Dietary influence on serum copper and zinc concentrations in horses

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Summary

Serum copper and zinc were determined in five groups of horses on four different farms. All but one of the farms were feeding rations that met or exceeded recommended levels of the trace minerals. Three of the groups were experiencing problems potentially related to copper deficits. Serum copper and zinc varied ($p < .05$) with time of year and herd, though not between feeds in within a herd. Trace minerals such as iron, aluminum and selenium present in the water, pasture or feed, respectively may have contributed to the variability in serum copper and zinc observed. When determining dietary adequacy of trace minerals in horses showing clinical signs potentially related to deficits, more than just the feed content of the minerals suspected need to be evaluated.

Einfluß der Ernährung auf die Konzentrationen von Kupfer und Zink im Serum

In 5 Gruppen von Pferden, die aus 4 Farmen stammten, wurden die Kupfer- und Zinkgehalte im Serum bestimmt. Mit einer Ausnahme wurden Rationen gefüttert, die den gängigen Empfehlungen zur Spurenelementversorgung entsprachen. Drei Gruppen zeigten Probleme, die möglicherweise mit einem Kupferdefizit in Zusammenhang standen. Die Kupfer- und Zinkgehalte im Serum variierten ($p < 0,05$) in Abhängigkeit von Jahreszeit und Herde, aber nicht infolge der Fütterung innerhalb einer Herde. Spurenelemente wie Eisen, Aluminium und Selen im Trinkwasser, Gras oder Futter haben möglicherweise zu den beobachteten Variationen der Kupfer- und Zinkgehalte im Serum beigetragen. Bei Untersuchungen zur Ausgewogenheit der Spurenelementversorgung sollte daher bei klinischen Erscheinungen, die mit einem bestimmten Element in Zusammenhang stehen können, eine umfassendere Analyse erstellt werden.

Introduction

In the past 15 years, dietary copper and zinc in horses have received considerable scientific attention. Field reports have suggested a correlation between low copper and/or zinc intakes and developmental orthopedic disease in foals (*Bridges* et *al.*, 1984; *Knight* et *al.*, 1985). Most controlled studies of copper and zinc supplementation have focussed upon broodmares and foals (*Baucus* et *al.*, 1987; *Breedveld* et *al.*, 1987; *Bridges* and *Harris*, 1988; *Bridges* and *Moffitt*, 1990; *Burton* and *Hurtig*, 1991; *Coger* et *al.*, 1987; *Hurtig* et *al.*, 1990; *Knight* et *al.*, 1988; *Ott* and *Asquith*, 1987; *Thomas* et *al.*, 1987).

In general, dietary copper and zinc intake by horses is considered to be important, though what constitutes "adequate" intake is controversial (*Cymbaluk* and *Christensen*, 1986; *Cymbaluk* et *al.*, 1981, 1981a; *Glade*, 1986; *Hintz*, 1985; *NRC*, 1989).

Diagnosis of mineral deficiencies commonly involves analyses of mineral content of the diet, blood and tissue samples and presence or absence of clinical signs (*Ralston*, 1991). While feed analyses are commonly employed both in field and controlled experiements, analyses of only the copper and zinc of feeds used is not sufficient. Many other minerals may interfere with the absorption/utilization of the trace minerals (*Cymbaluk* et *al.*, 1981b; *Lawrence* et *al.*, 1987; *Schryver* et *al.*, 1986; *Stowe*, 1980). In addition, feed analyses may not accurately reflect actual dietary content of the trace minerals due to either inaccurate sampling or laboratory error (*Ralston*, 1991; *Ralston* and *LeRoy*, 1991).

Hepatic and renal concentrations of copper and zinc are fairly accurate measures of dietary adequacy (*Cymbaluk* and *Christensen*, 1986; *Mills*, 1987), however use of these tissues is impractical in most field situations. Individual serum samples may not be diagnostic for deficits of copper and perhaps zinc due to variability, both within individuals and over time (*Baucus* et *al.*, 1987; *Smith* et *al.*, 1983; *Thomas* et *al.*, 1987). Serum ceruloplasmin correlates with serum copper but apparently does not confer greater information regarding copper status than does serum copper in this species (*Smith* et *al.*, 1983). Mean serum concentrations of copper or zinc in a herd may reflect adequacy, especially if clinical signs associated with deficits or excesses of the mineral are present (*Mills*, 1987; *Ralston*, 1991). Typical responses to copper deficits in other species are considered to be reduced ceruloplasmin with concommitant reduction in neutrophil function, cardiac enlargement and fragility, skeletal abnormalities and anemia (*Mills*, 1987). In horses, copper deficits have been associated with skeletal abnormalities, arterial rupture and perhaps pigmentation loss (*Bridges* et *al.*, 1984; *Bridges* and *Harris*, 1988; *Burton* and *Hurtig*, 1991; *Hildebran* and *Hunt*, 1986; *Hurtig* et *al.*, 1990; *Knight* et *al.*, 1985, 1988; *Mills*, 1987). Zinc deficiency has not been proven in horses to date. Zinc excess has been associated with conditioned copper deficits in foals and elevated serum zinc concentrations (*Bridges* et *al.*, 1984).

