incubated for 60 min at 37°C. Concentration effect ($P < 0.01$), IP₆ effect ($P < 0.01$), Concentration × IP₆ interaction ($P < 0.01$).

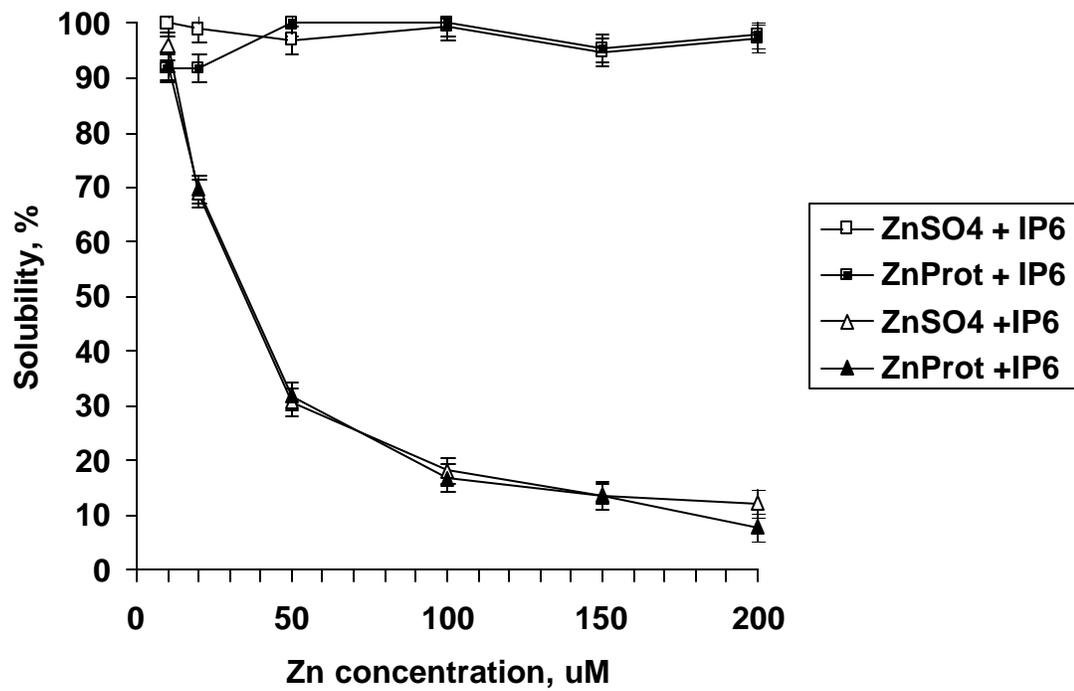


Figure 4. Solubility of increasing concentrations of Zn as ZnSO₄ or ZnProt in the absence or presence of 200 μM IP₆ and μM Ca. Concentration effect ($P < 0.01$), IP₆ effect ($P < 0.01$), Concentration × IP₆ interaction ($P < 0.01$).

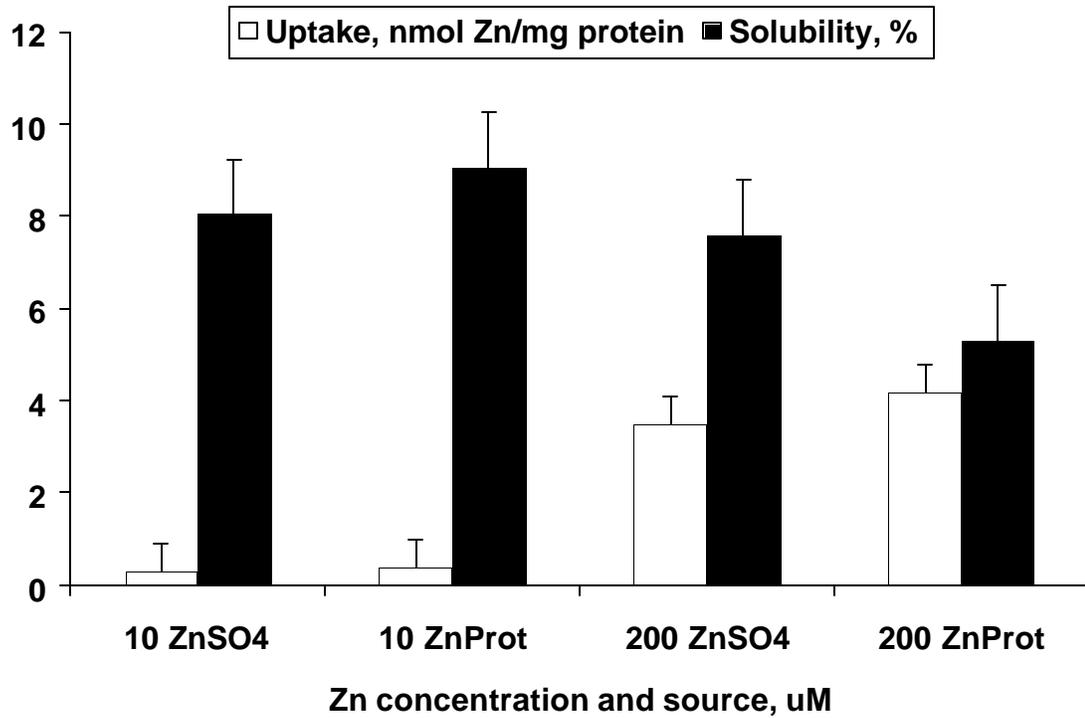


Figure 5. Uptake and solubility of Zn from aqueous fractions of ruminal and intestinal digestions containing 10 or 200 μM added Zn as ZnSO₄ or ZnProt. Cells were incubated for 60 min at 37°C. Uptake: Concentration effect ($P < 0.01$).

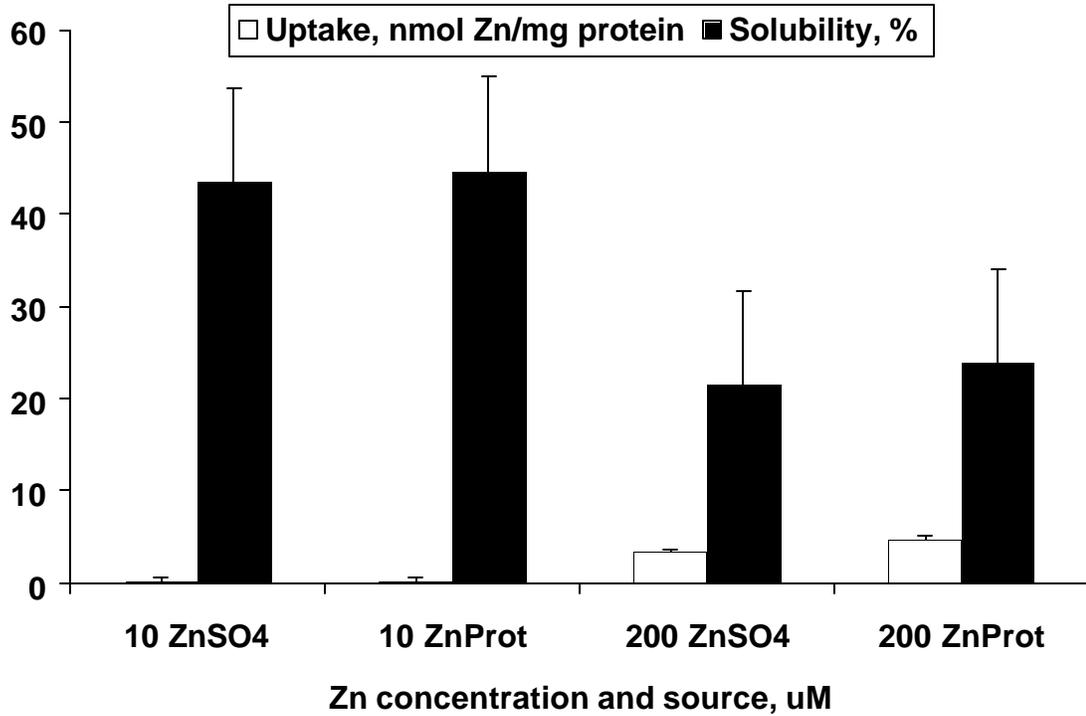


Figure 6. Uptake and solubility of Zn from aqueous fractions of intestinal digestions containing 10 or 200 μM added Zn as ZnSO₄ or ZnProt. Cells were incubated for 60 min at 37°C. Uptake: Concentration effect ($P < 0.01$), Source effect ($P < 0.04$), Concentration \times source interaction ($P < 0.04$). Solubility: Concentration effect ($P < 0.05$).

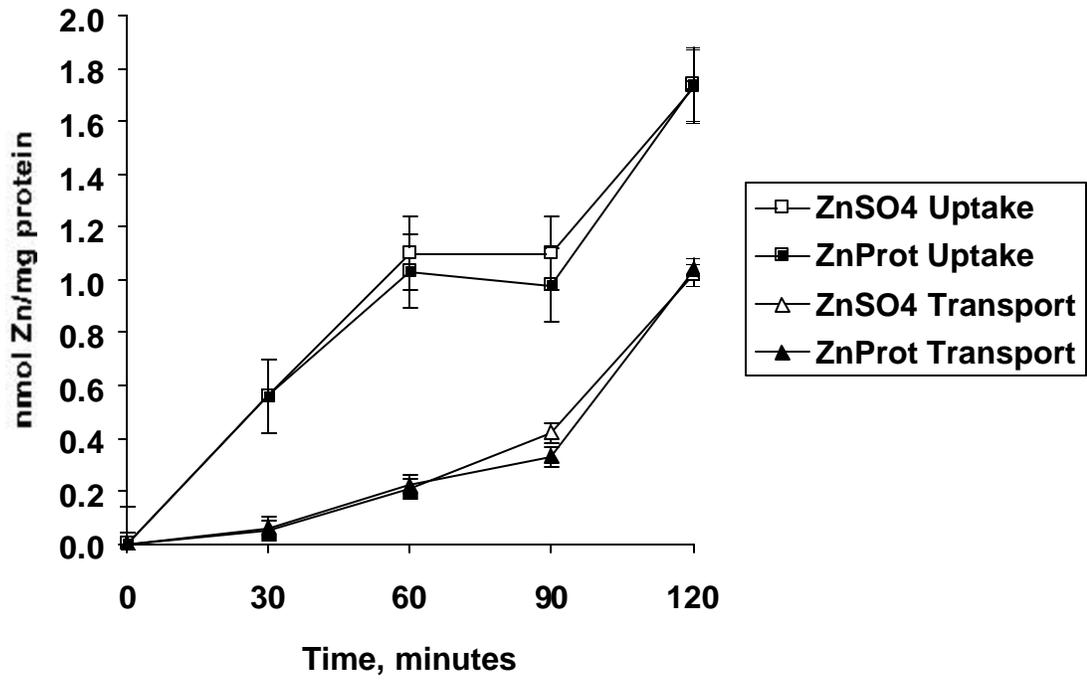


Figure 7. Uptake and transport of Zn from buffer containing 20 μM Zn as ZnSO_4 or ZnProt incubated for increasing times at 37°C. Uptake: Time effect ($P < 0.01$). Transport: Time effect ($P < 0.01$).

