



# Absorption and Retention of Trace Minerals in Adult Horses

E. L. WAGNER<sup>\*1</sup>, PAS, G. D. POTTER<sup>\*</sup>, PAS, E. M. ELLER<sup>\*</sup>, P. G. GIBBS<sup>\*</sup>, PAS, and D. M. HOOD<sup>†</sup>

<sup>\*</sup>Department of Animal Science, Texas A&M University, College Station 77843 and <sup>†</sup>Veterinary Physiology and Pharmacology, Texas A&M University, College Station 77843

## Abstract

Absorption and retention of Cu, Mn, and Zn were compared when feeding diets supplemented with oxide, sulfate, or organic-chelated forms of the minerals. Six mature Miniature Horses were used in the replicated 3 × 3 Latin square-designed experiment. The experiment was conducted in three 17-d periods, consisting of 10 d of diet adaptation followed by a 7-d total fecal and urine collection. Horses were fed a diet of 40% coastal Bermudagrass and 60% concentrate formulated to meet the energy, protein, Ca, and P requirements for maintenance of mature horses. Copper, Mn, and Zn were supplemented to provide 140 to 180% of the NRC (1989) recommended dietary concentrations for these minerals and were supplied in oxide, sulfate, and organic-chelate forms. Following total collections of feces and urine, feed, fecal, and urine samples were processed and analyzed for Cu, Mn, and Zn concentrations by atomic absorption spectrophotometry. Absorption of Cu, Mn, and Zn in all forms was low compared with previous studies, but systemic retention of these minerals was high. The cause of this disparity is unknown, although experimental error is unlikely. There were no differences in the absorption or retention of Cu, Mn, and Zn among the three forms of mineral supple-

*ments. Mature, idle horses may not be appropriate animals in which to evaluate absorption of various forms of trace minerals.*

(Key Words: Trace Minerals, Absorption, Oxide, Sulfate, Organic-Chelate.)

## Introduction

Copper, Mn, and Zn are important nutrients in the diets of horses, with integral roles in such processes as bone matrix formation and hoof strength (Jackson, 1998). These minerals can be supplemented in the diet as inorganic oxides, sulfates, or organic chelates with the mineral bound to an amino acid or peptide complex. Differences in the absorption and incorporation of these mineral supplement forms have been noted in several livestock species (Baker and Ammerman, 1995a,b; Henry, 1995). Ashmead et al. (1985) suggested the differences in absorption are due to the specific ion-friendly conditions required by inorganic minerals compared with the less energy-demanding uptake of chelated minerals bound to and absorbed with their low molecular weight, amino acid complex. The latter transport method is hypothesized to result in an increased rate of absorption and incorporation of minerals into body tissues.

Differences in absorption, retention, and bioavailability among ox-

ide, sulfate, and organic-chelate mineral supplements for non-equine species have been reported (Henry et al., 1986, 1989; Spears, 1989; Baker et al., 1991; Kegley and Spears, 1994; Sandoval et al., 1997), whereas other research has not been as conclusive (Apgar et al., 1995; Apgar and Kornegay, 1996). Research in horses on the various forms of trace minerals has been limited. When comparing an inorganic trace mineral supplement to an inorganic-organic blend, there was no difference in liver mineral concentration (Siciliano et al., 2001a) or hoof wall characteristics (Siciliano et al., 2001b) in mature horses. In another study, yearlings supplemented with a proteinate trace mineral mix had greater hoof growth rates than those fed an inorganic supplement, but there was no difference in hoof wall strength or skeletal growth characteristics between groups (Ott and Johnson, 2001). These studies have illustrated a need for a direct comparison of the absorption and retention of oxide, sulfate, and organic-chelate trace mineral supplements in horses. Furthermore, there is a tendency in the equine industry to feed these minerals at amounts greater than NRC (1989) recommendations, yet there have been few studies examining the efficiency of absorption at or near these values.

## Materials and Methods

Six mature Miniature Horse geldings with an average BW of 90 kg

<sup>1</sup>To whom correspondence should be addressed: blwagner@neo.tamu.edu

**TABLE 1. Ingredient composition in experimental diets (DM basis).**

Item	Percentage
Coastal Bermudagrass	40.0
Shell corn	25.5
Oats	25.5
Soybean meal	5.0
Molasses	3.0
Salt	0.5
Mineral mix	0.5

**TABLE 2. Nutrient concentrations in experimental diets (DM basis).**

Item <sup>a</sup>	Oxide	Sulfate	Chelate
DE, Mcal/d	2.9	2.9	2.9
CP, %	13.4	13.4	13.4
Ca, %	0.5	0.5	0.5
P, %	0.3	0.3	0.3
Cu, ppm	17.8	18.0	18.0
Mn, ppm	66.2	68.4	66.3
Zn, ppm	63.9	64.0	64.0

