JOURNAL OF ANIMAL SCIENCE
The Premier Journal and Leading Source of New Knowledge and Perspective in Animal Science

Copper and zinc balance in exercising horses fed 2 forms of mineral supplements

E. L. Wagner, G. D. Potter, P. G. Gibbs, E. M. Eller, B. D. Scott, M. M. Vogelsang and R. L. Walzem

J ANIM SCI 2011, 89:722-728.
doi: 10.2527/jas.2010-2871 originally published online November 12, 2010

Copper and zinc balance in exercising horses fed 2 forms of mineral supplements

E. L. Wagner,*[1,2] G. D. Potter,* P. G. Gibbs,*[3] E. M. Eller,*[4] B. D. Scott,* M. M. Vogelsang,* and R. L. Walzem†

*Department of Animal Science, and †Department of Poultry Science, Texas A&M University, College Station 77843

ABSTRACT: Studies comparing the absorption and retention of various forms of trace minerals in horses have yielded mixed results. The objective of this study was to compare Cu and Zn absorption and retention in exercising horses where the mineral was supplemented in the sulfate or organic chelate form. Nine mature horses were used in a modified switchback design experiment consisting of seven 28-d periods. Horses were fed a diet consisting of 50% concentrate and 50% hay that was balanced to meet the energy, protein, Ca, and P requirements for horses performing moderate-intensity exercise. Horses were subjected to a controlled mineral repletion-depletion diet sequence before feeding the experimental diet to standardize mineral status across horses. The experimental diet was designed to provide 90% of the 1989 NRC for Cu and Zn, with supplemental mineral provided in the inorganic sulfate form ($CuSO_4$ and $ZnSO_4$) or the organic chelate form (Cu-Lys and Zn-Met). Feed, fecal, urine, and water samples collected during a total collection during the last 4 d of the experimental diet periods were analyzed to determine apparent absorption and retention of Cu and Zn from the 2 mineral forms. A formulation error caused horses receiving the organic chelate diet to consume about 3 times the amount of Cu and Zn compared with those fed the sulfate-supplemented diet. Copper and Zn intake and fecal excretion were greater ($P < 0.05$) for horses consuming the organic chelate-supplemented diet. Apparent absorption values for all horses were negative. Apparent Cu absorption and retention as a percentage of intake were greater for horses fed the organic chelate diet ($P < 0.05$). It is unknown why excretion of Cu and Zn by the horses during the total collection exceeded the mineral intake. Although Cu-Lys seemed to be better absorbed than $CuSO_4$ and absorption of Zn-Met and $ZnSO_4$ were not different, these results are tempered by the observation of abnormally high fecal and urinary excretion values for Cu and Zn in the present study.

Key words: absorption, copper, horse, organic chelate, sulfate, zinc

J. Anim. Sci. 2011. 89:722–728
doi:10.2527/jas.2010-2871

INTRODUCTION

Elemental Cu and Zn are commonly added to formulated horse diets to correct or prevent deficiencies and related disorders. Most equine diets need supplementation to meet the NRC (1989) minimum requirements of 10 mg/kg of Cu and 40 mg/kg of Zn for all classes of horses. Mineral supplements can be divided into 2 groups: inorganic salts, typically including a sulfate or oxide group, and organic chelates, where the mineral is covalently bound to an AA or proteinate complex. It is thought that organic chelate minerals are absorbed as intact molecules via mechanisms for absorption of their constituent AA. Organic chelate minerals require fewer steps to be absorbed, making the process more efficient compared with the inorganic mineral salts, which are dependent on changes in pH and the presence of carrier proteins as they are absorbed against a concentration gradient (Ashmead et al., 1985).

Oxide-bound minerals are considered less bioavailable than sulfates and organic chelates, but studies are inconclusive as to whether sulfates or organic chelates offer an advantage in availability and utilization (Baker and Ammerman, 1995a,b). Some studies with equids have found no difference in utilization of minerals from organic and inorganic sources (Siciliano et al., 2001a,b;

[1]Corresponding author: elw0001@auburn.edu
[2]Present address: Department of Animal Sciences, 210 Upchurch Hall, Auburn University, AL 36849.
[3]Present address: Texas AgriLife Extension System, Texas A&M University, College Station, TX 77843.
[4]Present address: College of Agriculture and Life Sciences, North Carolina State University, Carthage, NC 28327.
Received January 28, 2010.
Accepted November 2, 2010.