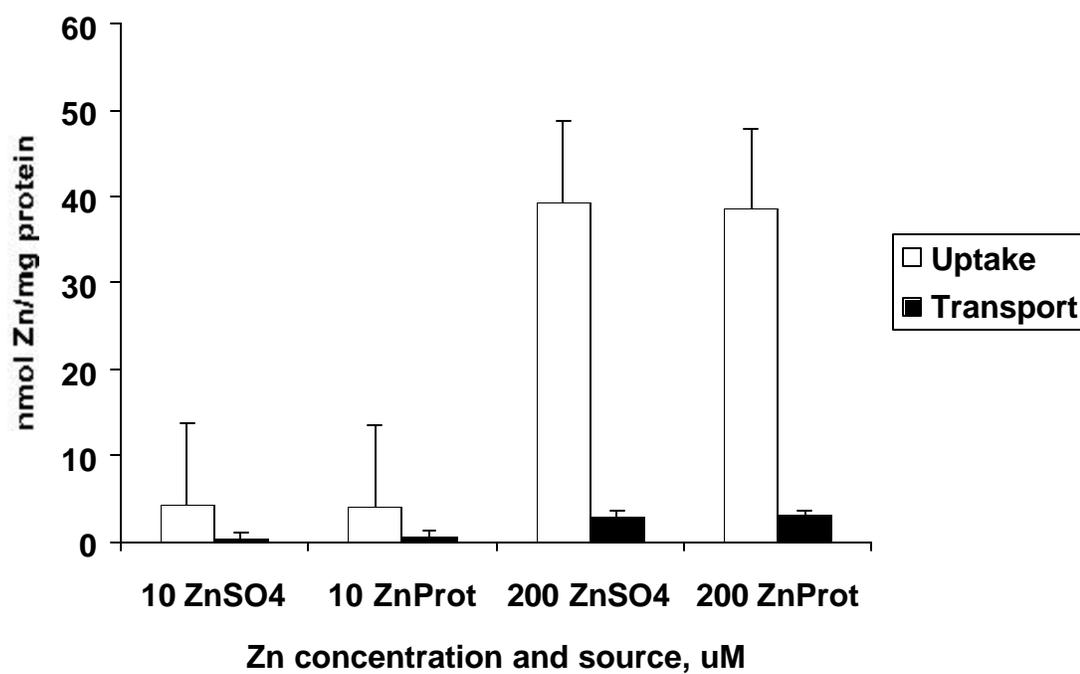


Figure 8. Uptake and transport of Zn from buffer containing increasing Zn concentrations as ZnSO₄ or ZnProt, during a 120 min incubation at 37°C. Uptake: Concentration effect ($P < 0.01$). Transport: Concentration effect ($P < 0.01$).

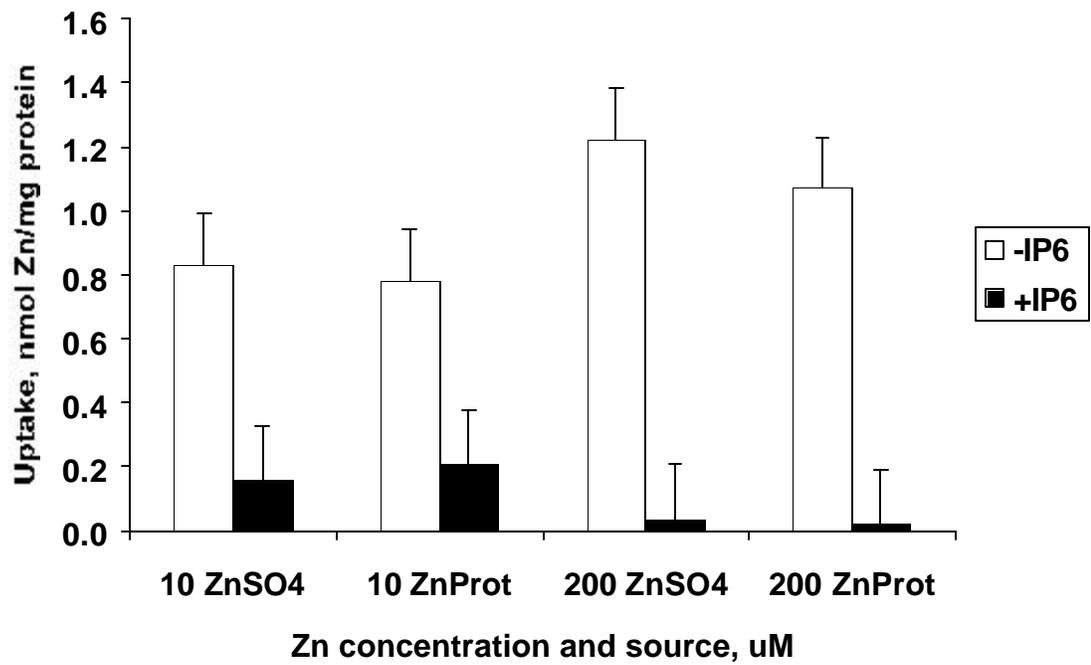


Figure 9. Uptake of Zn from buffers containing 10 or 200 μM Zn as ZnSO₄ or ZnProt, in the presence or absence of 200 μM IP₆ and μM Ca. Cells were incubated for 120 min at 37°C. IP₆ effect ($P < 0.01$), Concentration \times IP₆ interaction ($P < 0.05$).

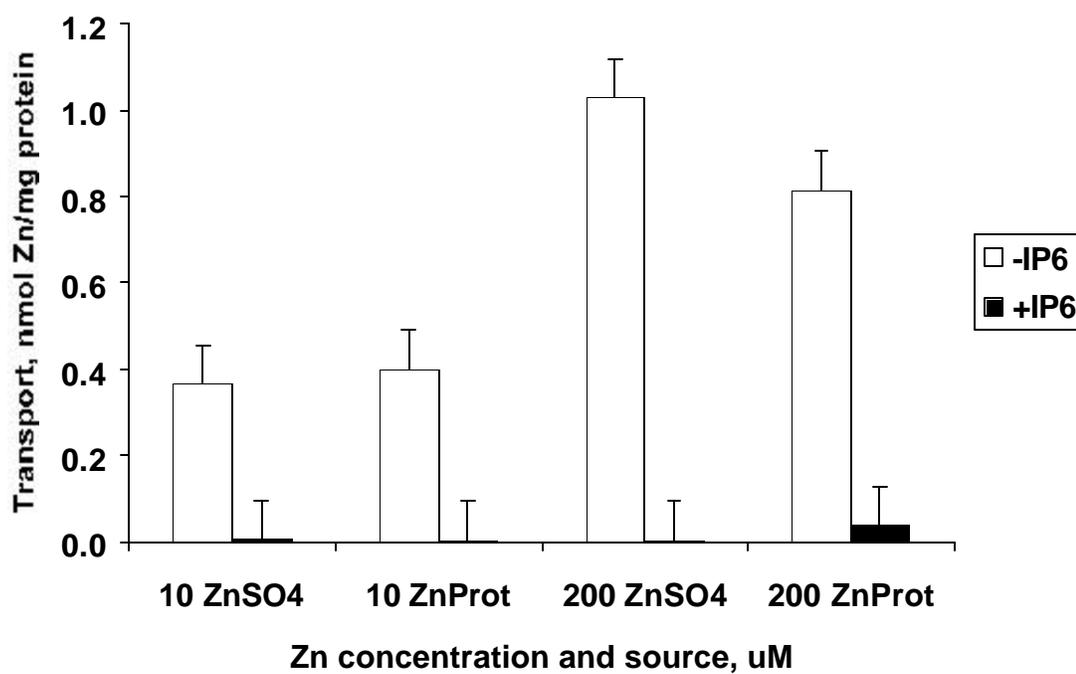


Figure 10. Transport of Zn from buffers containing 10 or 200 μM Zn as ZnSO₄ or ZnProt, in the presence or absence of 200 μM IP₆ and μM Ca. Cells were incubated for 120 min at 37°C. Concentration effect ($P < 0.01$), IP₆ effect ($P < 0.01$), Concentration \times IP₆ interaction ($P < 0.01$).

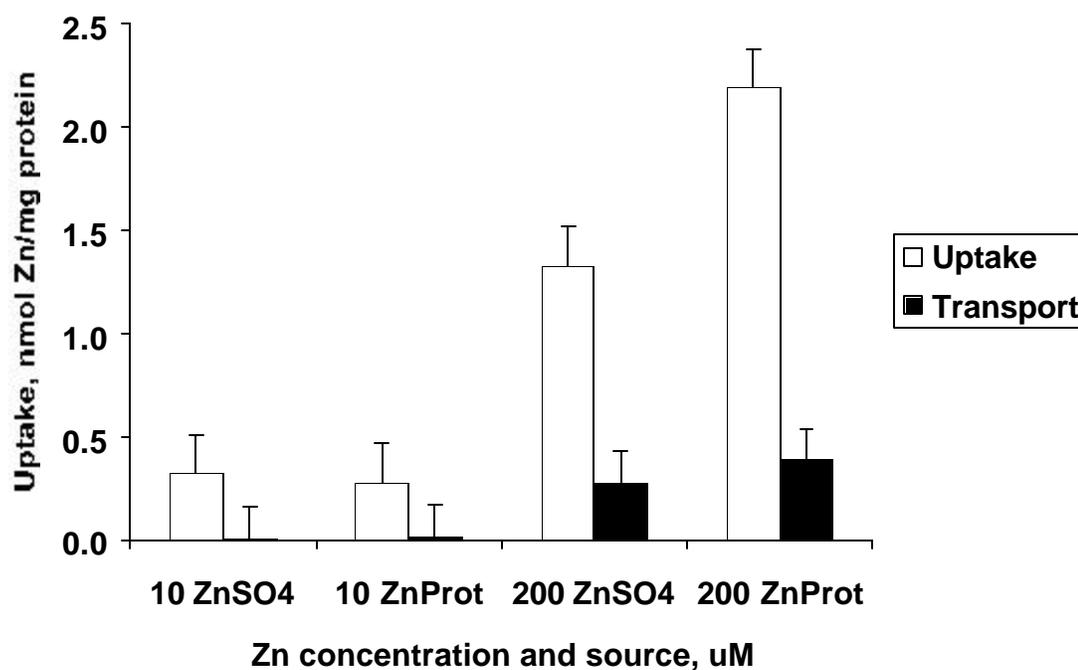


Figure 11. Uptake and transport of Zn from aqueous fractions of ruminal and intestinal digestions containing 10 or 200 μM added Zn as ZnSO_4 or ZnProt . Cells were incubated for 120 min at 37°C . Uptake: Concentration effect ($P < 0.01$), Source effect ($P < 0.05$), Concentration \times source interaction ($P < 0.03$). Transport: Concentration effect ($P < 0.05$).

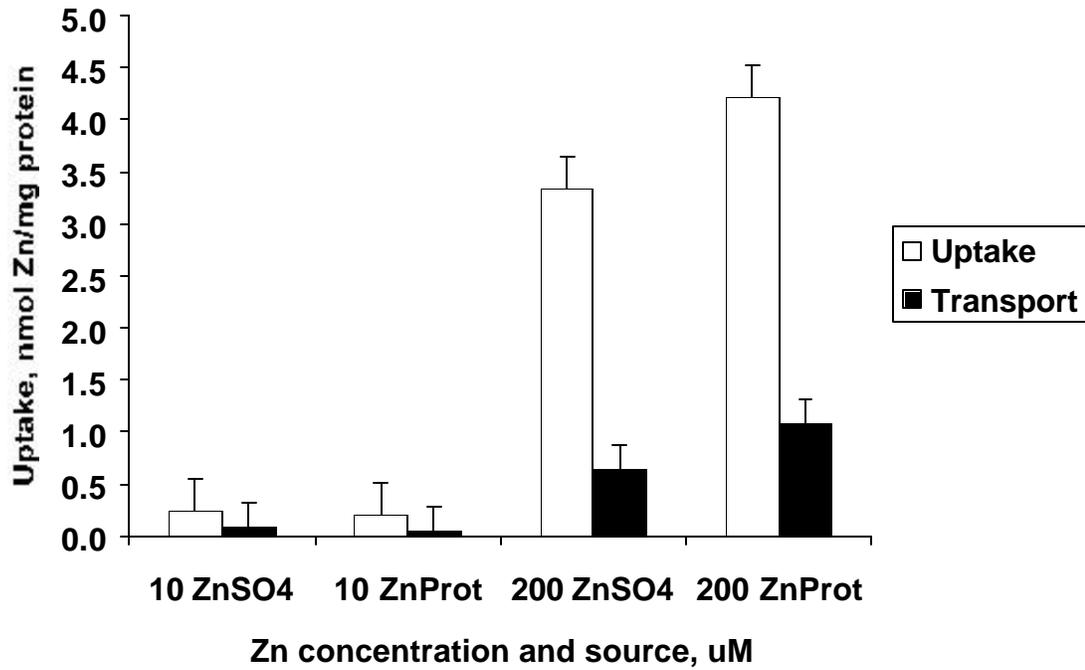


Figure 12. Uptake and transport of Zn from aqueous fractions of intestinal digestions containing 10 or 200 μM added Zn as ZnSO_4 or ZnProt . Cells were incubated for 120 min at 37°C . Uptake: Concentration effect ($P < 0.01$). Transport: Concentration effect ($P < 0.01$).

