SHORT COMMUNICATION

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Effect of chelated mineral supplementation on the absorption of Cu, Fe, K, Mn and Zn in horse hair

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The supplement effect of Cu, Fe, K, Mg, Mn and Zn chelated with glycine on the absorption of these elements by animals was evaluated. The evaluation was carried out by comparing the mineral concentrations found in hair of the animals before and after 47 days of supplementation with a combination of glycine-mineral chelates. Five horses known to be clinically well were used for this study. Instrumental neutron activation analysis (INAA) was applied to the determination of Cu, Fe, K, Mg, Mn and Zn concentrations in the horse hair samples. It was observed that the supplementation helps the Fe, K and Zn absorption by the animal organism. The data obtained suggest that the hair is a suitable material to investigate Fe, K and Zn in mineral metabolism in the animal organism.

Introduction

Minerals including Fe, Cu, Mg, K, Mn and Zn work as co-enzymes to make blood, bone, other tissues and body fluids better. They are involved in neural transmission and enzyme and hormone production, consequently in the general performance of the animals.[1]

The development of organic trace mineral supplements has centered around the theory that they are more bioavailable than inorganic forms. In the chelate or complex form the mineral would be protected from forming complexes with other dietary components that inhibit absorption and thus allowed for greater absorption by the body.[2] PAIK[3] carried out a series of experiments to determine the effects of chelated minerals on the performance of pigs, chickens and dairy cows. Minerals (Cu, Mn, Zn) chelated with methionine were used and it was concluded that the chelated minerals were effective in improving the performance of animals when the chelated minerals at the lowest supplementary level was compared to inorganic minerals, especially at the pharmacological levels. ROJAS et al.[4] compared Zn and Cu absorption in the serum, liver, pancreas, kidney, bone, hair and neck muscle of 32 yearling cattle supplemented with zinc-methionine and with $ZnSO_4$ and ZnO. Analyses of mineral contents in hair samples have been also used to evaluate both the differences in the concentrations of essential elements in diets and the health status of domestic animals.[5,6]

The present work aims at evaluating supplement effect using a combination of Cu, Fe, Mg, Mn and Zn chelated with glycine and glycine-potassium complex on the absorption of these elements by animal and also to know which element in the hair is a suitable subject for investigation on mineral metabolism. This combination of glycine-mineral chelates and glycine-potassium complex is usually given to horses that participate in competitions. This evaluation was carried out by comparing mineral concentrations found in hair before and after 47 days animals have been supplemented. Five horses known to be clinically well were used for this study. The horse hair samples were cut in the lateral part from the neck. Instrumental neutron activation analysis (INAA) followed by gamma-ray spectrometry was applied to estimate the concentrations of Cu, Fe, K, Mg, Mn and Zn in hair samples.

Experimental

Management of animals and sample collection

Experimentation took place at the horse livestock field of the Military Police of São Paulo State. Five horses, at the mean age of 8 years were randomly chosen from the group of animals that have participated in competitions. Every animal was clinically well.

In order to study the effect of supplementation with a combination of glycine-mineral chelates and glycine-potassium complex two horse hair samples were collected from each animal. The first hair samples were collected from the lateral part of the neck, before supplementation. After all the animals individually received 24 g organic mineral supplement per day during 47 days. Both ration and source of the water were just the same. This supplement was a combination of glicyne-mineral chelates with the following mineral composition: Cu (0.4%); Fe (1.0%); Mg (2.5%); Mn (0.7%) and Zn (1.4%) and complex with K (4.6%). In this case, the horses received additional minerals in excess of the amount currently recommended.

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Table 1. Cu, Fe, K, Mg, Mn and Zn concentrations (in μg/g) obtained in horse hair before and after chelate mineral supplementation

Animal	Cu		Fe		K		Mg		Mn		Zn	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	22	10	22	24	3.9	11.4	1453	580	5.1	2.6	74	101
2	9	17	25	32	8.0	14.0	279	347	4.7	8.4	99	133
3	14	9	13	23	5.7	21.0	490	562	2.0	3.1	89	104
4	11	10	22	32	1.0	8.3	221	185	2.5	2.9	98	118
5	23	8	10	27	5.4	20.1	837	293	3.7	2.1	70	95

The precision of the data estimated from counting statistics were 9, 4, 10, 9, 2, and 1% for Cu, Fe, K, Mg, Mn and Zn, respectively.

