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LIMING AND PLANT AGING INFLUENCE ON MICRONUTRIENT UPTAKE BY *BRACHIARIA DECUMBENS* FORAGE

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ABSTRACT

Brachiaria decumbens is the main forage in pastures of several Brazilian regions. The effects of liming and plant age on micronutrient uptake by the forage of a degraded *Brachiaria decumbens* pasture under restoration process, were studied in São Carlos – SP, southeastern Brazil, under altitude tropical climate. Experimental design was a random block (100 m²), with 6 replications and 3 treatments. Each block received the following treatment: (a) 0 t/ha of limestone with NK; (b) 2 t/ha of limestone applied on soil surface with NK and maintenance of 1 t/ha per annum; (c) 8 t/ha of limestone applied once on soil surface with NK. Forage samples were collected 14 cm above soil surface, each 36 days in the rain season. Instrumental neutron activation analysis (INAA) followed by gamma-ray spectrometry was the analytical method used to determine the micronutrient content. In some cases, Co Fe, Mn and Zn were negatively affected by increasing limestone doses. The opposite effect was observed for Cl. Decreases of Cl, Co and Mo uptake in forage were enhanced with plant aging.

1. INTRODUCTION

Grass forage is the most economic, and in many times the only feed source for bovines. *Brachiaria decumbens* is largely used as forage in pastures of several Brazilian tropical regions. This forage is adapted to soils with low fertility and relatively high acidity. According to Valle and Miles[1] the pastures of *Brachiaria* occupy more than 40 million hectares in Brazil. Nevertheless, worse management practices have resulted in pasture degradation. Thus, to restore or to maintain good tropical grass pastures it is necessary to know the adequate fertilizer supply, mainly nitrogen, and limestone use to overcome soil acidity. Liming may increase the forages productivity, optimize the use of essential nutrients and, also it supplies Ca and Mg [2]. The performance of phosphorus and of nitrogen as nutrients to restore or to maintain grass pastures under tropical climate is pointed out in literature [3-5].

Leaves are the first part grazed by animals so their chemical composition is important to know the nutritional value. Usually, main forage value is given by its protein content [2]. However, tissue mineral composition also plays an important role in animal nutrition.

In present study, instrumental neutron activation analysis (INAA) [6-8] followed by gamma-ray spectrometry was applied to estimate the concentrations of Cl, Co, Fe, Mn, Mo and Zn in the aboveground part of *Brachiaria decumbens*. This forage was grown-up on a degraded pastureland, submitted to a recovery process and maintained under intensive management, where different limestone doses were applied to the soil surface and, fertilizer was top dressed after each cutting in the rain season. Analysis of variance was used to verify differences among treatments and if element concentrations present in *Brachiaria* tissue were affected by limestone doses and plant aging.

2. EXPERIMENTAL

2.1. Sampling protocol, collection and sample treatment

The field trial was performed at the experimental farm of Embrapa Southeast Cattle, São Carlos-SP, Brazil, on a 16 years old *Brachiaria decumbens* pasture, grown on a dystrophic Hapludox (Oxisol). Limestone and phosphorus were applied at the beginning of this study.

Experimental design was a random block, with 6 replications and 3 treatments. Each 100 m² block received following treatment: (a) 0 t/ha of limestone with NK, tagged as T0; (b) 2 t/ha of limestone applied on soil surface with and maintenance of 1 t/ha per annum, (T2M); (c) 8 t/ha of limestone applied once, in November 1999, on soil surface with NK, (T8). NK, fertilizing as 100 kg N as ammonium sulphate and 100 kg K₂O-KCl, after each cutting (4 to 5 times a year in the rainy season, with a re-growth period of around 33 days).

Forage samples were collected in the following dates: April 2001, December 2002 and March 2004, that is, 17, 37 and 52 months respectively, after the limestone input on soil surface. Aboveground part of the plants, composed by leaves and slender stems, was considered in this work. Samples were collected 14 cm above soil surface, from continuous 40 m²/ plot. Total number of collected samples was 54.

Parts of the samples destined for analysis, were dried at 60°C during 72 hours under forced air circulation. Dried materials were ground in a Willey mill and passed through a 20-mesh sieve (0.84 mm).

For irradiation, 200 mg of each sample were transferred to polyethylene envelopes, which were cleaned prior to use by leaching with a diluted HNO₃ (1:5).

2.2. Preparation of standards

Standard solutions of Cl, Co, Fe, Mn, Mo and Zn (Spex Certiprep) were used to prepare the standards. Aliquots (50-100 µl) of these solutions were pipetted on small sheets of analytical filter paper (Whatman N° 42). After drying, these filter papers were placed into polyethylene bags. Standards contained: Cl (246 µg), Co (2.5 µg), Fe (245 µg), Mn (4.8 µg), Mo (24.5 µg), and Zn (24.5 µg).