CHAPTER 5

Uptake and transport of zinc from zinc sulfate and zinc propionate by Caco-2 cells^{1,2}

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ABSTRACT: Experiments were conducted to evaluate effects of time, Zn concentration, inositol hexaphosphate (**IP₆**) addition, and simulated ruminal and intestinal digestion on the uptake and transport of Zn from ZnSO₄ and Zn propionate (**ZnProp**) by Caco-2 cells grown on plastic (uptake only) or on membrane inserts (uptake and transport). Increasing incubation time from 2 to 120 min and Zn concentration from 10 to 200 μM increased ($P < 0.01$) Zn uptake and transport. In the presence of IP₆ and Ca, Zn uptake and transport were affected by a concentration × IP₆ interaction ($P < 0.01$). In the absence of antagonists, uptake and transport increased as Zn concentration increased; however when antagonists were added, uptake and transport were nearly completely attenuated. Zinc uptake by cells grown on inserts tended to be influenced by a source × concentration interaction ($P < 0.07$). At 10 μM Zn, uptake was not different; however, at 200 μM, uptake from ZnSO₄ was greater than from ZnProp. Following simulated ruminal and intestinal digestions, Zn uptake was greater from digestions containing 200 μM added Zn than from those containing 10 μM. In cells grown on plastic, Zn uptake following ruminal and intestinal digestion tended ($P < 0.10$) to be greater from ZnProp than from ZnSO₄. Transport and solubility of Zn following ruminal and intestinal digestion were not affected by Zn source or concentration. Following intestinal digestion alone, Zn uptake by cells grown on inserts tended to be affected by a source × concentration interaction ($P < 0.09$). Zinc uptake from digestions containing 10 μM added Zn was not affected by Zn source; however, when 200 μM Zn was added, uptake was greater from ZnSO₄ than from ZnProp. Zinc uptake by cells grown on plastic and Zn transport by cells grown on inserts were greater ($P < 0.01$) from digestions containing 200 μM added Zn than from those containing 10 μM added Zn, but were not affected by Zn source.

Solubility of Zn following intestinal digestion was affected by a source \times concentration interaction ($P < 0.01$). When 10 μM Zn was added to digestions, Zn from ZnSO_4 was more soluble than from ZnProp; however, when 200 μM Zn was added, Zn from ZnProp was more soluble than from ZnSO_4 . In the absence of inhibitors or in the presence of 200 μM IP_6 and Ca, uptake and transport of Zn from ZnSO_4 and ZnProp were similar; however, following simulated ruminal digestions, Zn solubility and uptake tended to be greater from ZnProp than from ZnSO_4 .

Key Words: zinc, propionate, Caco-2, uptake, transport

Introduction

Bioavailability of Zn from organic and inorganic sources has been the subject of numerous *in vivo* experiments (Spears, 1996). The organic Zn compound Zn propionate (**ZnProp**) is a complex formed by combining 1 mol Zn from a soluble Zn salt with 2 mol propionate. Zinc propionate has been demonstrated to be more bioavailable in dogs (Brinkhaus et al., 1998; Wedekind and Lowry, 1998), and chicks (Kemin Industries, 1995) than inorganic Zn. However, *in vitro*, Zn uptake by monkey kidney fibroblasts (CV-1) and epithelial cells (T-84) was not different between inorganic and organic Zn sources (Buetler et al., 1998). Observed differences have been hypothesized to result from enhanced absorption and(or) solubility of organic relative to inorganic Zn sources. If Zn complexes are degraded or ionized during the digestive process, uptake would likely be similar to that found with inorganic salts. Minimal research to date has specifically addressed absorption characteristics of organic and inorganic Zn sources.

Caco-2, a transformed human colonic cell line, has been used as an *in vitro* model to evaluate nutrient and drug metabolism by intestinal absorptive epithelium (Han et al., 1994). Monolayers of Caco-2 cells spontaneously differentiate into highly polarized cells that possess many of the properties of mature intestinal absorptive epithelial cells (Hidalgo et al., 1989). Characteristics of Ca (Giuliano and Wood, 1991), Cu (Reeves et al., 1996), Fe (Alvarez-Hernandez et al., 1991; Halleux and Schneider, 1991), Mn (Finley and Monroe, 1997) and Zn (Raffaniello and Wapnir, 1991; Raffaniello et al., 1992) uptake and transport have been evaluated using Caco-2 cells.

A series of experiments were conducted to compare the uptake and transport of Zn from ZnSO₄ and ZnProp by Caco-2 cells.

Materials and Methods

Materials. Dulbecco's modified Eagle medium (**DMEM**), fetal calf serum (**FCS**), medium supplements except antibiotics and test reagents were purchased from Sigma Chemical Company (St. Louis, MO). Gentamicin and fungizone were purchased from GIBCO (Grand Island, NY). ⁶⁵Zinc (185 GBq/g) was obtained from New England Nuclear (Boston, MA). All other supplies were purchased from Fisher Scientific Company (Pittsburgh, PA) unless specifically stated otherwise.

Caco-2 cells. The TC-7 clone of the Caco-2 human colonic adenocarcinoma cell line (HTB-37) was generously donated by Dr. Monique Rousset (INSERM, Cedex, France). Cells were grown in 75-cm² plastic flasks (Falcon, Becton Dickinson & Company, Franklin Lakes, NJ) in high glucose complete Dulbecco's modified minimal essential medium (**cdMEM**; pH 7.0) containing DMEM (D7777), heat inactivated fetal calf serum (**FCS**; 10%, v/v), NaHCO₃ (44 mM), HEPES (15 mM), non-essential amino acids (1%), L-glutamine (4 mM), and antibiotics (50 mg gentamicin and 0.5 mg fungizone/L) in an atmosphere of air:CO₂ (95:5) at 37°C and 90% humidity. Stock cultures were seeded at a density of 5,333 cells/cm², and harvested by treatment with Hank's balanced salt solution without Ca or Mg and containing trypsin (0.25%) and EDTA (2 mM) for re-seeding when the monolayer was 70-80% confluent. Suspended cells were transferred either to 12-well cluster dishes (Falcon, Becton Dickinson & Company, Franklin Lakes, NJ) to assess uptake alone or track-etched polyethylene terephthalate membrane filters (0.4 µm pore size, Falcon, Becton Dickinson & Company, Franklin Lakes, NJ) in 6-well cluster dishes (Falcon, Becton Dickinson & Company, Lincoln Park, NJ) to examine Zn uptake and transport together. Medium was changed

every second day and the day before cultures were used for experiments. Differentiated cultures were used 10 to 14 d after reaching confluency between passages 79 and 91 (Ellwood et al., 1993).

Uptake experiments. Preliminary experiments were conducted to determine solubility of the Zn sources in the uptake buffer and to determine the optimum pH for uptake and transport. Results indicated no difference in solubility between the Zn sources and that solubility of both sources was nearly 100%. Zinc uptake was maximal at pH 6.0 and declined steadily as pH was either reduced to 5.0 or increased to 7.5. Test solutions were prepared and utilized in an identical manner for uptake experiments alone and uptake and transport experiments together. Solutions were prepared fresh prior to the experiment by adding an aliquot of ZnSO₄ or ZnProp (Kemin Industries, Des Moines, IA) stock solution and ⁶⁵Zn to a volume of uptake buffer (pH 6.0; 130 mM NaCl, 10 mM KCl, 10 mM 2-[N-morpholino]ethanesulfonic acid, 5 mM glucose, and 1 mM MgSO₄) to provide the appropriate Zn concentration(s) and 9.25 kBq ⁶⁵Zn/mL. To determine the effect of dietary inhibitors on Zn uptake, 200 μM IP₆ and 200 μM Ca were added to uptake solutions. Stock solutions of IP₆ (1000 μM) and Ca (1000 μM Ca as CaCl₂·2H₂O) were prepared in uptake buffer, and then appropriate aliquots were added to test solutions following addition of Zn treatments and a 15 min incubation period. Final test solutions were allowed to equilibrate for 15 min at 37°C prior to the experiment. Spent medium was aspirated and each well was washed twice with 1 mL uptake buffer at 37°C. Test solutions (1 mL) were added to each well and cultures were incubated at 37°C for the appropriate time. After incubation, test solutions were removed and each well was washed three times with ice-cold wash solution (pH 7.0; 150 mM NaCl, 10 mM HEPES,

and 5 mM tetrasodium EDTA). Preliminary experiments comparing the effectiveness of multiple washes on removal of non-specifically bound ^{65}Zn determined that more than 3 washes resulted in no further reduction in the ^{65}Zn content of cell suspensions. Cellular material was collected by adding 0.5 mL harvest solution (pH 7.0; 1.7 mM SDS and 1.0 mM tetrasodium EDTA) to each well, scraping the surface with a rubber policeman, and transferring the material to microfuge tubes for solication and storage. The process was then repeated. Cellular material was homogenized by sonication (50 Sonic Dismembrator, Fisher Scientific, Pittsburgh, PA) for 2 to 3 sec. Aliquots of each cell suspension (0.4 mL) and test solution (0.1 mL) were transferred to 5 mL-polypropylene tubes (Sarstedt, Newton, NC) for gamma counting (Cobra II, Packard Instrument Company, Meriden, CT). The quantity of Zn taken up by the cells was determined by first expressing specific radioactivity of the cell suspensions as a percent of the total specific radioactivity in the uptake buffer. Then the percent of the specific radioactivity taken up by the cells was multiplied by the non-radioactive Zn concentration in the uptake buffer. The ratio of radioactive Zn to non-radioactive Zn was assumed to remain constant. Total protein content of each cell suspension was determined by colorimetric assay (Bio-Rad Laboratories Incorporated, Hercules, CA) using a plate reader (PowerWave_X, Bio-Tek Instruments, Winooski, VT). Cell suspensions were diluted 1:5 with harvest solution and aliquots (0.01 mL) of each dilution and BSA standard were pipetted into 96-well dishes (Costar, Corning Incorporated, Corning, NY) and combined with assay reagent. Data were expressed as nmol Zn/mg protein to normalize data among samples.

Transport experiments. Test solutions were prepared as described above, however phenol red (500 μ M) was added to the uptake buffer to facilitate a measure of monolayer integrity. Phenol red is not metabolized or transported by Caco-2 cells, thus appearance of phenol red in the basolateral buffer would be indicative of either paracellular transport or compromised monolayer integrity. A preliminary experiment determined that basal Dulbecco's modified minimal essential medium (**bDMEM**; pH 7.0, containing 44 mM NaHCO₃ and 15 mM HEPES) and cDMEM maintained monolayer integrity more effectively than uptake buffer during a 120 min incubation at 37°C. Since cDMEM contains several potential Zn ligands (FCS and glutamine), bDMEM was selected as the basolateral media and BSA (2.5 mg/mL) was added to provide a single ligand for Zn transported across the basolateral membrane. Previous research has demonstrated that apical-to-basolateral Zn transport was markedly increased by the addition of BSA to the basolateral media up to 2.5 mg/mL (Fleet et al., 1993). Spent medium was aspirated and each insert and well was washed twice with uptake buffer (1 or 2 mL, respectively) at 37°C. Test solution was added to each insert (1 mL) and bDMEM + BSA was added to each well (2 mL). After incubation, inserts were removed and placed into new 6-well cluster dishes containing 2 mL ice-cold wash solution per well. Aliquots of each basolateral buffer were transferred to 5-mL polypropylene tubes for gamma counting (1 mL) and to 96-well dishes for determination of phenol red content (150 μ L). Phenol red content of basolateral buffers was determined colorimetrically after the addition of 20 μ L 1 M NaOH. Collection and gamma counting of cellular material and determination of total protein were determined as described for the uptake experiments.

