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Multielement determination in cattle hair infested with *Boophilus microplus* by instrumental neutron activation analysis

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Instrumental neutron activation analysis (INAA) was applied to estimate the concentrations of Ca, Co, Cr, Cu, Fe, K, Mg, Mo, Mn, Na, Se and Zn in hair samples from Holstein-Friesan male calves, which were infested with *Boophilus microplus larvae* and, also from healthy animals. These results were obtained in order to evaluate if is there a significant difference between the trace element concentrations in hair samples from infested and healthy animals.

Introduction+

Essential trace elements play an important role in regulating biological processes as agents which inhibit or activate various enzyme systems.[1] The variation of some trace elements in the animal hair can reflect the consumption of these ones in the diet,[2] and then the trace element analysis can be used as an indicator of animal health status.

In the present work, Instrumental Neutron Activation Analysis (INAA) followed by gamma-ray spectrometry was applied to estimate the concentrations of some essential trace elements in hair samples from Holstein-Friesan male calves, which were infested with 1 g *Boophilus microplus larvae* (Acari: Ixodidae). Also hair samples from healthy animals of the control group were analyzed in order to evaluate if is there a significant difference between the trace element concentrations in hair samples from infested and healthy animals.

The *Boophilus microplus* are found in the grass and in this way they can infest the cattle producing deleterious effects such as anaemia, reduction of growth, increase of mortality and others. In consequence of this fact, there was, in 1989, a loss of 1 billion of dollars in the Brazilian bovine production, according to SPATH.[3]

INAA is a method particularly attractive for analyzing this kind of material, because it involves a minimum of sample handling and it is therefore less prone to errors. This method has been applied to the analysis of biological materials by several researchers[4–7] during the last years.

The accuracy of elemental standards used in this work was confirmed by Ca, Co, Cr, Cu, K, Mg, Mn, Na and Se determination in the NIST 1566a Oyster Tissue and, Fe, Mo, Zn in the NIES-CRM-10C Rice Flour. Relative errors were lower than 6.6% for both certified reference materials. The precision of the analysis of animal hair was evaluated by five replicate determinations of each element in each sample.

Experimental

Sample collection and preparation

Nine cattle hair samples were provided by the Parasitology Department of Biomedical Science Institute of São Paulo University. Samples were collected from the back of five Holstein-Friesan male calves, identified as A-89; A-91; A-94; A-98; A-99, aged six months, that were infested with 1 g of *Boophilus microplus larvae,* and four healthy animals of the same specie and age, identified as A-88; A-92; A-95; A-96.

The cattle hair samples were first rinsed once with a 2% Triton X100 solution and three times with water to remove coarse external dirt. The cattle 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, at last with acetone P.A.(Merck), and dried at 45 °C for 30 hours.

Aliquots of hair samples weighing about 200 mg were sealed in clean polyethylene bags for irradiation.

Preparation of standards

Standard solutions of the elements were prepared by dissolution of the high purity metals, oxides or salts of the elements in suitable reagents. Aliquots (20–100 μl) taken from such solutions were pipetted on analytical filter paper (Whatman N°. 42) for irradiation. After drying, these filter papers were placed into clean polyethylene bags.

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Four sets of standards containing the following elements were prepared: 1.) Cu (52.9 µg), Mg (550 µg); 2.) Mn (4.5 µg); 3.) Ca (1735 µg), K (395 µg), Mo (46 µg), Na (67.4 µg); 4.) Co (980 ng), Cr (2.4 µg), Fe (205 µg), Se (9 µg), Zn (24.3 µg).

Irradiations and counting

Two types of irradiation were carried out at the IEA-R1 nuclear research reactor. In one case, the sample and standards (sets 1 and 2) were irradiated together in nylon containers, for a period of 3 min. and after a decay time of 2 min, the following radionuclides were measured: ^{66}Cu, ^{27}Mg, while the ^{56}Mn radionuclide was measured after 90 min. In the second irradiation, the sample and standards (sets 3 and 4) were irradiated together in aluminium containers, for a period of 8 h. Measurements were carried out after 2 days of decay time to detect ^{47}Ca, ^{42}K, ^{99}Mo, ^{24}Na and after, at least, 7 days to detect ^{60}Co, ^{51}Cr, ^{59}Fe, ^{75}Se and ^{65}Zn. The thermal neutron flux utilized ranged from 10^{11} to 10^{13} $n \cdot cm^{-2} \cdot s^{-1}$.

Measurements were done using an ORTEC EG&G high resolution solid state Ge detector, type POP TOP, Model 20190 with a resolution (FWHM) of 1.85 keV for the 1332 keV peak of ^{60}Co. This detector is coupled to an EG&G ORTEC ACE8K card. Data reduction was carried out using IBM/PC microcomputer and VISPECT2 software in Turbo Basic language for spectral analysis.

Results and discussion

Concentration of trace elements in cattle hair from infested and healthy animals are presented in Table 1 and Table 2, respectively. These results are means of five determinations for each animal with their respective standard deviations.