<sup>a</sup>DE and CP values were calculated from NRC (1989). Mineral values were obtained from laboratory analyses.

were used in a replicated 3 × 3 Latin square-designed experiment. The experiment was conducted in three 17-d periods, divided into a 10-d diet adaptation period followed by a 7-d total collection. The diet treatments consisted of a control diet (40% hay:60% concentrate) balanced to contain sufficient energy, protein, Ca, and P for maintenance of mature horses. In each of the three experimental diets, Cu, Mn, and Zn were supplemented to provide 130 to 160% of the NRC (1989) recommended dietary concentrations for the minerals (Tables 1, 2, and 3). In one diet, supplementation was supplied by the oxide form, the second by the sulfate form, and the third by minerals in the organic-chelated form, provided as mineral-proteinates. All of the trace mineral supplements were provided by a commercial supplier. Concentrate was hand-mixed weekly and offered to the horses as a textured feed.

Horses were maintained on drylots between feedings. At feeding, horses were brought to individual concrete-floored stalls and offered feed from non-metal containers. Drylots were cleaned daily to remove waste and reduce the risk of coprophagy. The protocol for animal treatment was reviewed and approved by the Texas A&M University Agricultural Animal Care and Use Committee. Horses were placed in the drylot and fed to project standards for a minimum of 30 d prior to the beginning of the re-

search period. During this time, horses were offered the basal diet.

Horses were offered feed at 1.5% BW daily in non-metal feeders, with free access to tap water provided in non-metal buckets. Actual daily DM consumption averaged 1.2% of BW. In the final 2 d of the 10-d adaptation period, horses were placed in individual wooden metabolism crates to begin adaptation and to eliminate any coprophagy. A 7-d total collection period in the metabolism crates followed, allowing for separate and total collection of feces and urine. Crates were constructed such that the horses stood on non-reactive, plastic-coated grates, allowing for urine collection in plastic trays beneath the

crates. Urine was collected as it was produced, and a 50% aliquot was retained. Fecal matter was collected in plastic buckets and weighed at 3-h intervals; a 10% aliquot was saved. Daily concentrate and hay samples were obtained from each treatment group. Water intake was also measured, and tap water was sampled each collection period.

Sample aliquots were frozen and stored for later analyses. Feed and feces were dried for determination of DM, ground in a Wiley mill, and stored in air-tight, mineral-free plastic containers. Nitric-perchloric acid digestions were performed in triplicate on feed and fecal samples. Feed, fecal, urine, and water samples were ana-

**TABLE 3. Trace mineral contributions in the diets of horses fed various forms of trace minerals.**

Item	Total daily intake	Contributed by supplement	Contributed by supplement
	— (mg/100 kg BW) —		(%)
Cu			
Oxide	16.20	6.81	42.04
Sulfate	17.62	8.23	46.71
Organic-chelate	16.78	7.83	46.66
Mn			
Oxide	87.72	53.20	60.65
Sulfate	86.13	53.31	61.89
Organic-chelate	90.69	54.98	60.62
Zn			
Oxide	67.01	33.42	49.87
Sulfate	70.43	35.34	50.18
Organic-chelate	65.06	32.63	50.15

TABLE 4. Daily Cu absorption and retention of horses fed various forms of trace minerals.

Item	Oxide		Sulfate		Organic-chelate	
	Mean	SE	Mean	SE	Mean	SE
Intake, mg/100 kg BW	16.20	1.56	17.62	1.80	16.78	1.41
Fecal excretion, mg/100 kg BW	15.45	1.77	16.17	1.44	16.16	1.34
Absorbed, mg/100 kg BW	0.75	0.45	1.44	1.16	0.62	0.96
% of intake	5.32	2.78	6.55	6.14	2.76	5.85
Urine, mg/100 kg BW	0.07	0.01	0.08	0.03	0.07	0.02
Retained, mg/100 kg BW	0.69	0.45	1.38	1.16	0.56	0.96
% of intake	4.93	2.78	6.19	6.14	2.40	5.84
% of absorbed	101.12	4.42	87.03	10.63	100.68	4.04

lyzed for Cu, Mn, and Zn using atomic absorption spectrophotometry.

Resulting data were analyzed for treatment, period, and horse effects by analysis of variance procedures appropriate for the Latin square design, using STATA statistical software (Stata-Corp, 2001).

## Results and Discussion

Mean mineral concentrations in the diets as offered were 14, 73, and 56 ppm of Cu, Mn, and Zn, respectively, on a DM basis. These feeding rates represent 140 to 180% of the NRC (1989) values for the minerals studied. Daily intake, absorption, and retention of Cu, Mn, and Zn are shown in Tables 4, 5, and 6. Zinc data from one horse in Period 2 was omitted because of sample contamination. Absorption, absorption as a per-

centage of intake, retention, and retention as a percentage of intake for Zn were significantly greater ( $P<0.05$ ) in Period 1 compared with Periods 2 and 3. No other period effects were noted, nor were there any diet  $\times$  period interactions.