Solubility experiments. Test solutions were prepared fresh prior to each experiment by adding an aliquot of ZnSO₄ or ZnProp stock solution and ⁶⁵Zn to a volume of uptake buffer to provide appropriate Zn concentrations and 9.25 kBq ⁶⁵Zn/mL. To determine the effect of dietary inhibitors on Zn solubility, stock solutions of IP₆ and Ca were added to the test solutions to provide 200 μM IP₆ and 200 μM Ca and test solutions were allowed to equilibrate for 15 min. Test solutions were then vortexed and an aliquot was transferred to 5-mL polyethylene tubes for gamma counting. Test solutions were then allowed to equilibrate at room temperature for 1 hr and centrifuged at 8,160 × g for 5 min (5415C, Eppendorf Scientific Incorporated, Westbury, NY). Following centrifugation, a second aliquot was transferred to 5-mL polyethylene tubes for gamma counting. Solubility was determined from the specific radioactivity of aliquots collected before and after centrifugation.

Simulated ruminal digestion. Buffered mineral medium (McDougall, 1948) was prepared fresh prior to the experiment. Rumen fluid was collected from fistulated steers and strained through 8 layers of cheesecloth into a pre-warmed (39°C), insulated container for transport to the laboratory. Upon arrival, pH was measured and rumen fluid was strained again through 8 layers of cheesecloth. Rumen inoculum and mineral medium were combined in a 2:1 ratio and anaerobic conditions were maintained by bubbling CO₂ through the mixture. Urea was added to provide a final concentration of 0.05% and the solution was mixed well under CO₂. Aliquots (10 mL) were transferred into 50 mL centrifuge tubes containing 0.118 g substrate (74% corn, 11% soybean meal, 10% oat straw, and 5% CaCl₂·2H₂O). Zinc sulfate or ZnProp and ⁶⁵Zn were combined prior to the experiment as described above and added to provide either 10 or 200 μM Zn

and 18.5 kBq/mL in the final intestinal digestion (only 8 mL from each ruminal digestion solution was required for each intestinal digestion solution). Tubes were mixed gently, blanketed in CO₂, and stoppers with one-way valves were installed to preserve an anaerobic environment. Tubes were then incubated in a waterbath for 18 h at 39°C, and were mixed gently periodically during the incubation. After incubation, digestion mixtures were immediately transferred to 15-mL tubes (Sarstedt, Newton, NC) and used in a simulated intestinal digestion procedure described below.

Simulated intestinal digestion. Two grams of substrate (85% corn, 10% soybean meal, and 5% CaCl₂·2H₂O) were diluted in 160 mL of saline (120 mM NaCl and 150 μM butylated hydroxytoluene) and then homogenized for 3 min at 80 rpm (Tissumizer, Tekmar Company, Cincinnati, OH). The pH of the solution was lowered to 2.1 using 1 M HCl, and porcine pepsin was added to provide a final concentration of 2 mg/mL. The solution was homogenized for 3 min at 80 rpm and aliquots (8 mL) were transferred into 15 mL-tubes (Sarstedt, Newton, NC). Zinc sulfate or ZnProp and ⁶⁵Zn were combined prior to the experiment as described above and added to provide either 10 or 200 μM Zn and 18.5 kBq/mL in the final digestion volume (10 mL). Digestion solutions from simulated ruminal digestions entered the intestinal phase of the digestion process with the addition of porcine pepsin and reduction in pH. From that point all tubes, regardless of origin (ruminal or intestinal) were treated in an identical manner. Three replicate digestion tubes were maintained for each treatment combination. Solubility of endogenous Zn and the contribution of added ZnSO₄ and ZnProp to soluble Zn concentration were determined in parallel digestion tubes. Endogenous Zn was determined in triplicate digestion tubes containing the meal alone with no added Zn. The

contribution of added Zn was determined by adding either 10 or 200 μM Zn from each source to triplicate digestion tubes without ^{65}Zn . Tubes were wrapped in laboratory film (Parafilm, American National Can, Chicago, IL) and incubated in a shaking water bath (95 rpm; YB-531, American Scientific) for 60 min at 37°C. After incubation, the pH was raised to 6.0 with 1 M NaHCO_3 , and bile extract, pancreatin and pancreatic lipase (all of porcine origin) were added to each tube to provide final concentrations of 2.4, 0.4, and 0.2 mg/mL, respectively. The pH was then adjusted to 6.9 with 1 M NaHCO_3 and the final volume was brought to 10 mL with saline. Tubes were blanketed with nitrogen gas, wrapped in laboratory film and incubated in a shaking water bath (95 rpm) for 120 min at 37°C.

Following the final incubation, aliquots (4.5 mL each) from each digestion were transferred into ultracentrifuge tubes (Beckman Quick-Seal, Beckman Coulter Incorporated, Fullerton, CA). Tubes were then capped and centrifuged at $142,646 \times g$ for 40 min at 4°C (Beckman L7-65, 50.3 Ti rotor, Beckman Coulter Incorporated, Fullerton, CA). Aqueous fractions were collected by puncturing the side of the tubes with 18 ga needles (Precision Glide, Becton Dickinson and Company, Franklin Lakes, NJ) attached to 10 mL syringes (Becton Dickinson and Company, Franklin Lakes, NJ) and filtered through syringe filters (0.2 μm pores, polysulfone membrane, Acrodisc, Pall Gelman Laboratory, Ann Arbor, MI) into 5-mL polypropylene tubes. Aliquots (0.1 mL) of each test solution prior to and after ultracentrifugation were transferred into 5-mL polypropylene tubes for quantification of ^{65}Zn . Solubility of Zn in samples containing ^{65}Zn was determined from the specific radioactivity of each sample prior to and after ultracentrifugation. Solubility of endogenous Zn and Zn contributed by added ZnSO_4 and

ZnProp was determined were analyzed by flame atomic absorption spectrophotometry (AA-6701F, Shimadzu, Kyoto, Japan). Remaining aqueous fractions were pooled among replicate digestions from each concentration \times source combination. Pooled aqueous fractions were used as is for each experiment, and were placed directly onto cell monolayers.

Statistical analysis. All variables were tested in at least triplicate and each experiment was repeated twice. Results are presented as pooled means between replicates. Time-dependent data were analyzed by ANOVA using the Mixed procedure of SAS (Version 6.12, SAS Institute Inc., Cary, NC) as described by Littell et al. (1998). The model for time-dependent data included time, Zn source and time \times Zn source interaction. Remaining data were analyzed by ANOVA using the General Linear Models procedure of SAS. The models included Zn source, Zn concentration, and Zn source \times Zn concentration interaction. All data are presented as means \pm SE, and significance was declared at $P < 0.05$.

Results

Uptake experiments. The effect of time on Zn uptake was examined by incubating cultures with uptake buffer containing 20 μM Zn as ZnSO_4 or ZnProp while increasing incubation times from 5 to 120 min. Increasing incubation times resulted in corresponding increases ($P < 0.01$) in Zn uptake (Figure 1). To determine the effect of Zn concentration on Zn uptake, cultures were incubated for 60 min with uptake buffer containing incremental increases in Zn concentration from 10 to 200 μM Zn as ZnSO_4 or ZnProp. Zinc uptake increased ($P < 0.01$) at a decreasing rate as Zn concentration increased from 10 to 50 μM Zn, then as Zn concentration increased from 50 to 200 μM ,

uptake was nearly linear (Figure 2). Zinc uptake in time- and concentration-dependent experiments was not affected by Zn source.

Interactions with IP₆ and Ca are known to decrease Zn bioavailability, thus solubility and uptake of Zn from ZnSO₄ and ZnProp at concentrations ranging from 10 to 200 μM were examined in the presence of 200 μM IP₆ and 200 μM Ca. Zinc uptake was affected by a concentration × IP₆ interaction ($P < 0.01$; Figure 3). In the absence of inhibitors, Zn uptake increased with increasing Zn concentration in the uptake buffer. Addition of IP₆ and Ca reduced uptake at all Zn concentrations; however reductions were more pronounced as Zn concentration increased. Zinc solubility in the presence of IP₆ and Ca was affected by a concentration × IP₆ interaction ($P < 0.01$). In the presence of IP₆ and Ca, Zn solubility diminished as Zn concentration increased (Figure 4); however in the absence of antagonists, Zn solubility was near 100% for both Zn sources. Uptake and solubility of Zn were not different between ZnSO₄ and ZnProp.

Complete diets contain several potential ligands that may positively or negatively influence Zn absorption. Solubility and uptake of Zn from each source were evaluated following ruminal and intestinal digestions in series and intestinal digestion alone with 10 or 200 μM added Zn as ZnSO₄ or ZnProp in the digestion solutions. Following ruminal and intestinal digestion, Zn uptake was 6-fold greater ($P < 0.01$) from digestions containing 200 relative to 10 μM Zn and numerically ($P < 0.10$) was 37.5% greater from digestions containing ZnProp than those containing ZnSO₄ (Figure 5). When exposed to the intestinal digestion alone, uptake was 15-fold greater ($P < 0.01$) from aqueous fractions of digestions containing 200 μM Zn than digestions containing 10 μM added Zn; however, Zn uptake was not affected by source (Figure 6). Zinc solubility was

affected by a concentration \times source interaction ($P < 0.01$; Figure 6). When 10 μM Zn was added to digestion solutions, Zn from ZnSO_4 was 12.9% more soluble than Zn from ZnProp; however when 200 μM Zn was added, Zn from ZnProp was 7.8% more soluble than Zn from ZnSO_4 .

Transport experiments. Preliminary experiments determined that transport of Zn into the basolateral buffer from 20 μM Zn was minimal prior to 30 min. Thus, the effect of incremental increases in incubation time from 30 to 120 min was investigated with 20 μM Zn in the uptake buffer. Main effect means for Zn uptake increased ($P < 0.01$) with incremental increases in incubation time from 30 to 90 min; however, increasing incubation time from 90 to 120 min resulted in no further increase in uptake (Figure 7). Increasing incubation time from 30 to 60 min increased ($P < 0.01$) Zn transport; however, subsequent increases in incubation time did not affect transport. The effect of increasing Zn concentration in the uptake buffer from 10 to 200 μM Zn on uptake into and transport across cell monolayers was evaluated by incubating cultures for 120 min. Zinc uptake and transport were 5- and 16-fold greater ($P < 0.01$), respectively, when cultures were incubated with 200 μM Zn than with 10 μM Zn (Figure 8). Uptake and transport of Zn in time- and concentration-dependent experiments were not significantly affected by Zn source.

Zinc bioavailability in the presence of dietary inhibitors was examined by adding 200 μM IP_6 and Ca to the uptake buffer and measuring uptake into and transport of Zn across monolayers following incubation for 120 min. Uptake (Figure 9) and transport (Figure 10) of Zn were affected by a concentration \times IP_6 interaction ($P < 0.01$). In the absence of inhibitors, Zn uptake and transport were 5- and 19-fold greater with 200 μM

Zn in the uptake buffer than 10 μM Zn. However, in the presence of IP_6 and Ca, uptake and transport of Zn were greatly reduced, but were not affected by Zn concentration. Zinc uptake also tended to be influenced by a source \times concentration ($P < 0.07$) interaction. With 10 μM Zn in the uptake buffer, uptake was not different, however with 200 μM Zn in the uptake buffer, Zn uptake from ZnSO_4 was 11.8% greater than from ZnProp. Zinc transport was not affected by Zn source.

Bioavailability of Zn was estimated following simulated ruminal and intestinal digestions processes. Uptake of Zn from aqueous fractions of digestions containing 200 μM added Zn was 9-fold greater ($P < 0.01$) than those containing 10 μM added Zn, but was not different between ZnSO_4 and ZnProp (Figure 11). Zinc transport following simulated ruminal and intestinal digestions was not affected by Zn source or concentration. Zinc uptake following intestinal digestion alone tended to be influenced by a source \times concentration interaction ($P < 0.09$). When incubated with aqueous fractions from digestion solutions containing 10 μM added Zn, uptake of Zn was not different; however, when 200 μM Zn was added, uptake was 12.5% greater from ZnSO_4 than from ZnProp (Figure 12). Transport of Zn was greater from aqueous fractions of digestions containing 200 μM added Zn than from those containing 10 μM added Zn, but was not affected by Zn source.