Table 1. Exercise and dietary treatments by period

Period	Exercise	Diet
1	Conditioning	Depletion diet (no Cu and Zn supplementation)
2	Conditioning	Repletion diet [Cu and Zn at 100% of NRC (1989)]
3	Standard workload	Depletion diet
4	Standard workload	Experimental diet [Cu and Zn at 90% of NRC (1989)]
5	Standard workload	Repletion diet
6	Standard workload	Depletion diet
7	Standard workload	Experimental diet

Baker et al., 2005). Other studies found an advantage for organic supplementation in some classes of horses, but not in all of the measures of interest (Ott and Asquith, 1994; Ott and Johnson, 2001). Also, mineral balance studies have yielded mixed results (Miller et al., 2003; Baker et al., 2005; Wagner et al., 2005).

The objective of this study was to compare absorption and retention of Cu and Zn in exercising horses with the mineral being supplemented in the sulfate or organic chelate form. By feeding these mineral forms in a marginally deficient diet, it was thought that any advantage in one form over another would become more apparent.

MATERIALS AND METHODS

Horses were maintained at the Texas A&M University Horse Center, and all procedures were approved by the Institutional Animal Care and Use Committee.

Eight mature mares and one gelding of various breeds (2 Arabians, 2 Paints, and 5 Quarter Horses) were used in a modified switchback experimental design. Horses ranged in age from 4 to 14 yr, with a mean age of 8.2 yr. Before and during the experiment, horses were dewormed and vaccinated in accordance with the standard operating procedures of the facility.

The experiment was conducted in seven 28-d periods as outlined in Table 1. Periods 1 and 2 served as exercise conditioning periods, where horses were acclimated to the exercise equipment as well as adapted to the exercise demands of the subsequent periods. The concentration of dietary Cu and Zn changed each period, such that the horses were subjected to a standardized repletion/depletion cycle before receiving the appropriate experimental diet. Horses were blocked by age and breed and randomly assigned to 1 of 2 groups for the initial period where the experimental diets were offered (period 4), and received the other treatment diet during the second experimental diet period (period 7).

Horses were initially offered a diet consisting of 50% concentrate and 50% hay at 1.5 to 2% of BW divided between 2 feedings daily. During the first 3 periods, the amount of feed offered was adjusted such that the horses maintained an approximate BCS of 5 to 6 (Henneke et al., 1983), and this amount was held constant for the remainder of the project.

The diet was formulated to provide DE at 175% of maintenance, with CP, Ca, and P balanced to appropriate nutrient:DE ratios (NRC, 1989). Other nutrients were supplied to meet or exceed NRC requirements. Diets were formulated to supply various concentrations of Cu and Zn each period, such that the horses would receive no supplemental Cu and Zn (depletion diet; periods 1, 3, and 6), 100% of the NRC (repletion diet; periods 2 and 5), or the experimental diet of Cu and Zn supplemented to meet 90% of the NRC (1989) recommendations (periods 4 and 7; Table 2). In this manner, horses were subject to controlled repletion and depletion of body mineral stores before planned feeding of a marginally deficient Cu and Zn diet, so that a systemic demand for Cu and Zn existed during the feeding of the experimental diets in periods 4 and 7. Composition of the concentrates can be found in Table 3. Mineral supplementation in the repletion phases was provided by $CuSO_4$ and $ZnSO_4$, whereas supplementation in the experimental phases was provided either by the sulfate forms or organic chelate sources in the form of Cu-Lys

Table 2. Calculated energy and nutrient composition of diets (DM basis)[1]

			Experimental diet	
Item	Depletion diet	Repletion diet	Sulfate	Organic chelate
DE, Mcal/kg	3.05	3.05	3.05	3.05
CP, %	13.2	13.2	13.2	13.2
Ca, %	0.42	0.42	0.42	0.42
P, %	0.32	0.32	0.32	0.32
Cu, mg/kg	6.69	9.74	8.73	8.69
Zn, mg/kg	29.18	37.18	33.54	33.58

[1]Diets consisted of 50% concentrate and 50% bermudagrass hay.