There was no significant difference ( $P>0.05$ ) across treatments for mineral absorption, absorption as a percentage of intake, retention, retention as a percentage of intake, or retention as a percentage of absorption for Cu, Mn, or Zn. Absorption of Cu, Mn, and Zn were all very low in this study and were not consistent with Cu and Zn absorption values previously reported by Cymbaluk et al. (1981) and Hoyt et al. (1995). Dietary Cu and Zn concentrations in those studies were similar to diets in the present study. If the horses in the present study were in a high state of mineral intake prior to the study, low absorption efficiency would be ex-

pected, as the route of excretion of Cu, Mn, and Zn from the body is through bile, in a form unavailable for reabsorption (Chesters, 1997, Harris, 1997 and Leach and Harris, 1997). However, there was no evidence of that possibility when the data were grouped by period. In fact, Zn absorption was greater in Period 1 compared with subsequent periods. Efficiency of retention of absorbed Cu, Mn, and Zn approached or exceeded 100%, although this is a reflection of the typically minimal urinary excretion of these minerals rather than low absorption efficiency. It has been well documented that the major route of excretion of Cu, Mn, and Zn is through the gastrointestinal tract, with very little mineral filtered out by the kidneys (Chesters, 1997; Harris, 1997; Leach and Harris, 1997).

One possible cause of lesser absorption is poor availability of the mineral from the supplements. At least

TABLE 5. Daily Mn absorption and retention of horses fed various forms of trace minerals.

Item	Oxide		Sulfate		Organic-chelate	
	Mean	SE	Mean	SE	Mean	SE
Intake, mg/100 kg BW	87.72	8.13	86.13	8.65	90.69	6.05
Fecal excretion, mg/100 kg BW	76.76	9.40	78.02	7.14	76.43	5.92
Absorbed, mg/100 kg BW	10.95	1.97	8.11	3.27	14.26	3.79
% of intake	13.60	2.76	8.59	3.51	15.52	3.62
Urine, mg/100 kg BW	0.09	0.03	0.07	0.02	0.21	0.11
Retained, mg/100 kg BW	10.86	1.99	8.04	3.26	14.04	3.84
% of intake	13.50	2.77	8.52	3.51	15.30	3.69
% of absorbed	97.79	1.68	100.46	1.69	96.91	2.25

TABLE 6. Daily Zn absorption and retention of horses fed various forms of trace minerals.

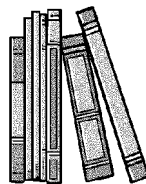
Item	Oxide		Sulfate		Organic-chelate	
	Mean	SE	Mean	SE	Mean	SE
Intake, mg/100 kg BW	67.01	7.02	70.43	6.59	65.06	4.86
Fecal excretion, mg/100 kg BW	57.91	6.31	60.85	5.14	57.86	3.74
Absorbed, mg/100 kg BW	9.10	2.36	9.58	2.24	7.21	1.82
% of intake	13.85	3.50	12.84	2.78	10.58	2.58
Urine, mg/100 kg BW	0.34	0.09	1.32	0.90	0.31	0.05
Retained, mg/100 kg BW	8.77	2.28	8.25	2.50	6.90	1.83
% of intake	13.38	3.44	11.11	3.21	10.10	2.63
% of absorbed	95.62	0.89	81.90	11.43	91.11	4.98

42% of the mineral in each diet was supplied by the supplement (Table 3). Using the initial diet balance calculations, a percentage of mineral was subtracted from the daily feed intake corresponding to the amount of mineral provided in the commercial supplement. The same amount of mineral was subtracted from the daily fecal mineral excretion, thereby assuming zero absorption of minerals from the supplement. Mean daily intake, excretion, absorption, absorption as a percentage of intake, retention, retention as a percentage of intake, and retention as a percentage of absorption were calculated for each mineral. These calculations indicate that mineral absorption efficiencies were low before supplemental mineral was added, and there were no significant differences noted among the forms of minerals. The exact cause of low absorptive efficiency in this study is not known, but it does not appear to be an experimental error. Dry matter digestibility was normal, averaging 71.73%, and recovery of controls and spiked samples analyzed 98 to 100% of the minerals studied.

## Implications

In this study, there were no significant differences in absorption and retention of Cu, Mn, or Zn when fed as the oxide, sulfate, or organic-chelate. The low absorption and retention values observed in this study offer several points for consideration in the ap-

plication of trace mineral supplementation for horses. Current NRC (1989) recommendations for Cu, Mn, and Zn are apparently sufficient for meeting the needs of mature, idle horses. More knowledge and control of potential trace mineral saturation and depletion in experimental horses is needed to design mineral balance studies in the future. Also, the mature, idle horse may not be a satisfactory model to use in trace mineral absorption studies. Horses that are in a state of potential increased systemic demand, such as during lactation, growth, or intense exercise may be a more appropriate model. Examining trace mineral absorption in these animals may determine whether the chemical form affects the efficiency of absorption and retention.