Discussion

Zinc uptake and transport by Caco-2 cell monolayers increased as incubation time and Zn concentration in the uptake buffer increased; however, neither was affected by Zn source. Uptake in time-dependent experiments was nearly linear as incubation time increased, while uptake in concentration-dependent experiments displayed characteristics

indicative of both saturable and non-saturable components of Zn absorption. These observations are consistent with those of Raffaniello et al. (1992) and Finley et al. (1995) who observed linear increases in Zn uptake during incubations of 50 min and 25 h, respectively. Zinc transport responded to increasing incubation time in a manner similar to that reported by Raffaniello et al. (1992). Transport increased at an increasing rate as incubation times increased, suggesting a lag in Zn transport with respect to Zn uptake. Binding of Zn to one of several potential intracellular ligands likely accounts for the lag in Zn transport. In contrast, Fleet et al. (1993) observed a curvilinear response in Zn transport as Zn concentration of the uptake buffer increased from 10 to 1000 μM , indicating the presence of both saturable and non-saturable transport mechanisms. The saturable component was calculated to have a K_t of 226 μM . Only two Zn concentrations were used in the current transport experiments, 10 and 200 μM , precluding examination of transport kinetics; however, based on the data of Fleet et al. (1993) saturation of any potential transport mechanism was unlikely. Lack of differences in uptake with respect to Zn source is consistent with the findings of Buetler et al. (1998). The authors observed no difference in uptake of ^{65}Zn by cultured monkey kidney fibroblasts (CV-1) or intestinal epithelial cells (T-84) when supplied as ZnCl_2 , Zn methionine, or ZnProp. Furthermore, using gel filtration chromatography, Buetler et al. (1998) reported similar elution ^{65}Zn peaks for ZnCl_2 , reagent Zn methionine and ZnProp, suggesting that if indeed complexes were formed, they readily dissociate in solution.

Phytate is a common component of plant seeds and is particularly high in oilseeds and cereal grains (Baker and Ammerman, 1995). The effect of IP_6 and Ca on Zn solubility and bioavailability has been investigated extensively. Reduced absorption of

Zn in the presence of IP₆ has been demonstrated in several monogastric species (Baker and Ammerman, 1995), and is generally accepted as the mechanism by which Zn bioavailability is attenuated. In the current experiments, Zn solubility the presence of IP₆ and Ca declined from 70% to 5% as Zn concentration in the uptake buffer increased incrementally from 10 to 200 μM. This observation agrees with Han et al. (1994) who reported a 92% reduction in Zn solubility following the addition of a 10-fold molar excess of IP₆ to a Ca-free uptake buffer. The current experiment included 200 μM IP₆ and 200 μM Ca representing IP₆:Zn molar ratios ranging from 20:1 to 1:1. In vivo, molar phytate:Zn ratios above 12 to 15 are considered detrimental to Zn status when Zn is near the required level (Baker and Ammerman, 1997). Depressed Zn bioavailability observed in the presence of phytate and Ca, and the associated reduction in solubility proposed to be responsible, makes the results of the current experiments intriguing. Lonnerdahl et al. (1984) suggested that Ca might block the phytate binding sites, leaving Zn available for absorption. Given this suggestion and the 1:1 phytate:Ca molar ratio used in the current experiment, it is reasonable to assume that competition for binding sites prevents Zn from associating with phytate at low Zn concentrations, allowing greater Zn solubility. As Zn concentration increases, Zn would become increasingly competitive for binding sites, thereby reducing Zn solubility. Zinc uptake by monolayers grown on inserts in the presence of IP₆ and Ca was greater from ZnSO₄ than from ZnProp, at 200 but not 10 μM added Zn. Since Zn solubility in the presence of IP₆ and Ca was not influenced by Zn source and Zn uptake in the absence of inhibitors was similar between sources, the reason for differences in Zn uptake is unclear. It is possible that a portion of the Zn from ZnProp bound to IP₆ and Ca remained soluble, but was unavailable for uptake.

As components of the diet are degraded, Zn is presented to enterocytes as smaller Zn-binding ligands, primarily peptides, amino acids, and nucleotides, and perhaps as free Zn (Cousins, 1995). Depending on their chemical nature and complexation constants, organic chelators may affect bioavailability either positively or negatively (Baker and Ammerman, 1997). The effect of dietary ligands, such as citrate, cysteine, EDTA, histidine, and picolinate on Zn absorption has varied depending on experimental conditions (Wapnir, 1989). Uptake of Zn by cells grown on plastic following ruminal and intestinal digestions was greater from digestions containing ZnProp than from those containing ZnSO₄. Zinc solubility following ruminal and intestinal digestion was not statistically different between Zn sources; however, numerically Zn from ZnProp appeared to be more soluble than Zn from ZnSO₄. This small difference in solubility may account for greater uptake of Zn from digestions containing ZnProp. Zinc uptake and transport by cells grown on inserts was not affected by Zn source. Since identical digestion processes and substrates were used in both systems, differences in Zn uptake with respect to Zn source are difficult to explain. When uptake was assessed alone, cells were incubated for 60 min as compared to 120 min for uptake and transport experiments. It is possible that Zn from ZnProp is absorbed more rapidly than Zn from ZnSO₄, then, as incubation time increases, differences in absorption become insignificant. Fundamental differences in the cell systems used (i.e. cells grown on plastic for uptake and on semi-permeable membranes for uptake and transport experiments) may also account for observed differences. Following intestinal digestion alone, Zn uptake by cells grown on plastic was not affected by Zn source; however, Zn uptake by cells grown on inserts tended to be greater from ZnSO₄ than from ZnProp. Transport of Zn was not significantly

affected by Zn source; however, numerically, Zn transport appeared to be greater from ZnProp. Zinc solubility at 10 μ M added Zn was greater from ZnSO₄ than from ZnProp; however, when 200 μ M Zn was added, ZnProp was more soluble. Tendencies for lower uptake and greater transport of Zn from ZnProp than from ZnSO₄ suggests that different uptake and transport mechanisms may exist. Given the greater solubility of Zn from ZnProp at high Zn concentrations, it is possible that all or part of the Zn from ZnProp is translocated via paracellular transport or simple diffusion. As described above, differences in cell systems and incubation times may also partially explain observed differences in uptake and transport of Zn following intestinal digestion. In vivo observations suggest that ZnProp may be more bioavailable than ZnO in dogs (Brinkhaus et al., 1998; Wedekind and Lowry, 1998) and ZnSO₄ in chicks (Kemin Industries, 1995). Numerous physiological and dietary factors in vivo are likely responsible for the differences between in vitro and in vivo models.

These data suggest that increasing incubation time and Zn concentration increase Zn uptake and transport by Caco-2 cells. Solubility, uptake and transport are clearly reduced by the addition of IP₆ and Ca, and depression in Zn solubility is more severe as Zn concentration increases. In the absence of antagonists or in the presence of a limited number of antagonists at high concentration, Zn source appears to have a minor effect on solubility, uptake and transport. However, following simulated ruminal and(or) intestinal digestion processes, solubility and uptake of Zn from ZnProp appeared to be greater than from ZnSO₄ when each were added at high concentration.

Implications

Commercially available organic Zn sources have been touted as being more bioavailable than inorganic sources. Enhanced absorption has been proposed to be a mechanism by which organic Zn sources may elicit responses in growth, reproduction, or health in various species. Results of the current experiment suggest that in the absence of dietary antagonists, inorganic and organic Zn sources are absorbed to a similar extent and by a similar, if not identical, mechanism. However, in the presence of potential inhibitors, solubility, uptake and transport of Zn from ZnProp may be greater than from ZnSO₄. Potential differences in post-absorptive metabolism also warrant further investigation in an attempt to explain physiological responses to organic Zn supplementation.

Literature Cited

- Alvarez-Hernandez, X., G. M. Nichols, and J. Glass. 1991. Caco-2 cell line: a system for studying intestinal iron transport across epithelial cell monolayers. *Biochim. Biophys. Acta* 1070:205-208.
- Ammerman, C. B., D. H. Baker, and A. J. Lewis. 1995. *Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins*. Academic Press, San Diego, CA.
- Baker, D. H., and C. B. Ammerman. 1995. Zinc bioavailability. In: *Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins*. pp 367-398. Academic Press, San Diego, CA.
- Beutler, K. T., O. Pankewycz, and D. L. Brautigam. 1998. Equivalent uptake of organic and inorganic zinc by monkey kidney fibroblasts, human intestinal epithelial cells, and perfused mouse intestine. *Biol. Tr. Elem. Res.* 61:19-31.
- Brinkhaus, F., J. Mann, C. Zorich, and J. Greaves. 1998. Bioavailability of zinc propionate in dogs. *J. Nutr.* 128:2596S-2597S.
- Cao, J., P. R. Henry, R. Guo, R. A. Holwerda, J. P. Toth, R. C. Littell, R. D. Miles, and C. B. Ammerman. 2000. Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. *J. Anim. Sci.* 78:2039-2054.
- Chesters, J. K. 1997. Zinc. In: B. L. O'Dell and R. A. Sunde (eds.) *Handbook of Nutritionally Essential Trace Elements*. pp 185-230. Marcel Dekker, Inc. New York, NY.

- Cousins, R. J. 1996. Zinc. In: L. J. Filer and E. E. Ziegler (eds.) Present Knowledge in Nutrition, 7th Edition. pp 293-306. International Life Science Institute-Nutrition Foundation, Washington, DC.
- Ellwood, K. C., C. Chatzidakis, and M. L. Failla. 1993. Fructose utilization by the human intestinal epithelial cell line, Caco-2. Proc. Soc. Exp. Biol. Med. 202:440-446.
- Erickson, R. H., J. R. Gum, Jr., M. M. Lindstrom, D. McKean, and Y. S. Kim. 1995. Regional expression and dietary regulation of rat small intestinal peptide and amino acid transporter mRNAs. Biochim. Biophys. Res. Comm. 216:249-257.
- Finley, J. W., M. Briske-Anderson, P. G. Reeves and L. K. Johnson. 1995. Zinc uptake and transcellular movement by Caco-2 cells: studies with media containing fetal bovine serum. J. Nutr. Biochem. 6:137-144.
- Finley, J. W., and P. Monroe. 1997. Mn absorption: the use of Caco-2 cells as a model of the intestinal epithelia. Nutr. Biochem. 8:92-101.
- Fleet, J. C., A. J. Turnbull, M. Bourcier, and R. J. Wood. 1993. Vitamin D-sensitive and quinacrine-sensitive zinc transport in human intestinal cell line Caco-2. Am. J. Physiol. 264:G1037-G1045.
- Giuliano, A. R., R. T. Franceschi, and R. J. Wood. 1991. Characterization of the vitamin D receptor from the Caco-2 human colon carcinoma cell line: effect of cellular differentiation. Arch. Biochem. Biophys. 285:261-269.
- Giuliano, A. R., and R. J. Wood. 1991. Vitamin D-regulated calcium transport in Caco-2 cells: unique in vitro model. Am. J. Physiol. 260:G207-G212.

- Gunshin, H., B. Mackenzie, U. V. Berger, Y. Gunshin, M. F. Romero, W. F. Boron, S. Nussberger, J. L. Gollan, and M. A. Hediger. 1997. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* 388:482-488.
- Halleux, C., and Y. Schneider. 1991. Iron absorption by intestinal epithelial cells. 1. Caco-2 cultivated in serum-free medium, in polyethyleneterephthalate microporous membrane, as an in vitro model. *In Vitro Cell. & Dev. Biol.* 27:293-302.
- Han, O., M. L. Failla, A. D. Hill, E. R. Morris, and J. C. Smith. 1994. Inositol phosphates inhibit uptake and transport of iron and zinc by a human intestinal cell line. *J. Nutr.* 124:580-587.
- Hempe, J. W., and R. J. Cousins. 1992. Cysteine-rich intestinal protein and intestinal metallothionein: an inverse relationship as a conceptual model for zinc absorption in rats. *J. Nutr.* 122:89-95.
- Hidalgo, I. J., T. J. Raub, and R. T. Borchart. 1989. Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. *Gastroenterology* 96:736-749.
- Kemin Industries. 1995. Bioavailability of zinc from organic and inorganic sources using the chick bioassay technique. Tech. Bull. No. 11575. Kemin Industries, Des Moines, IA.
- Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. *J. Anim. Sci.* 76:1216-1231.