Table 3. Composition of the depletion, repletion, and experimental concentrates[1,2]

	Concentrate			
			Experimental concentrate	
Ingredient, %	Depletion	Repletion	Sulfate	Organic chelate
Ground corn	53.55	53.55	53.55	53.25
Whole oats	16.00	16.00	16.00	16.00
Wheat middlings	11.00	11.00	11.00	11.00
Soybean meal	10.00	10.00	10.00	10.00
Vegetable oil	3.50	3.50	3.50	3.50
Sweet 80 fat	2.50	2.50	2.50	2.50
Salt	1.00	1.00	1.00	1.00
$CaCO_3$	0.80	0.80	0.80	0.80
$CuSO_4$	—	0.0025	0.0015	—
$ZnSO_4$	—	0.0045	0.0025	—
CuPLEX 50	—	—	—	0.08
Zinpro 40	—	—	—	0.23
Liquid binder	1.50	1.50	1.50	1.50
Preservative	0.10	0.10	0.10	0.10
Flavoring	0.05	0.05	0.05	0.05

[1]Concentrate consisted of 50% of the total diet, and the other 50% consisted of bermudagrass hay.

[2]Copper and zinc supplements were added at the expense of ground corn. Organic chelate sources were Cu-Lys and Zn-Met (CuPLEX 50 and Zinpro 40, Zinpro Corp., Eden Prairie, MN). All concentrates were manufactured by a commercial feed mill (Martindale Feed Mill, Valley View, TX), and the information on Sweet 80 fat, liquid binder, preservative, and flavoring was not provided.

and Zn-Met (CuPLEX 50 and Zinpro 40, Zinpro Corp., Eden Prairie, MN). All concentrates were manufactured by a commercial feel mill (Martindale Feed Mill, Valley View, TX).

Daily hay and concentrate amounts were divided into 2 separate feedings and offered at 600 and 1800 h. Horses were fed individually in concrete-floored stalls from nonmetal containers. Refusals, if any, were weighed and recorded. When not being fed, horses were group-housed in dry-lot pens with ad libitum access to municipal tap water. Pens were cleaned twice daily to prevent coprophagy.

During the standard workload phases, horses were exercised on a mechanical walker (Trojan Manufacturing Co., Iowa Park, TX) to create a DE demand of 175% of maintenance. The horses were conditioned to perform an average daily workload of 3.93 kg·km·10^{-3} with a DE demand of approximately 29 Mcal, based on an established formula (Anderson et al., 1983). Horses were exercised on the mechanical walker 5 d/wk, with a workout consisting of 3 min warm-up at the walk, 10 min trotting at 3.3 m/s, three 7-min bouts of cantering at 5.5 m/s, 10 min trotting and a 3 min cool-down at the walk, with 3-min walk breaks between trotting and cantering bouts, for a total of 10.99 km of nonwalking exercise.

A 4-d total collection for determination of Cu and Zn balance was conducted d 25 through d 28 of periods 4 and 7. Horses were confined to individual tie stalls and fitted with a urine collection harness to allow for separate and total collection of feces and urine. Frequent inspections of horses during collection reduced the risk of sample contamination and included thorough sweeping of floor surfaces to remove dirt and hair. To alleviate the effects of confinement, horses were walked and lightly trotted for 30 to 45 min on a mechanical walker each day of the total collection.

Fecal samples were placed in plastic buckets immediately after defecation. Every 3 h, feces for each horse were weighed and recorded, with a 10% aliquot added to a daily collection container. Contaminated fecal matter was weighed separately and weight recorded, but not added to the daily sample. Daily fecal samples were stored in plastic freezer-quality bags and frozen at −20°C for later analyses.

Urine was collected after every void to prevent contamination. Urine volume was measured in a plastic graduated cylinder and recorded, with a 10% aliquot added to a daily collection bottle. Any contaminated urine was measured and volume recorded, but not added to the daily composite. Urine initially was refrigerated in mineral-free collection bottles. At the end of each collection period, daily urine samples were strained through 3 layers of cheesecloth and individually frozen in mineral-free polypropylene tubes at −20°C until further analysis. Collection and measuring equipment was rinsed with distilled water between uses and thoroughly cleaned and rinsed between collection periods.

