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Application of neutron activation analysis to evaluate the health status of equines by means of Cu, Fe, Mn and Zn determinations in their hair

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Instrumental neutron activation analysis (INAA) was applied to evaluate the clinical status of equines, belonging to the Military Police of São Paulo State, by means of Cu, Fe, Mn and Zn determinations in their hair. Comparison of the results obtained in these analyses with reference values indicated Zn deficiency in the equines, Fe is in the minimum limit and the elements Cu and Mn are within the normal range.

Introduction

Micro or trace minerals are involved in nerve transmission and in the production of enzymes and hormones in the organisms of the animal. The analysis of the mineral composition of the hair of domestic animals has been proposed as a method to evaluate their nutritional status. However, SIPPEL et al.[1] believed that horse hair analyses may be useful, but that the method should be considered experimental. WYSOCKI and KLETT[2] concluded that levels of Ca and P in the diet are not correlated with the amounts in the ponies' hair. CAPE and HINTZ[3] verified that many factors can influence the mineral content from equine hair such as: age, month of collection, hair color and changes in nutrition. In the case of nutrition, an increase of Mo concentration was observed in the hair of the ponies fed with excessive amounts of this element.

In the present work, instrumental neutron activation analysis (INAA) followed by gamma-ray spectrometry was applied to estimate the concentrations of Cu, Fe, Mn and Zn in hair samples from ten equines, belonging to Military Police of São Paulo State. The comparison of the results obtained in these analyses with reference values was considered to evaluate the health status of animals in relation to the elements analysed.

INAA is a method that involves a minimum sample handling and has been applied to the analysis of biological materials by several researchers[4–7] during the last years.

Experimental

Sample collection and preparation

Equine hair samples were collected from ten animals belonging to Military Police of São Paulo State, were assigned randomly. Samples were collected from the neck of animals coded as P23; 115; 146; 338; 347; 358; 401; 568; 710 and 732.

The equine hair samples were first rinsed once with a 2% Triton X100 solution and three times with water to remove coarse external dirt. The equine hair was cut into approximately 3 mm pieces and it was then washed twice with a 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 150 mg were sealed in clean polyethylene bags for irradiation.

Preparation of standards

Standard solutions of the Cu, Fe, Mn and Zn were prepared by dissolution of the high purity metals, oxides or salts of the elements in suitable reagents. Aliquots (50–100 µl) taken from such 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); Mn (4.49 µg) and Zn (24.3 µg). The accuracy of elemental standards used in this work was confirmed by Cu, Mn determination in the NIST 1566a Oyster Tissue and Fe, Zn in the NIES-CRM-10C Rice Flour.

Irradiation and counting

Two types of irradiation were carried out at the IEA-R1m nuclear research reactor. In one case, the sample and standards (Cu, Mn) were irradiated together in nylon container, for 4 minutes and after a decay time of 2 minutes the ^{66}Cu radionuclide (in 1039 keV) was measured in the sample and in the Cu standard afterwards. The ^{56}Mn radionuclide (in 846 keV) was measured after 90 minutes of decay time. In the second irradiation, the sample and standards (Fe, Zn) were irradiated together in aluminum container, for a period of 8 hours. Measurements were carried out after, at least, 7 days to detect ^{59}Fe (in 1099 keV) and ^{65}Zn (in 1115 keV). The thermal neutron flux utilized ranged from 10^{11} to 10^{13} $n\cdot cm^{-2}\cdot s^{-1}$.

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Table 1. Cu, Fe, Mn and Zn concentrations (in µg/g) obtained in equine hair by INAA

Animal	Cu	Fe	Mn	Zn
P23	9.0	21	2.77	120*
115	35.6**	42	5.42	116*
146	10.2	20	2.59	101*
338	6.0	33	1.75	120*
347	8.9	15*	2.63	95*
358	8.4	30	2.64	113*
401	54.5**	32	4.27	126*
568	22.2**	33	4.19	109*
710	9.0	24	3.04	110*
732	6.5	21	4.53	103*
Ref.values[8] (min.–max.)	(2.42–20)	(18–156)	(0.05–6.51)	(128–187)

* Low.
** High.
Error for each value, in case of Cu, Fe, Mn and Zn is 10, 3, 3 and 1%, respectively.