- Lonnerdahl, B., A. Cederblad, L. Davidson, and B. Sandstrom. 1984. The effect of individual components of soy formulas and cow's milk formula on zinc bioavailability. *Am. J. Clin. Nutr.* 40:1064-1070.
- Matsui, T., and H. Yano. 1998. Formulation of low pollution feeds for animal pollution. In: *Proceedings of the 8th World Conference on Animal Production: Symposium Series 1*, Seoul, Korea. pp 110-119.
- McDougall, E. I. 1948. Studies on ruminant saliva. I. The composition and output of sheep saliva. *Biochem. J.* 3:15-18.
- McMahon, R. J., and R. J. Cousins. 1998. Mammalian zinc transporters. *J. Nutr.* 128:667-670.
- Raffaniello, R. D., S. Lee, S. Teichberg, and R. A. Wapnir. 1992. Distinct mechanisms of zinc uptake at the apical and basolateral membranes of Caco-2 cells. *J. Cell. Physiol.* 152:356-361.
- Raffaniello, R. D., and R. A. Wapnir. 1991. Zinc-induced metallothionein synthesis by Caco-2 cells. *Biochem. Med. Metab. Biol.* 45:101-107.
- Reeves, P. G., M. Briske-Anderson, and S. M. Newman, Jr. 1996. High zinc concentrations in culture media affect copper uptake and transport in differentiated human colon adenocarcinoma cells. *J. Nutr.* 126:1701-1712.
- Spears, J. W. 1996. Organic trace minerals in ruminant nutrition. *Anim. Feed Sci. Technol.* 58:151-163.
- Tacnet, F., D. W. Watkins, and P. Ripoche. 1990. Studies of Zn transport into brush-border membrane vesicles isolated from pig small intestine. *Biochim. Biophys. Acta* 1024:323-330.

- Tacnet, F., F. Lauthier, and P. Ripoche. 1993. Mechanisms of zinc transport into pig small intestine brush-border membrane vesicles. *J. Physiol.* 465:57-72.
- Wedekind, K. J., and S. R. Lowry. 1998. Are organic zinc source efficacious in puppies? *J. Nutr.* 128:2593S-2595S.

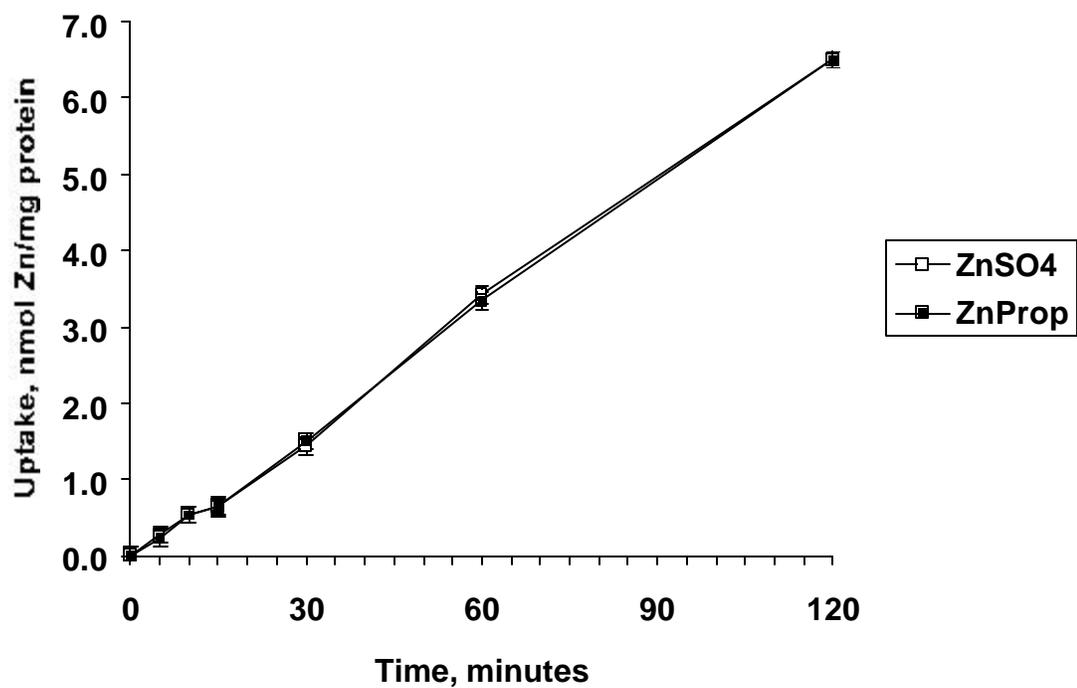


Figure 1. Uptake of Zn from buffer containing 20 μM Zn as ZnSO₄ or ZnProp incubated for increasing times at 37°C. Time effect ($P < 0.01$).

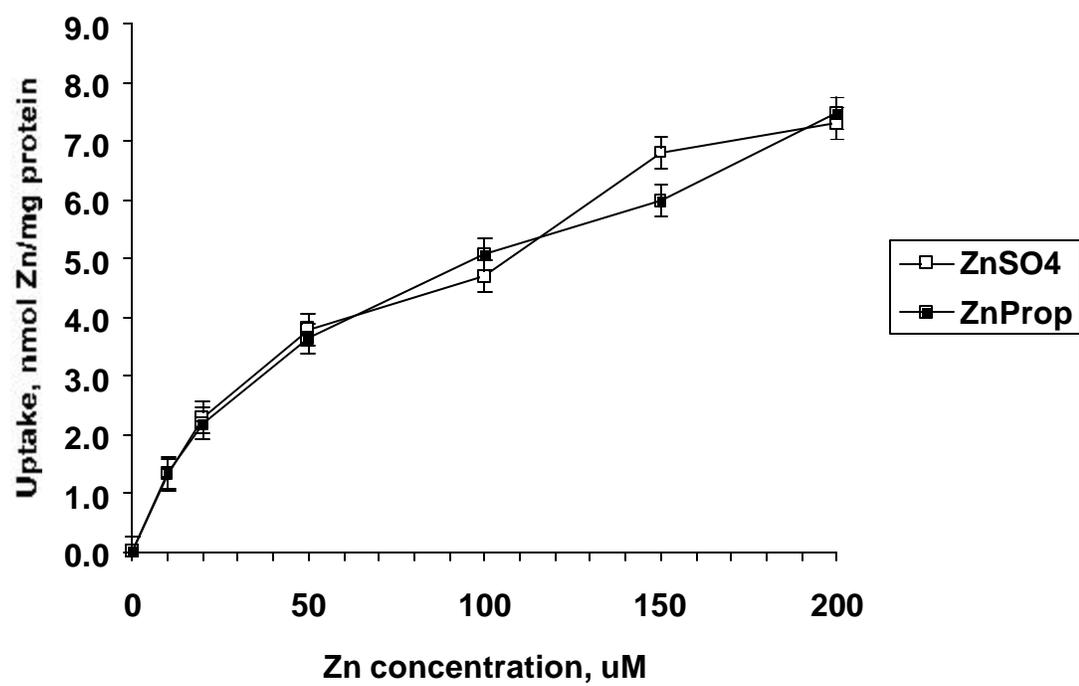


Figure 2. Uptake of Zn from buffer containing increasing Zn concentrations as ZnSO₄ or ZnProp, during a 60 min incubation at 37°C. Concentration effect ($P < 0.01$).

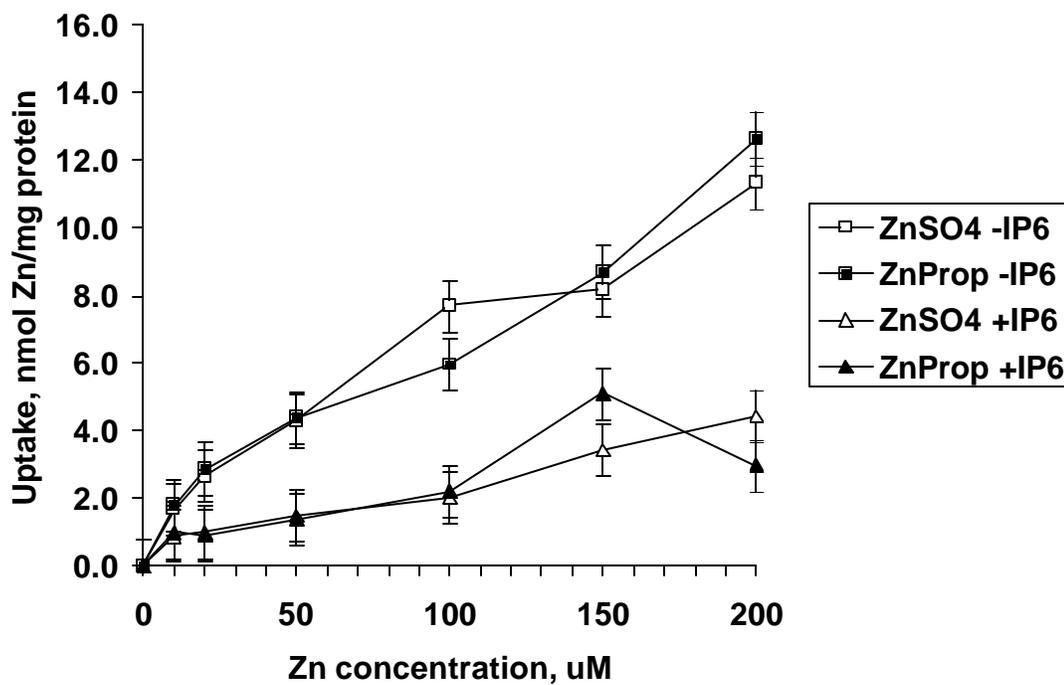


Figure 3. Uptake of Zn from buffers containing increasing concentrations of Zn as ZnSO₄ or ZnProp, in the presence or absence of 200 μM IP₆ and μM Ca. Cells were incubated for 60 min at 37°C. Concentration effect ($P < 0.01$), IP₆ effect ($P < 0.01$), Concentration × IP₆ interaction ($P < 0.01$).

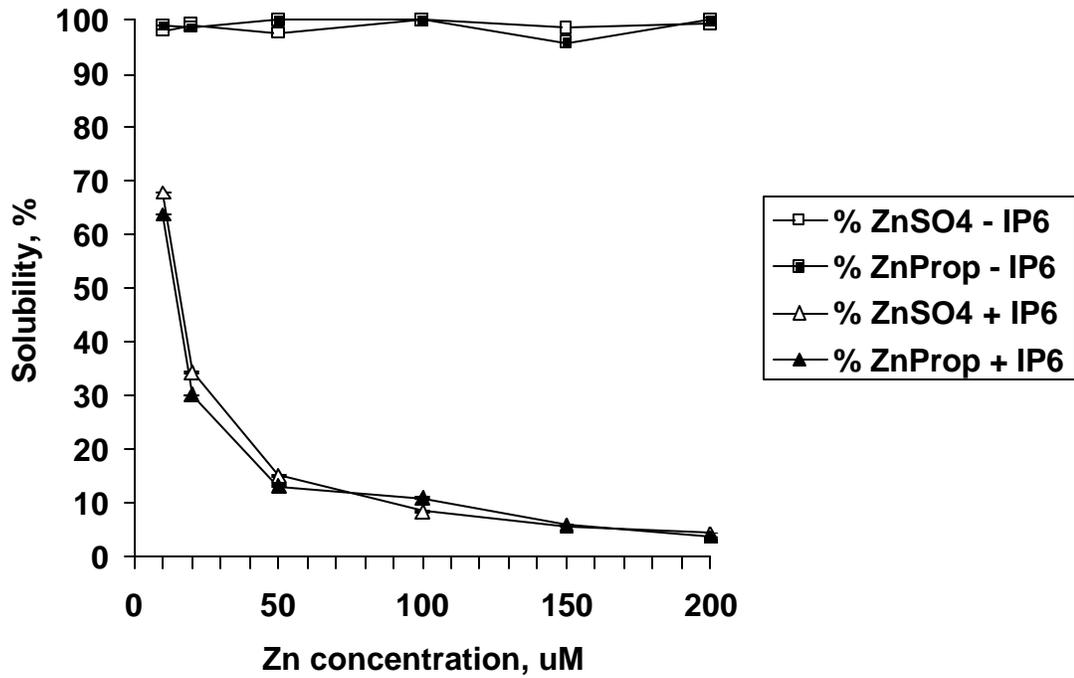


Figure 4. Solubility of increasing concentrations of Zn as ZnSO₄ or ZnProp in the absence or presence of 200 μM IP₆ and μM Ca. Concentration effect ($P < 0.01$), IP₆ effect ($P < 0.01$), Concentration \times IP₆ interaction ($P < 0.01$).