Concentrate and hay samples were obtained daily at feeding time during the total collection and stored in plastic bags for later analyses. Water intake was measured and recorded. One water sample per collection period was collected from the same source as the drinking water of the horses. The samples were frozen at −20°C until further analysis. Additional concentrate and hay samples were collected at random during the nonexperimental diet phases of the project and stored for later analyses.

In the laboratory, feed and fecal samples were dried at 65°C for a minimum of 72 h and ground in a Wiley mill with a 2-mm screen. Dried samples were stored in sealed, mineral-free plastic containers. Composites of hay, concentrate, and fecal samples were generated from individual daily samples to provide 1 sample per horse per treatment.

Copper and Zn concentrations of feed, fecal, urine, and water samples were determined by the Soil, Water and Forage Testing Laboratory at Texas A&M University, College Station, TX. Before shipment to the laboratory, submitted samples were placed in mineral-free polypropylene tubes, and assigned a sample ID number so as to blind laboratory technicians to sample identity. Corn stalk standard samples were similarly processed, labeled, and inserted randomly among the experiment samples to serve as control samples. Mineral content of the feed and fecal samples was determined by inductively coupled plasma analysis of a nitric acid digest (Havlin and Soltanpour, 1989). Water and urine samples were digested using a modified Kjeldahl procedure (Parkinson and Allen, 1975) before inductively coupled plasma analysis.

Resulting data were analyzed using STATA statistical software (College Station, TX). Mineral intake, excretion, and apparent absorption values were evaluated using ANOVA, with period, diet, and the interaction of diet and period in the model for all results reported herein. Differences were considered significant at $P < 0.05$ and trends noted at $P < 0.10$.

RESULTS AND DISCUSSION

One horse developed hind limb lameness during period 5 requiring several months of rest and was removed from the project. All other horses remained sound and healthy through the course of the project. One horse refused to consume concentrate during the final total collection. Consequently, all intake and mineral balance data for this horse during period 7 were omitted from statistical analyses.

Mean analyzed mineral concentrations of the depletion and repletion diets on a DM basis are presented in Table 4. During the depletion phases, the diet contained 50 to 56% of NRC (1989) values for Cu and 78 to 83% of Zn. Zinc content of the repletion diet was slightly greater than the requirement of 40 mg/kg, analyzing at 102 to 113% of the target value. Copper content of the repletion diet varied during the course of the project. During the first repletion period, Cu concentration of the diet was similar to the requirement of 10 mg/kg. Copper concentration of the diet fed during the second repletion period was 7.05 mg/kg, or 71% of the requirement. At the onset of the project, sufficient quantities of feed were ordered such that all depletion and repletion period concentrates should have come from single, uniform batches. However, damage to feed bags necessitated reordering of the repletion and depletion phase concentrates during the study. Variations in mineral concentration can be attributed to inherent variation in trace mineral content in grains and forages.

Table 4. Analyzed Cu and Zn concentrations of depletion and repletion diets[1] (DM basis) where mineral was provided in the sulfate form

Period	Diet	Cu, mg/kg	Zn, mg/kg
1	Depletion	5.05	33.35
2	Repletion	10.75	45.50
3	Depletion	5.05	33.35
5	Repletion	7.05	42.35
6	Depletion	5.60	31.00

[1]Diets consisted of 50% concentrate and 50% bermudagrass hay.

Mean mineral concentration of the sulfate diet was 6.28 mg/kg of Cu and 35.99 mg/kg of Zn on a DM basis, whereas the chelate diet contained 23.62 mg/kg of Cu and 90.67 mg/kg of Zn on a DM basis. Mineral content of the diets seemed to be different. A calculation error in the formulation of the organic chelate diet resulted in the Cu-Lys and Zn-Met being included at 10 times the rate needed. Further complicating the differences between expected and observed dietary mineral concentrations was variation in the mineral content of the hay. All hay was purchased in 1 lot and sampled before the beginning of the project for the purpose of diet formulation. Pre- and postproject hay analysis was performed by the same laboratory. The initial hay samples tested 6 mg/kg of Cu and 28 mg/kg of Zn on a DM basis, whereas grab samples taken throughout the project, including total collections, averaged 4.5 mg/kg of Cu and 24.14 mg/kg of Zn.