In order to evaluate if is there a significant difference between the trace element concentrations in hair samples from infested and healthy animals the statistical t-test was applied, at the significance level of 0.05. From the t-distribution table for 7 degrees of freedom the critical value of $|t|$ is 2.36 ($p = 0.05$). The experimental values of $|t|$ are shown in Table 3.

Table 1. Elemental concentrations obtained in hair by INAA from infested cattle

Element (energy, keV)*	Unit	Concentration (Mean ± SD) A-89	A-91	A-94	A-98	A-99
Ca (1296)	mg/kg	1199 ± 244	2422 ± 184	2131 ± 250	1810 ± 355	2422 ± 279
Co (1332)	µg/kg	128 ± 10	38 ± 7	50 ± 16	87 ± 23	66 ± 9
Cr (320)	µg/kg	299 ± 22	231 ± 50	239 ± 47	1876 ± 308	316 ± 49
Cu (1039)	mg/kg	11 ± 2	24 ± 2	15 ± 2	20 ± 3	9 ± 2
Fe (1098)	mg/kg	25 ± 2	21 ± 4	27 ± 5	26 ± 7	15 ± 2
K (1525)	mg/kg	435 ± 29	879 ± 74	580 ± 22	200 ± 23	2692 ± 184
Mg (1013)	mg/kg	226 ± 11	287 ± 13	394 ± 30	271 ± 41	404 ± 38
Mo (140)	µg/kg	841 ± 27	851 ± 116	829 ± 59	942 ± 180	514 ± 101
Mn (846)	µg/kg	6859 ± 1531	2752 ± 486	1866 ± 272	5625 ± 929	6178 ± 571
Na (1368)	mg/kg	60 ± 7	63 ± 7	60 ± 5	17 ± 3	613 ± 62
Se (264)	µg/kg	541 ± 61	710 ± 77	579 ± 46	728 ± 63	601 ± 68
Zn (1115)	mg/kg	222 ± 19	150 ± 12	174 ± 9	134 ± 16	159 ± 10

* Gamma-ray energy utilized in this work.

Table 2. Elemental concentrations obtained in hair by INAA from healthy cattle

Element (energy, keV)*	Unit	Concentration (Mean ± SD) A-88	A-92	A-95	A-96
Ca (1296)	mg/kg	935 ± 40	956 ± 99	838 ± 141	978 ± 90
Co (1332)	µg/kg	44 ± 2	47 ± 8	42 ± 1	44 ± 9
Cr (320)	µg/kg	nd	163 ± 27	166 ± 41	186 ± 34
Cu (1039)	mg/kg	17 ± 4	9 ± 2	25 ± 4	10 ± 2
Fe (1098)	mg/kg	18 ± 3	16 ± 3	19 ± 3	20 ± 2
K (1525)	mg/kg	100 ± 5	190 ± 29	106 ± 7	265 ± 26
Mg (1013)	mg/kg	180 ± 20	179 ± 16	148 ± 15	171 ± 28
Mo (140)	µg/kg	687 ± 135	621 ± 69	682 ± 83	615 ± 133
Mn (846)	µg/kg	2637 ± 469	3071 ± 424	2330 ± 193	2549 ± 595
Na (1368)	mg/kg	8 ± 2	15 ± 4	8 ± 2	24 ± 3
Se (264)	µg/kg	437 ± 43	382 ± 56	437 ± 64	471 ± 58
Zn (1115)	mg/kg	135 ± 8	114 ± 10	117 ± 4	111 ± 5

* Gamma-ray energy utilized in this work.
nd – not determined.

Table 3. Experimental values of $|t|$

Element	$t_{experimental}$
Ca	4.09
Co	1.64
Cr	0.98
Cu	0.12
Fe	1.92
K	1.56
Mg	3.61
Mn	1.78
Mo	1.70
Na	1.16
Se	4.45
Zn	2.77

$p = 0.05$

For the elements Ca, Mg, Se and Zn, the experimental values of $|t|$ are higher than the critical value. Therefore, for these elements, there is a significant difference between the results obtained for healthy and infested animals.

On the other hand, for Co, Cr, Cu, Fe, K, Mn, Mo and Na the experimental values of $|t|$ are lower than critical value $|t|$, so the concentrations of these elements can be considered the same between these two groups of animals.

These results show the infested animals present higher Ca, Mg, Se and Zn concentrations than control group. As the infested animals were suffering an exploitation by the parasites, a reduction in microelements would be expected. These results may be derived from an inflammation process in the dermas site of tick's attachment what would increase the blood supply and consequently the avail of microelements. This effect may also explain the large variations in the concentrations of Co, Cr, Cu, K, Mn and Na obtained among different hair samples from the group of infested animals. This affirmation is just a supposition and more investigations will be necessary to explain the alterations observed in infested animals.

In conclusion, the results show that instrumental neutron activation analysis presents precision lower than 15% for most elements and it is sensitive enough to detect even small variations in the elemental concentrations.

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