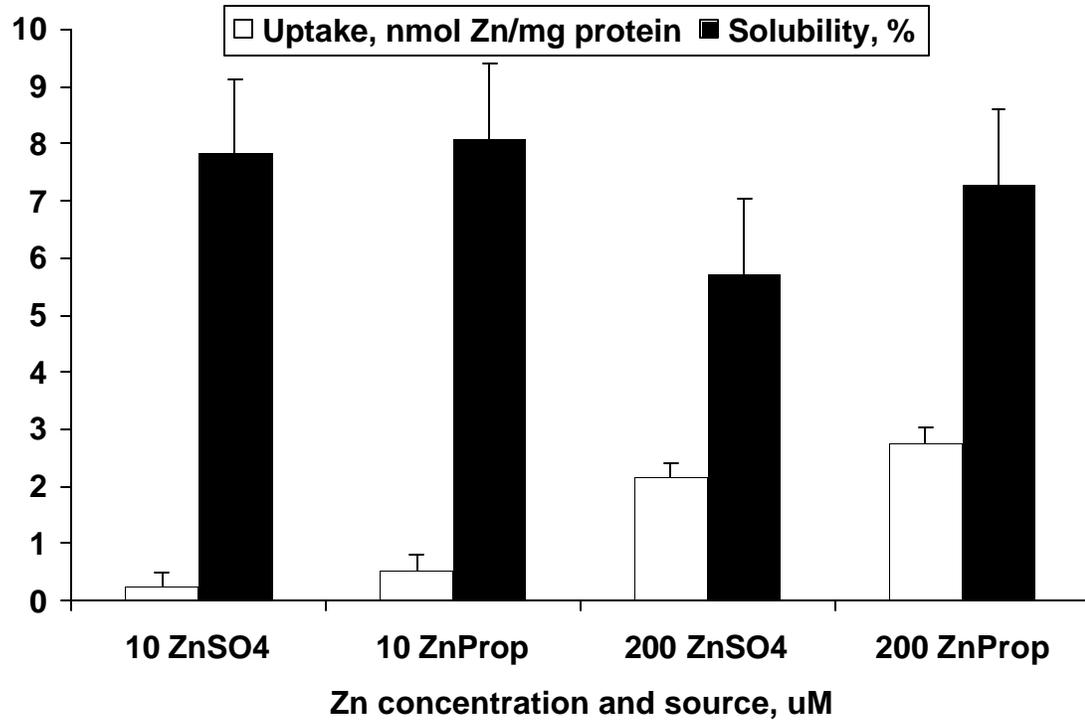


Figure 5. Uptake and solubility of Zn from aqueous fractions of ruminal and intestinal digestions containing 10 or 200 μM added Zn as ZnSO₄ or ZnProp. Cells were incubated for 60 min at 37°C. Uptake: Concentration effect ($P < 0.01$), Source effect ($P < 0.10$).

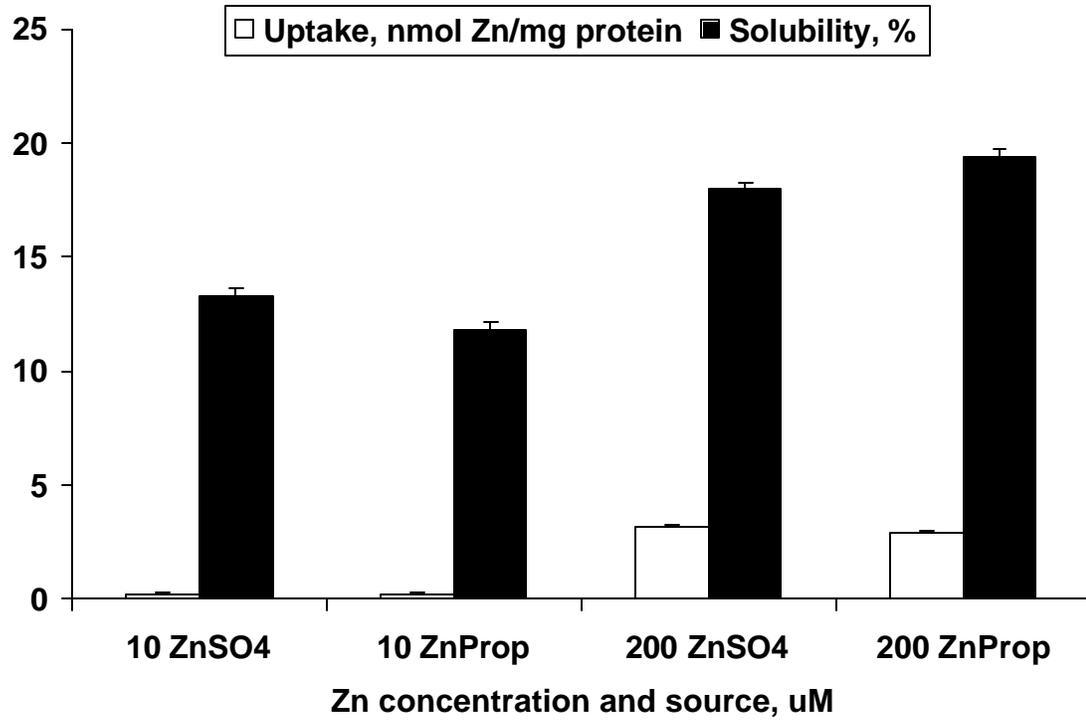


Figure 6. Uptake and solubility of Zn from aqueous fractions of intestinal digestions containing 10 or 200 μM added Zn as ZnSO₄ or ZnProp. Cells were incubated for 60 min at 37°C. Uptake: Concentration effect ($P < 0.01$). Solubility: Concentration effect ($P < 0.01$), Concentration \times source interaction ($P < 0.01$).

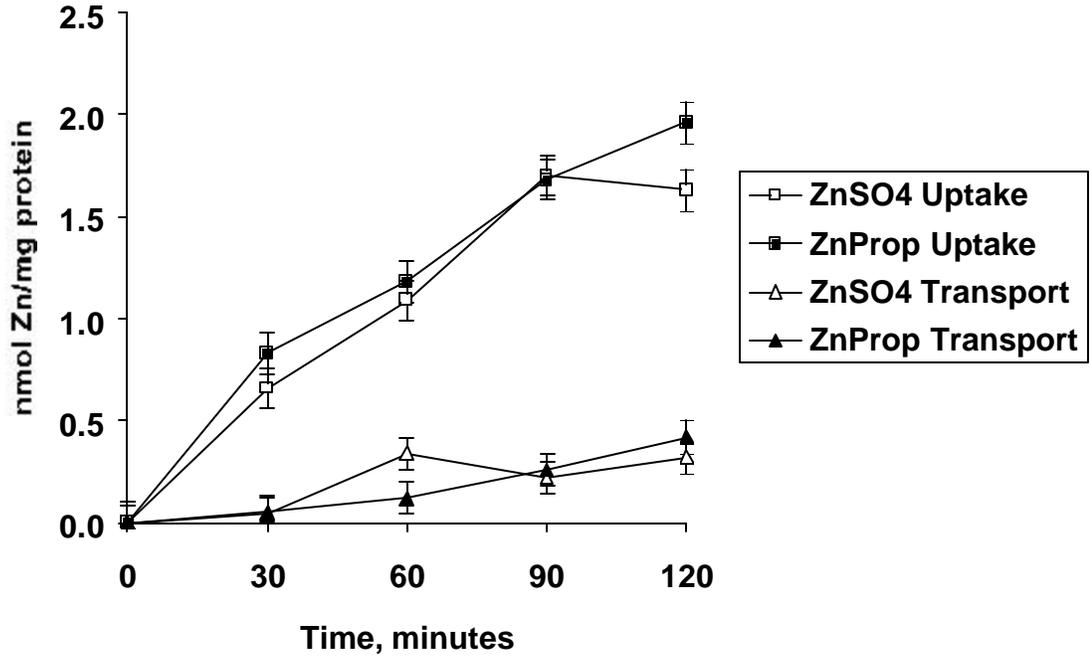


Figure 7. Uptake and transport of Zn from buffer containing 20 μ M Zn as ZnSO₄ or ZnProp incubated for increasing times at 37°C. Uptake: Time effect ($P < 0.01$). Transport: Time effect ($P < 0.01$).

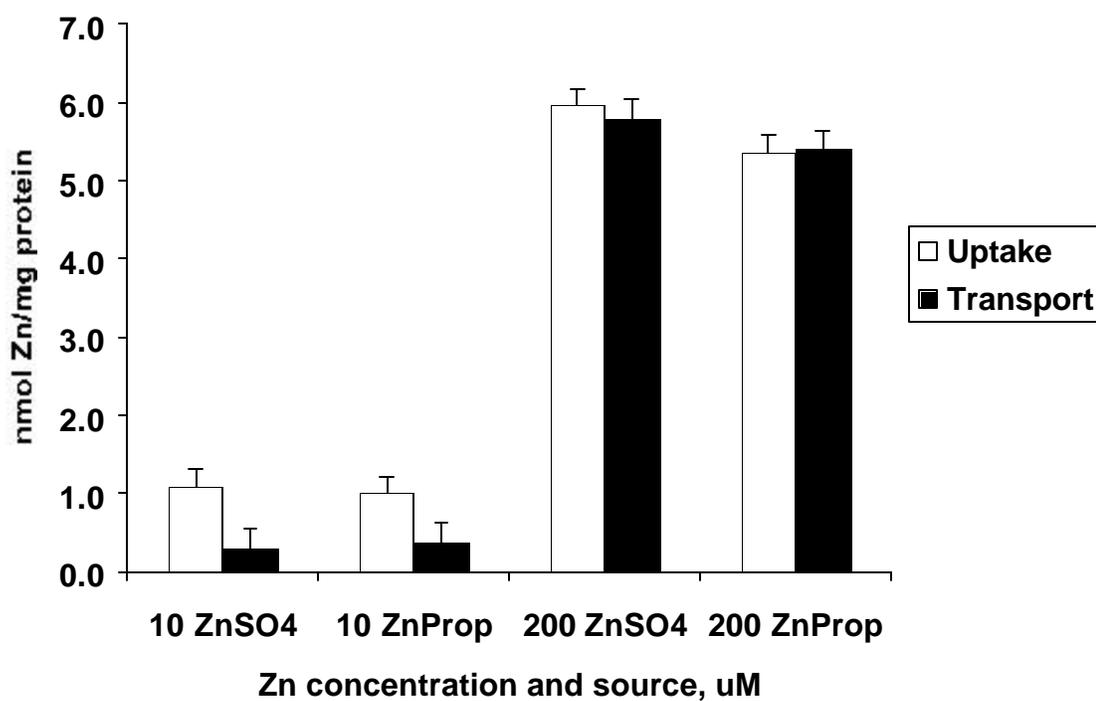


Figure 8. Uptake and transport of Zn from buffer containing increasing Zn concentrations as ZnSO₄ or ZnProp, during a 120 min incubation at 37°C. Uptake: Concentration effect ($P < 0.01$). Transport: Concentration effect ($P < 0.01$).