This survey was prompted by the report of potentially copper responsive problems (an increased incidence of developmental orthopedic disease, arterial rupture in periparturient mares, pigmentation loss and/or anemia) on three farms. Results of serum copper and zinc analyses were compared to diet mineral intakes.

[1] Horse Chow 100, Pure Pride 100, Purina Mills, Inc., St. Louis, Mo.

Materials and Methods

Five groups of horses on four farms were sampled. Herd 1 consisted of 9 standardbred and one anglo-arabian mare used to test two types of commercial pelleted ration[1] at Cook College, Rutgers University. The horses were fed

Tab. 1: Ration, water pasture concentrations of copper (ppm), zinc (ppm) and iron (ppm). Ration and pasture values are on a 100 % dry matter basis.

Farm	Ration concentration of			Water* Iron (ppm)	Pasture		
	Copper	Zinc	Iron		Copper	Zinc	Iron
1	16	78	75	9.0	NA	NA	NA
2A**	11	79	180	.07	4	20	142
2B**	11–19	79–84	180–200	.07	4	20	142
3	19	54	177	.02	ND	ND	ND
4	5	14	61	.02	7	20	261

* Water, copper and zinc were less than 0,02 ppm on all farms
** Rations with different copper/zinc content fed in crossover NA = not available to horses sampled; ND = not determined

measured amounts of grain and grass hay and did not have access to pasture during the sampling period (January to June, 1990). Trace mineral salt was available free choice though intakes were not recorded. Mean packed cell volume in these mares was 33 ± .8 % which was low ($p < .05$) relative to other herds evaluated by our laboratory. Herd 2A contained 10 warmblood and 10 arabian horses at a private training facility being used to compare two types of extruded feed[2]. Measured amounts were fed daily with alfalfa hay. Trace mineral salt was available free choice but intakes were not monitored. These horses were maintained in stalls, with limited (2 – 3 hrs per day) access to pasture. Mean packed cell volume for these horses was 36.5 ±.5 % and there were no problems attributable to copper or zinc imbalances observed. Herd 2B consisted of 8 nature Arabian mares maintained on pasture on farm 2 (herd 2B). Four of these mares were fed the same hay, water and extruded feed[3] as in herd 2A, the other four were fed a sweet feed[4] instead of extruded feed. Mean packed cell volume in these mares was 37.2 ± 1.5 % but two of the mares developed periocular pigmentation loss early in the study. Herd 3 was on Standardbred breeding farm where the mares were maintained on pasture and fed pelleted concentrates[5] and alfalfa hay. This herd had an increase in arterial/ligamentous ruptures in periparturient mares (4/33) in 1990 relative to the past 10 years (0 in over 60 mares per year). Herd 4 was a group of thoroughbred broodmares and two year olds in training on a farm in New York which were fed free choice alfalfa hay, 4 to 6 kg oats, sea salt (50 gm), kelp (30 gm) and bee pollen (50 gm) per horse per day. No problems attributable to copper or zinc imbalances were observed in herd 4. Trace mineral salt was available free choice on both farms 3 and 4.

Representative water, feed and pasture samples (*Ralston,* 1991) were obtained from all farms. Amounts of hay and grain fed per horse per day were recorded. Blood for serum copper, zinc and blood packed cell volume[6] was drawn by venipuncture from horses in herds 1 and 2A before feeding in the morning at approximately 1 month intervals from January through May, 1990. The other herds were sampled for serum copper and zinc once in April – May, 1990.

Serum and water minerals were determined by atomic absorption. Argon spectroscopy[7] was used to determine mineral content of feeds. Ration copper and zinc intake were calculated based on the analyses and feed intakes.