The mean daily Cu and Zn absorption and retention for horses consuming the sulfate and organic-chelate experimental diets are presented in Tables 5 and 6. Total daily mineral intake, including mineral consumed from both feed and water, was used to calculate balance data. Mineral composition of the tap water during periods 4 and 7 averaged 0.156 mg/kg of Cu and 0.380 mg/kg of Zn. When combined with daily individual water intake data, 3.97% of daily Cu intake and 1.94% of daily Zn intake were attributed to water intake.

Copper intake and fecal excretion were greater ($P < 0.001$) for horses consuming the organic chelate diet. Although apparent absorption was not different between treatments, the difference ($P = 0.03$) in absorption as a percentage of intake reflected the disparity in Cu intake between diets. Urinary Cu excretion and apparent retention were not different. Copper retention between the diets was different when expressed as a percentage of intake ($P < 0.001$) but not as a percentage of the mineral absorbed. There was a trend ($P = 0.07$) for a period difference and a period × diet interaction ($P = 0.03$) for Cu absorbed as a percentage

Table 5. Apparent daily Cu absorption and retention of exercising horses fed mineral in the sulfate or organic chelate form

	Sulfate		Organic chelate		
Item	Mean	SEM	Mean	SEM	P-value
Intake, mg/100 kg of BW	10.53	0.62	35.73	1.27	<0.001
Fecal excretion, mg/100 kg of BW	13.15	0.66	38.59	1.89	<0.001
Absorbed, mg/100 kg of BW	−2.62	0.63	−2.85	0.89	0.991
% of intake	−26.14	6.83	−7.71	2.29	0.003
Urinary excretion, mg/100 kg of BW	3.43	1.02	3.46	1.50	0.947
Retained, mg/100 kg of BW	−6.05	1.19	−6.32	1.61	0.946
% of intake	−57.94	10.74	−17.21	4.19	0.001
% of absorbed	331.10	102.31	93.19	182.08	0.393

of intake, although no other differences or trends were noted for period or period × diet interactions regarding Cu balance.

Similarly, zinc intake and fecal excretion were greater ($P < 0.001$) for horses consuming the organic chelate-supplemented diet. No other differences were found between the diets for absorption, absorption as a percentage of intake, retention, retention as a percentage of intake, or retention as a percentage of Zn absorbed. There was a difference by period for urinary Zn excretion ($P = 0.03$), although no other period differences or trends were observed. Trends toward period × diet interactions were observed for Zn absorption ($P = 0.06$) and Zn retention ($P = 0.08$) but not for other measures of Zn balance.

The absorption and retention values in the present study are very different from those reported by other researchers. Ponies consuming 5.64 to 25.67 mg of Cu/100 kg of BW had fecal Cu excretion in the range of 2.88 to 14.18 mg of Cu/100 kg of BW, whereas urinary excretion remained constant at 0.12 mg of Cu/100 kg of BW (Cymbaluk et al., 1981). Schryver et al. (1980) also reported decreased urinary excretion of Zn (0.7 to 1.0 mg of Zn/100 kg of BW) in ponies consuming 36.8 to 310.7 mg of Zn/100 kg of BW, with a subsequent fecal Zn excretion of 34.3 to 264.7 mg/100 kg of BW. Ponies consuming an average of 11.65 mg of Cu/100 kg of BW while fed increasing concentrations of Zn had an average fecal excretion of 5.9 mg/100 kg of BW and urinary excretion of 0.05 mg/100 kg of BW (Hoyt et al., 1995). Urinary excretion of Zn for these ponies averaged 1.0 mg/100 kg of BW despite increasing intakes of Zn ranging from 76.6 to 606.8 mg/100 kg of BW.

The reduced absorption observed in the present study would normally indicate horses in a state of negative mineral balance, or deficient mineral intake. However, horses fed the organic chelate diet were consuming 2 to 3 times the NRC (1989) recommended intakes for Cu and Zn. Horses on both diets had unexpectedly increased concentrations of Cu and Zn in their feces, and mineral concentration in the urine exceeded other published values. On the surface, this might indicate widespread sample contamination during the total collection process. As detailed in the Materials and Methods section, typical procedures for a mineral balance study were followed. The urine collection harnesses did contain some rubber components, which have the potential to release Zn. However, the urine was in contact with these surfaces for a very brief time between the voiding of urine by the horse and the draining into the plastic collection vessel. This could have been a potential source of Zn in the urine, but it does not account for the unexpected concentration of Cu in the urine samples.