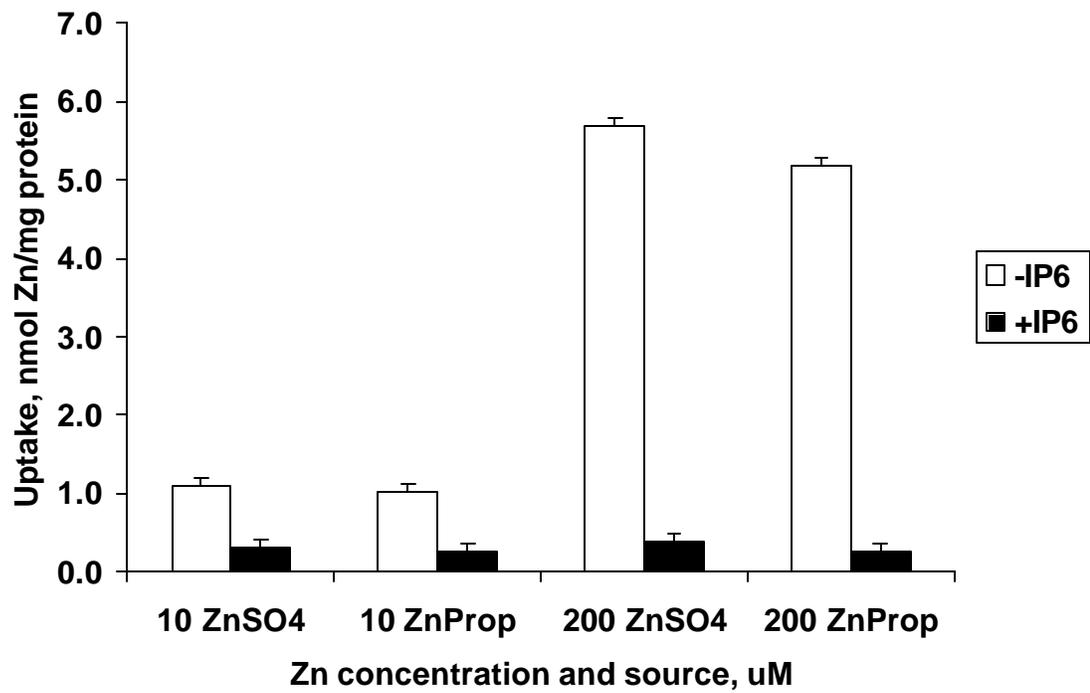


Figure 9. Uptake of Zn from buffers containing 10 or 200 μM Zn as ZnSO₄ or ZnProp, in the presence or absence of 200 μM IP₆ and μM Ca. Cells were incubated for 120 min at 37°C. Concentration effect ($P < 0.01$), IP₆ effect ($P < 0.01$), Source effect ($P < 0.01$), Concentration \times IP₆ interaction ($P < 0.01$) Concentration \times source interaction ($P < 0.07$).

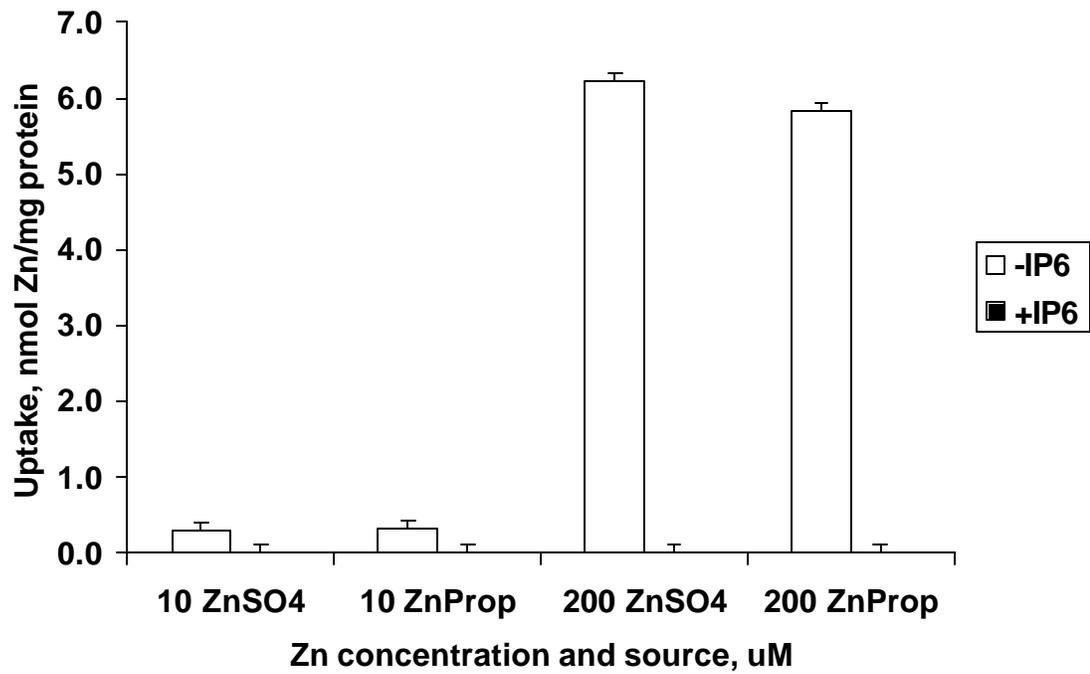


Figure 10. Transport of Zn from buffers containing 10 or 200 μM Zn as ZnSO₄ or ZnProp, in the presence or absence of 200 μM IP₆ and μM Ca. Cells were incubated for 120 min at 37°C. Concentration effect ($P < 0.01$), IP₆ effect ($P < 0.01$), Concentration \times IP₆ interaction ($P < 0.01$).

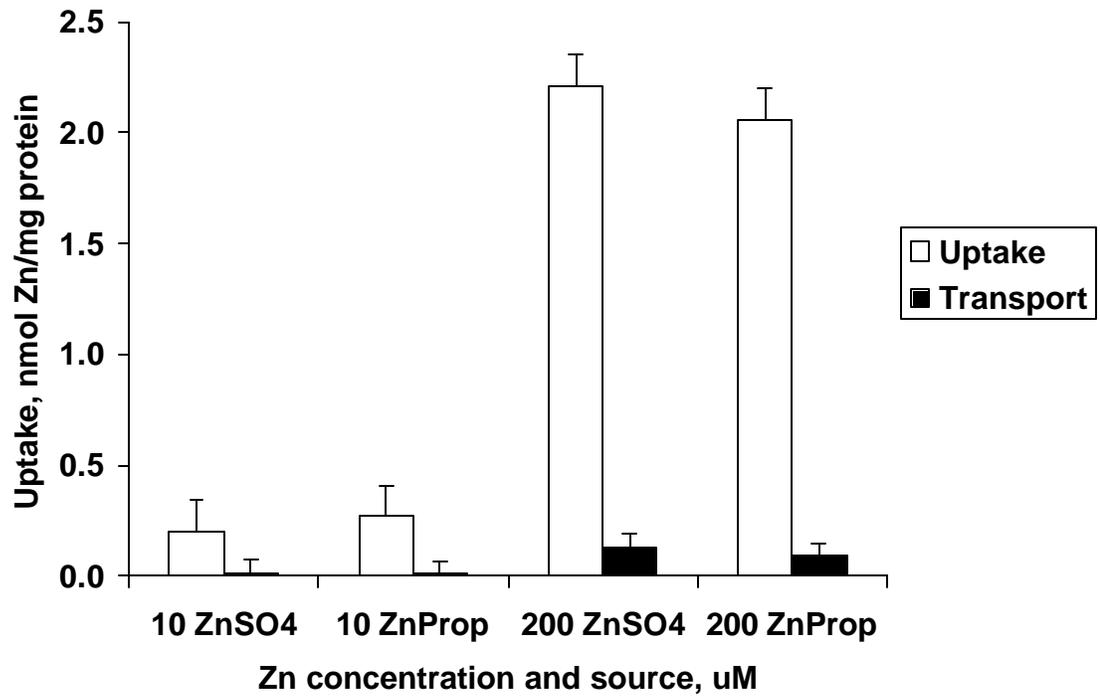


Figure 11. Uptake and transport of Zn from aqueous fractions of ruminal and intestinal digestions containing 10 or 200 μM added Zn as ZnSO_4 or ZnProp . Cells were incubated for 120 min at 37°C . Uptake: Concentration effect ($P < 0.01$).

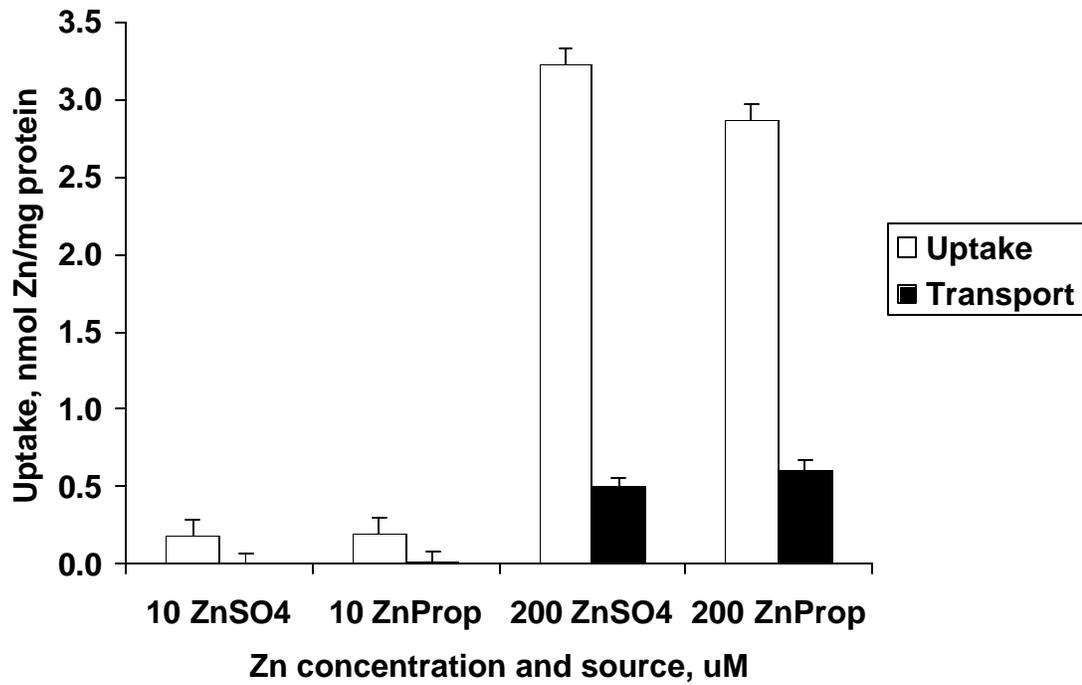


Figure 12. Uptake and transport of Zn from aqueous fractions of intestinal digestions containing 10 or 200 μM added Zn as ZnSO_4 or ZnProp . Cells were incubated for 120 min at 37°C . Uptake: Concentration effect ($P < 0.01$), Concentration \times source interaction ($P < 0.09$). Transport: Concentration effect ($P < 0.01$).

SUMMARY

A series of experiments was conducted to examine the absorption and post-absorptive metabolism of zinc (Zn) from inorganic and organic sources. Results of an in vivo experiment, using Holstein bull calves as models, suggested that, when Zn was supplemented at 20 mg Zn/kg DM for 98 d, Zn source had minimal impact on plasma or tissue Zn concentrations. However, when Zn was supplemented at 500 mg Zn/kg DM for 12 d, plasma and tissue Zn concentrations were greater in bulls that received ZnProt than in those that received ZnSO₄. These data suggested that when supplemented at high levels to a diet containing adequate Zn levels, Zn from ZnProt was absorbed and(or) retained to a greater extent than Zn from ZnSO₄. Subsequently, in vitro experiments were conducted to determine the uptake and transport of Zn from ZnSO₄, ZnProt or ZnProp by ruminal and omasal epithelium and Caco-2 cell monolayers. Results of these experiments suggested that when Zn was included in the mucosal buffer at low concentrations, or in the absence of antagonists, uptake and transport of Zn from inorganic and organic Zn sources were similar. However, following simulated ruminal and(or) intestinal digestion, uptake of Zn from the aqueous fractions of simulated digestion solutions appeared to be greater from organic than from inorganic Zn sources when Zn was included in the digestion solutions at 200 but not at 10 μM. Collectively these data suggest that, in the absence of antagonists, or in the presence of IP₆ and Ca only, uptake and transport of Zn from inorganic and organic Zn sources were similar. However, when added at high concentration in the presence of dietary antagonists, Zn uptake from organic sources appeared to be greater than from inorganic sources. Further research is certainly

warranted to more clearly define differences in Zn absorption that may exist between inorganic and organic Zn sources at different dietary levels.