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. Data reduction was carried out using IBM/PC microcomputer and VISPECT2 software in Turbo Basic language for spectral analysis.

Results and discussion

The accuracy of the results elemental standards were lower than 6.6% for the elements determined in both certified reference materials analysed.

Concentration of Cu, Fe, Mn and Zn in equine hair are presented in Table 1. These results are individual determinations. The relative errors of these individual determinations obtained using statistical counting errors were about 10, 3, 3, and 1% for Cu, Fe, Mn and Zn, respectively.

Comparison of the results obtained in these analyses with reference values indicated Zn deficiency in the equines, Fe is in the minimum limit and the elements Cu and Mn are within the normal range for horses.[8]

Zinc is an important essential element for numerous animal species. Most of this element is circumscribed to plasmatic proteins. Zinc activates some enzymes and is a component of a large number of important metalloenzymes, such as: DNA polymerase, alkaline phosphatase, ribonuclease, and others.[9] The zinc deficiency can cause consequences such as: growth retardation, skin lesions, skeletal abnormalities, etc. In this work, it was observed in the equines: roughness of hair, small skin exfoliation and hoof cracking. The symptoms mentioned could be related to the Zn deficiency in the horse hair (Table 1).

Most iron in the body is in the hemoglobin form, however the myoglobin, the cytochromes, and many other enzyme systems contain iron.[9] A deficiency of iron can cause: anemia, listlessness, reduced feed intake and weight gain. In this work, evident signs of apathy and fatigue were observed in some of the equines. Even though the organism may well control iron absorption the naturally available animal feeding sources of iron are very few. Besides it must also consider the calcium and phosphorus antagonism present in the diet.

Copper is an essential component of a number of enzymes. Various data suggest that the copper in specific biological processes can increase the hemoglobin formation, growth, hair pigmentation, and lactation.[9] From Reference 9, copper requirements are greatly increased by molybdenum and sulfur. The antagonistic action of molybdenum on copper metabolism is exacerbated when sulfur is also high. Thus, in this work, although the copper levels in the equine are within normality, it would be necessary to determine Mo and S, in the equine hair, to have a conclusion about the relations between two or more minerals.

Manganese is involved in the metabolism of fatty acids and in the synthesis of cholesterol. It is important for the health of articulations. Although the manganese concentrations obtained for the equine hair, in the present work, are within the normality it cannot say that there is an accumulation of Mn available for absorption. Some evidence suggests that high dietary of calcium and phosphorus may increase manganese requirements, therefore, it is necessary to consider the antagonistic effects among minerals involved and also the health status.

Conclusions

In conclusion, the results show that INAA is sensitive enough to detect even small differences in the Cu, Fe, Mn and Zn concentrations of equine hair. The data obtained can give an indication of health status of these animals, but for a more precise evaluation, it is necessary to analyse all essential minerals for life and also the minerals that are considered toxic.

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References

1. W. L. SIPPEL, J. FLOWERS, J. O'FARREL, Proc. Am. Assoc. Equine Pract., 10 (1964) 139.
2. A. A. WYSOCKI, R. KLETT, J. Anim. Sci., 32 (1971) 74.
3. L. CAPE, H. F. HINTZ, Am. J. Vet. Res., 43 (1982) No. 7, 1132.
4. C. RONNEAU, J. PATIGNEY-EVEUS, J. CARA, Biol. Trace Elem. Res., 26/27 (1990) 223.
5. M. SAIKI, M. B. A. VASCONCELLOS, V. A. MAIHARA, M. J. A. ARMELIN, D. I. T. FAVARO, S. O. ROGERO, Biol. Trace Elem. Res., 43/45 (1994) 517.
6. W. D. EHMANN, D. E. VANCE, J. Radioanal. Nucl. Chem., 203 (1996) 429.
7. M. J. A. ARMELIN, M. C. R. VIEIRA-BRESSAN, A. B. BENATTI, M. SAIKI, M. B. A. VASCONCELLOS, J. Radioanal. Nucl. Chem., 236 (1998) 77.
8. Data of Trace Mineral International Clinical and Environmental Laboratory. 6545 Gunpark Dribe Suite 240 Boulder, CO, USA.
9. Mineral Tolerance of Domestic Animals, National Academy of Sciences, Washington, D.C., 1980.