2.3. Irradiation and counting

Two types of irradiation were carried out at the IEA-R1 nuclear research reactor. In the first, sample and standards (Cl, Mn) were irradiated together in a nylon container for 2.5 minutes. After a decay time of 4 minutes the ^{37}Cl radionuclide (at 1642 keV) was measured in sample and in the Cl standard afterwards. The ^{56}Mn radionuclide (at 846 keV) was measured after 90 minutes of decay time. In the second irradiation, the sample and standards (Co, Fe, Mo and Zn) were irradiated together in an aluminum container for 8 hours. The ^{99}Mo radionuclide (at 140 keV) was measured after 3 days of decay, while ^{60}Co (at 1331 keV), ^{59}Fe (at 1099 keV) and ^{65}Zn (at 1115 keV) were measured after, at least, 10 days. The thermal neutron flux used ranging from 5×10^{11} to $5 \times 10^{12} \text{ n cm}^{-2}\text{s}^{-1}$.

The equipment to measure the gamma-radiation was Canberra hyperpure Ge detector (Model GX2020) coupled to an Integrated Signal Processor (Model 1510) and MCA System 100, both from Canberra. The detector 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 . The concentrations of elements were calculated by comparative method.

3. RESULTS AND DISCUSSION

Certified reference material NIST 1515 Apple Leaves was analyzed for quality control. Results showed a good agreement with the certified values, in most of the cases (Table 1).

Table 1. Concentrations of Cl, Co, Fe, Mn, Mo, and Zn obtained in NIST 1515 Apple Leaves by INAA

Element, unit	This work (mean \pm SD) ^a	Certified values
Cl, $\mu\text{g g}^{-1}$	560 ± 55	579 ± 23
Co, $\mu\text{g g}^{-1}$	0.096 ± 0.006	(0.09)
Fe, $\mu\text{g g}^{-1}$	79 ± 2	83 ± 5
Mn, $\mu\text{g g}^{-1}$	53 ± 4	54 ± 3
Mo, $\mu\text{g g}^{-1}$	0.14 ± 0.04	0.094 ± 0.013
Zn, $\mu\text{g g}^{-1}$	12.1 ± 0.6	12.5 ± 0.3

^aMean and standard deviation from 4 individual determinations.

In Table 2 are shown the concentrations of Cl, Co, Fe, Mn, Mo and Zn in *Brachiaria decumbens* forage treated with distinct limestone doses and collected at different plant ages, besides the output of variance analysis and Tukey test. Each value is the arithmetic mean and

standard deviation of forage element concentrations measured in 6 blocks. Standard deviation represents the uncertainty of analytical method and the variation observed of element concentrations within each treatment. Variance analysis was applied to the values of Table 2, with Tukey test [8], to verify if there is difference among element concentrations obtained in *Brachiaria* tissue, as affected by liming and plant age.

Table 2. Mean values and standard deviations of element concentrations in *Brachiaria decumbens* forage for different limestone doses and plant ages

Age (month)	Limestone Doses		
	T0	T2M	T8
Cl (g kg⁻¹)			
17	6.1 ± 0.7 aA	6.3 ± 0.8 aA	6.3 ± 0.9 aA
37	8.2 ± 0.7 bA	9.1 ± 0.6 bA	10.9 ± 0.7 bB
52	5.2 ± 0.8 aA	5.8 ± 0.8 aA	5.5 ± 0.5 aA
Fe (mg kg⁻¹)			
17	134 ± 15 aB	112 ± 8 aA	109 ± 6 aA
37	100 ± 7 aA	103 ± 5 aA	91 ± 4 bAB
52	154 ± 36 abA	111 ± 8 aB	107 ± 11 aB
Mn (mg kg⁻¹)			
17	220 ± 20 aA	169 ± 17 aB	143 ± 14 aB
37	166 ± 15 bA	155 ± 9 aAC	135 ± 6 aBC
52	142 ± 23 bA	163 ± 20 aA	148 ± 15 aA
Mo (µg kg⁻¹)			
17	381 ± 178 aA	427 ± 93 aA	441 ± 26 aA
37	499 ± 110 aA	416 ± 161 aA	426 ± 18 aA
52	< LD	< LD	< LD
Zn (mg kg⁻¹)			
17	27 ± 2 aA	21 ± 2 aB	19 ± 3 aB
37	32 ± 2 bA	26 ± 1 aB	21 ± 2 aC
52	22 ± 3 cA	23 ± 6 aA	20 ± 2 aA
Co (µg kg⁻¹)			
17	82 ± 21 aA	117 ± 33 aAB	71 ± 18 aAC
37	55 ± 6 bA	51 ± 5 bA	44 ± 7 bA
52	42 ± 14 bA	28 ± 9 bA	41 ± 17 bA

Means followed by same letter, small letter in vertical and capital letter in horizontal indicate no difference by Tukey test ($p < 0.05$). LD, Limit of Detection (Mo = 110 µg kg⁻¹).

Content of Mo in *Brachiaria* was affected only with plant aging. Significant effect ($p < 0.05$) occurred due to limestone doses and plant age on Cl, Co, Fe, Mn and Zn uptake by forage. Influence of limestone doses on micronutrient uptake occurred up to the 3 years after treatment start. According to the daily requirement of micronutrients by dairy cattle [9], increasing limestone doses did not induce deficiency or toxicity of any micronutrient in *Brachiaria decumbens* forage.

4. CONCLUSIONS

Content of Cl, Co, Fe, Mn and Zn present in *Brachiaria decumbens* are affected by limestone doses and plant aging, while the content of Mo is influenced only by the plant age. INAA is sensitive enough to determine Cl, Co, Fe, Mn, Mo, and Zn concentrations in this forage.

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