In the following day after the supplementation was stopped, hair samples were collected in the same part of body to verify if the mineral supplementation produced significant increase in the absorption of minerals by the animals.

Preparation of samples and standards

The horse hair samples were first rinsed once with a 2% Triton X100 solution and three times with water to remove coarse external dirt. The horse hair was cut into approximately 3 mm pieces and then it was washed twice with 2% Triton solution, three times with distilled and de-ionized water, finally with acetone P.A. (Merck), and dried at 45 °C for 30 hours.

Aliquots of hair samples weighing about 80 mg were sealed in clean polyethylene bags for irradiation.

Standard solutions of the Cu, Fe, K, Mg, Mn and Zn were prepared by dissolution of high purity metals, oxides or salts of elements in suitable reagents. Aliquots (50–100 μl) taken from those solutions were pipetted on analytical filter paper (Whatman No. 42) for irradiation. After drying, these filter papers were placed into polyethylene bags.

The standards contained: Cu (52.9 μg); Fe (205 μg); K (395 μg); Mg (550 μg); Mn (4.49 μg) and Zn (24.3 μg). Two certified reference materials were analyzed to verify the accuracy of the method. Cu, K, Mg and Mn were determined in the Oyster Tissue (NIST 1566[a]) and Fe and Zn were determined in the Rice Flour (NIES-CRM-10C).

Irradiation and counting

Two types of irradiation were carried out at the IEA-R1 nuclear research reactor. In one case, the sample and standards (Cu, K, Mg, Mn) were irradiated together in nylon container for 4 minutes and after a decay time of 2 minutes the ^{66}Cu and ^{27}Mg radionuclides (1039 and 1013 keV, respectively) were measured in the sample and in the standards. The ^{42}K and ^{56}Mn radionuclides (1524 and 846 keV, respectively) were measured after 90 minutes of decay time. In the second irradiation, the sample and standards (Fe, Zn) were irradiated together in an aluminum container for 8 hours. Measurements were carried out after, at least, 7 days of decay time to detect ^{59}Fe (1099 keV) and ^{65}Zn (1115 keV). The thermal neutron flux utilized ranged from 10^{11} to 10^{13} n·cm^{-2}·s^{-1}.

The equipment used to measure the gamma-radiation was a Canberra Model GX2020 hyperpure Ge detector coupled to model 1510 Integrated Signal Processor and MCA System 100, both from Canberra. The detector used had a resolution (FWHM) of 0.9 keV for 122 keV gamma-ray of ^{57}Co and 1.9 keV for 1332 keV gamma-ray of ^{60}Co.

Results and discussion

Four determinations were obtained for each one of the elements Cu, Fe, K, Mg, Mn and Zn in certified reference materials and arithmetic means values were calculated. The deviations of these arithmetic means from the certified values were lower than 9%.

The concentration of Cu, Fe, K, Mg, Mn and Zn in horse hair, determined by INAA are presented in Table 1. These results are individual determinations. The relative errors due to statistical counting errors were about 9, 4, 10, 9, 2 and 1% for Cu, Fe, K, Mg, Mn and Zn, respectively.

Differences between mineral concentrations of hair samples collected before and after supplementation (Table 1) were determined using the statistical *t* test appropriate for observations in pairs.[7] There were significant differences (p<0.05) in Fe, K and Zn concentrations between the samples suggesting that supplementation increases the absorption of these elements by the animal organism. However, it cannot be said that the increase in Fe, K and Zn concentrations was due to Fe-glycine , K-glycine, Zn-glycine or the combination of these six mineral complexes that make part of the supplement. On the other hand, the supplementation had not significant effect (p<0.05) on the Cu, Mg and Mn concentrations in the horse hair.

Conclusions

The results show that INAA is sensitive enough to determine Cu, Fe, K, Mg, Mn and Zn concentrations in the horse hair. The data obtained suggest that hair is a suitable subject for investigation of Fe, K and Zn on mineral metabolism in the animal.

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