## Literature Cited

- Apgar, G. A., and E. T. Kornegay. 1996. Mineral balance of finishing pigs fed copper sulfate or a copper-lysine complex at growth-stimulating levels. *J. Anim. Sci.* 74:1594.
- Apgar, G. A., E. T. Kornegay, M. D. Lindemann, and D. R. Notter. 1995. Evaluation of copper sulfate and a copper lysine complex as growth promoters for weanling swine. *J. Anim. Sci.* 73:2640.
- Ashmead, H. D., D. J. Graff, and H. H. Ashmead. 1985. Summary and implications. In *Intestinal Absorption of Metal Ions and Chelates*. p 213. Charles C. Thomas, Springfield, IL.
- Baker, D. H., and C. B. Ammerman. 1995a. Copper bioavailability. In *Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins*. p 127. Academic Press, Inc., San Diego, CA.
- Baker, D. H., and C. B. Ammerman. 1995b. Zinc bioavailability. In *Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins*. p 367. Academic Press, Inc., San Diego, CA.
- Baker, D. H., J. Odle, M. A. Funk, and T. M. Wieland. 1991. Research note: Bioavailability of copper in cupric oxide, cuprous oxide and in a copper-lysine complex. *Poultry Sci.* 70:177.
- Chesters, J. K. 1997. Zinc. In *Handbook of Nutritionally Essential Mineral Elements*. p 185. Marcel Dekker, Inc., New York, NY.
- Cymbaluk, N. F., H. F. Schryver, and H. F. Hintz. 1981. Copper metabolism and requirements in mature ponies. *J. Nutr.* 111:87.
- Harris, E. D. 1997. Copper. In *Handbook of Nutritionally Essential Mineral Elements*. p 231. Marcel Dekker, Inc., New York, NY.
- Henry, P. R. 1995. Manganese bioavailability. In *Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins*. p 239. Academic Press, Inc., San Diego, CA.
- Henry, P. R., C. B. Ammerman, and R. D. Miles. 1986. Bioavailability of manganese sulfate and manganese monoxide in chicks as measure by tissue uptake of manganese from conventional dietary levels. *Poultry Sci.* 65:983.
- Henry, P. R., C. B. Ammerman, and R. D. Miles. 1989. Relative bioavailability of manganese in a manganese-methionine complex for broiler chicks. *Poultry Sci.* 68:107.
- Hoyt, J. K., G. D. Potter, L. W. Greene, and J. G. Anderson, Jr. 1995. Copper balance in Miniature Horses fed varying amounts of zinc. *J. Eq. Vet. Sci.* 15(8):357.
- Jackson, S. G. 1998. Trace minerals for the performance horse known biochemical roles and estimates of requirements. *Aust. Eq. Vet.* 16(3):119.

- Kegley, E. B., and J. W. Spears. 1994. Bioavailability of feed-grade copper sources (oxide, sulfate or lysine) in growing cattle. *J. Anim. Sci.* 72:2728.
- Leach, R. M., and E. D. Harris. 1997. Manganese. In *Handbook of Nutritionally Essential Mineral Elements*. p 335. Marcel Dekker, Inc., New York, NY.
- NRC. 1989. *Nutrient Requirements of Horses*. (5th Ed.). National Academy Press, Washington, DC.
- Ott, E. A., and E. L. Johnson. 2001. Effect of trace mineral proteinates on growth and skeletal and hoof development in yearling horses. *J. Eq. Vet. Sci.* 21(6):287.
- Sandoval, M., P. R. Henry, C. B. Ammerman, R. D. Miles and R. C. Littell. 1997. Relative bioavailability of supplemental inorganic zinc sources for chicks. *J. Anim. Sci.* 75:3195.
- Siciliano, P. D., K. D. Culley, and T. E. Engle. 2001a. Effect of trace mineral source (inorganic vs. organic) on trace mineral status in horses. In *Proc. 17th Eq. Nutr. Phys. Symp.*, Lexington, KY. p 419. ENPS, Savoy, IL.
- Siciliano, P. D., K. D. Culley, T. E. Engle, and C. W. Smith. 2001b. Effect of trace mineral source (inorganic vs. organic) on hoof wall-growth rate, -hardness and -tensile strength. In *Proc. 17th Eq. Nutr. Phys. Symp.*, Lexington, KY. p 143. ENPS, Savoy, IL.
- 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.
- StataCorp. 2001. *Stata Statistical Software: Release 7.0*. Stata Corp., College Station, TX.