Data were compared between groups factoring the effects of feed, water iron and group on serum copper and zinc by GLM-ANOVA and Duncan's Multiple range Test. Differences between means were assessed by Duncans' between farms or between treatment groups on farms 1 and 2 by two tailed T-Tests. Significance was set at $p < .05$, with tendencies considered to be $p < .10$.

Results and Discussion

All horses were receiving rations that met or exceeded the National Research Council (1989) recommendations for

Tab. 2: Serum Copper and zinc (ppm). Values are means +/− SE.

Herd	N	Serum Copper	Serum Zinc
1	8*	1.03 +/− .03 a	.60 +/− .03 a
2 A	20*	1.66 +/− .03 c	.79 +/− .02 b
2 B	8	1.40 +/− .09 b	.65 +/− .04 a
3	33	1.32 +/− .05 b	.62 +/− .03 a
4	30	1.36 +/− .07 b	.73 +/− .03 b

a, b, c Means within a column with different superscripts differ, $p < .05$, GLM-ANOVA and Duncan's Multiple Range Test

[2] Manna Pro Elite-14, Manna Pro Corp., Los Angeles, Calif., Agway Performance, Agway Feeds, Inc.
[3] Manna Pro Elite-14, Manna Pro Corp., Los Angeles, Calif.
[4] Manna Pro Sweet 10, Manna Pro Corp., Los Angeles, Calif.
[5] Pure Pride 200
[6] QBC-V Veterinary Hematology Analyzer, Becton-Dickinson, Sparks, MD.
[7] Triple S Laboratory, Loveland, CO.

energy, protein, calcium and phosphorus for mature horses. Feed, water and pasture contents of copper, zinc and iron on the farms are given in Table 1.

Serum copper and zinc were lower ($p < .05$) in the mares on farm 1 than any of the other farms (Table 2). Serum copper was higher ($p < .01$) in herd 2A than any of the other herds, though ration intake was comparable to herds 1, 2B and 3. Serum copper varied over time in herd 2A ($p < .001$) but not herd 1 ($p = .89$) whereas serum zinc differed between sampling periods ($p < .001$) in both herds (Table 3). There were no differences ($p > .05$) between feed treatment groups in herds 1 or 2A. Feed content of copper and zinc alone did not explain the variation in serum levels of these two minerals ($p > .5$). However other sources of trace minerals may have affected copper and/or zinc availability in these herds. There was a tendency ($p = .07$) for iron content of water to correlate with reduced serum copper and zinc concentrations. Subsequent supplementation with copper sulfate (180 mg/horse/day) resulted in increased packed cell volume ($p < .05$) and serum copper in herd 1 (*Ralston,* 1992, unpublished data). The pasture on farm 2 contained 193 ppm aluminum which may have reduced availability of copper and zinc (*Schryver* et *al.,* 1986; *Ralston* and *LeRoy,* 1991), in herd 2B relative to the stall maintained herd 2A despite low forage availability during the study. Herd 3 mares had been fed a ration with increased selenium in 1989 - 1990. Though pasture grasses were not sampled on this farm, other pastures in the area tended to have 6 ppm copper or less on a dry matter basis (*Ralston* and *LeRoy,* 1990. unpublished data). Increased selenium intake may have increased metabolic excretion of copper (*Stowe,* 1980) and resulted in a conditioned copper deficit in this herd.

In the absence of other major mineral imbalances, free choice trace mineral intake may compensate for low dietary intakes of copper and zinc, as suggested by herd 4 data. More data is needed on factors which alter bioavailability of copper and zinc need in horses.

Tab. 3: Effect of sample date on serum copper and zinc in herds 1 and 2 A

Sample Date	Serum Copper (ppm)	Serum Zinc (ppm)
Herd 2 A*		
January	1.87 +/- .05 a	1.01 +/- .02 a
February	1.92 +/- .04 a	.94 +/- .02 a
April	1.29 +/- .05 b	.59 +/- .02 b
May	1.51 +/- .05 a, b	.61 +/- .02 a, b
Herd 1**		
January	1.05 +/- .05 a	.49 +/- .03 a
April	1.18 +/- .06 a	.61 +/- .03 b
May	1.06 +/- .06 a	.73 +/- .03 b

* Values are means +/- SE for 20 horses at each sampling time. There were no differences between treatment groups within the sampling times ($p > .15$)

** Values are means +/- SE for 8 horses at each sampling date. There were no differences (p >) between treatment groups within a time period

a, b, c Means within a column with different superscripts differ, $p < .01$, GLM-ANOVA and Duncan's Multiple Range Test

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