Another possible explanation for increased Zn and Cu concentrations in the urine could be a response to the exercise protocol of the horses. In a review paper focused on human athletes, Lukaski (2000) suggested changes in urinary Zn excretion can reflect increased

Table 6. Apparent daily zinc absorption and retention of exercising horses fed mineral in the sulfate or organic chelate form

	Sulfate		Organic chelate		
Item	Mean	SEM	Mean	SEM	P-value
Intake, mg/100 kg of BW	57.72	2.02	136.48	5.17	<0.001
Fecal excretion, mg/100 kg of BW	72.14	3.56	153.65	5.38	<0.001
Absorbed, mg/100 kg of BW	−14.43	4.54	−17.17	6.11	0.882
% of intake	−26.34	9.04	−13.63	5.23	0.164
Urinary excretion, mg/100 kg of BW	3.53	0.77	3.69	0.52	0.730
Retained, mg/100 kg of BW	−17.96	4.81	−20.85	6.21	0.854
% of intake	−32.48	9.67	−16.34	5.32	0.115
% of absorbed	41.05	99.10	114.44	15.55	0.517

skeletal muscle turnover and subsequent Zn loss from muscle cells. Urinary excretion of Zn along with sequestering of Zn in the liver seems to serve as a homeostatic mechanism regulating circulating concentrations of Zn in human athletes. Lukaski (2000) went on to summarize several studies that reported increased urinary Zn output in response to both acute exercise and long-term physical activity. A review by Campbell and Anderson (1987) also reported increases in urinary Zn losses, as well as urinary and fecal Cu losses, on exercise days compared with rest days for human athletes. Work by Kikukawa and Kobayashi (2002) noted an increase in urinary Cu and Zn after exercise, but there were no changes in concentrations of urinary Cu and Zn before exercise during the multi-month training program for air rescue trainees. There is a lack of information regarding changes in equine urinary mineral outputs in response to acute and long-term exercise. Based on studies in human athletes, it is plausible to expect an increase in equine urinary concentrations of Cu and Zn on exercise days compared with nonexercise days. However, horses in the present study were confined to tie stalls with limited exercise and restricted to 30-min walking and light trotting during the total collection period.

Previous trace mineral balance research from this laboratory compared absorption and retention of Cu and Zn in mature, idle horses and concluded that there were no differences in absorption among sulfate, oxide, and organic chelate forms of supplementation under that metabolic state (Wagner et al., 2005). That study also utilized a 10-d diet adaptation period, which may not have been of sufficient length for differences in absorption to become apparent. Other balance studies performed in horses had differing results. Miller et al. (2003) fed an unsupplemented concentrate, an inorganic supplemented concentrate, or an inorganic-organic blend supplemented concentrate to yearling geldings and found greater Cu digestibility, apparent Cu balance, Cu balance as a percentage of intake, and apparent Zn balance for horses consuming the organic supplement. In a study of similar design using mature horses, Baker et al. (2005) found that apparent Cu digestibility, Cu balance, apparent Zn digestibility, and Zn balance were greater for horses consuming the concentrate supplemented with the inorganic mineral sources.

With that research in mind, the present study was designed to determine if exercise would affect absorption and retention of Cu and Zn when the horses were fed a marginally deficient diet, with the mineral supplementation provided in the sulfate or organic chelate form. Controlled depletion and repletion periods were incorporated in the project design in an effort to standardize the mineral status of the horses before feeding of the experimental diets. Horses performed an average daily workload of 3.93 kg·km·10^{-3} with a DE demand of approximately 29 Mcal in an effort to create a state of increased mineral demand. A formulation error resulted in horses on the organic chelate diet consuming about 3 times the amount of Cu and Zn of that provided in the sulfate-supplemented diet. Fecal mineral excretion for the groups reflected this disparity in mineral intake. Apparent Cu absorption as a percentage of intake and retention as a percentage of intake seemed to be greater when the horses consumed Cu-Lys compared with $CuSO_4$. However, there were no differences in apparent Zn balance. Further investigation is needed to conclusively determine factors affecting Cu and Zn balance in the exercising horse.

LITERATURE CITED

Anderson, C. E., G. D. Potter, J. L. Kreider, and C. C. Courtney. 1983. Digestible energy requirements for exercising horses. J. Anim. Sci. 56:91–95.

Ashmead, H. D., D. J. Graff, and H. H. Ashmead. 1985. Summary and implications. Pages 213–232 in Intestinal Absorption of Metal Ions and Chelates. Charles C. Thomas, Springfield, IL.

Baker, D. H., and C. B. Ammerman. 1995a. Copper bioavailability. Pages 127–156 in Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins. D. H. Baker, C. B. Ammerman, and A. J. Lewis, ed. Academic Press Inc., San Diego, CA.

Baker, D. H., and C. B. Ammerman. 1995b. Zinc bioavailability. Pages 367–398 in Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins. D. H. Baker, C. B. Ammerman, and A. J. Lewis, ed. Academic Press Inc., San Diego, CA.

Baker, L. A., M. R. Wrigley, J. L. Pipken, J. T. Haliburton, and R. C. Bachman. 2005. Digestibility and retention of inorganic and organic sources of copper and zinc in mature horses. Pages 162–165 in 19th Equine Sci. Symp., Tuscon, AZ. Equine Sci. Soc., Champaign, IL.

Campbell, W. W., and R. A. Anderson. 1987. Effects of aerobic exercise and training on the trace minerals chromium, zinc and copper. Sports Med. 4:9–18.

Cymbaluk, N. F., H. F. Schryver, and H. F. Hintz. 1981. Copper metabolism and requirement in mature ponies. J. Nutr. 111:87–95.

Havlin, J. L., and P. N. Soltanpour. 1989. A nitric acid and plant digest method for use with inductively coupled plasma spectrometry. Commun. Soil Sci. Plant Anal. 14:969–980.

Henneke, D. R., G. D. Potter, J. L. Kreider, and B. F. Yeates. 1983. Relationship between condition score, physical measurements and body fat percentage in mares. Equine Vet. J. 15:371–372.

Hoyt, J. K., G. D. Potter, L. W. Greene, and J. J. G. Anderson. 1995. Copper balance in Miniature Horses fed varying amounts of zinc. J. Eq. Vet. Sci. 15:357–359.

Kikukawa, A., and A. Kobayashi. 2002. Changes in urinary zinc and copper with strenuous physical exercise. Aviat. Space Environ. Med. 73:991–995.

Lukaski, H. C. 2000. Magnesium, zinc, and chromium nutriture and physical activity. Am. J. Clin. Nutr. 72:585S–593S.

Miller, E. D., L. A. Baker, J. L. Pipken, R. C. Bachman, J. T. Haliburton, and G. O. Veneklasen. 2003. The effect of supplemental inorganic and organic forms of copper and zinc on digestibility in yearling geldings in training. Pages 107–112 in 18th Equine Nutr. Phys. Symp., East Lansing, MI. Equine Sci. Soc., Champaign, IL.

NRC. 1989. Nutrient Requirements of Horses. 5th rev. ed. Natl. Acad. Press, Washington, DC.

Ott, E. A., and R. L. Asquith. 1994. Trace mineral supplementation of broodmares. J. Eq. Vet. Sci. 14:93–101.

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. 21:287–292.

Parkinson, J. A., and S. E. Allen. 1975. A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. Commun. Soil Sci. Plant Anal. 6:1–11.

Schryver, H. F., H. F. Hintz, and J. E. Lowe. 1980. Absorption, excretion and tissue distribution of stable zinc and ^{65}zinc in ponies. J. Anim. Sci. 51:896–902.

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. Pages 419–420 in 17th Equine Nutr. Phys. Symp, Lexington, KY. Equine Sci. Soc., Champaign, 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. Pages 143–144 in 17th Equine Nutr. Phys. Symp., Lexington, KY. Equine Sci. Soc., Champaign, IL.

Wagner, E. L., G. D. Potter, E. M. Eller, P. G. Gibbs, and D. M. Hood. 2005. Absorption and retention of trace minerals in adult horses. Prof. Anim. Sci. 21:207–211.