

Effect of liming and fertilizer on mineral content and productivity of *Brachiaria Decumbens* grass forage

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To restore a degraded pasture of *Brachiaria decumbens*, located in São Carlos – SP, southeastern Brazil, under altitude tropical climate, an experiment was carried out to study the effects of limestone, buried or not buried in the soil, and fertilizer use on mineral content and forage yield, after 3 years of treatment. Limestone and phosphorus were applied once, one month before starting. NK were applied after each cutting, for fertilized plots, four to five times a year. Experimental design was a random block (100 m²), with 6 replications and 4 treatments. Each block received 4 t/ha of limestone, except the control. Forage samples were collected 14 cm above soil surface. Instrumental neutron activation analysis (INAA) followed by gamma-ray spectrometry was the analytical method used to determine the mineral contents. Dry matter yield was affected positively with liming when compared with the limestone control, but the effect of limestone use was more pronounced with the concomitant use of NK fertilizer. The contents of Ca, Cs, Fe, La, Mg, Rb, Sc, Sm and Th in forage were negatively affected with the NK use, perhaps due to a dilution effect, while a reverse were observed for K, Cl, perhaps due to input of KCl, besides Br, Mn and Se. It seems that limestone is not a key input to restore degraded tropical pastureland, grown on acid soils, when nitrogen is lacking. INAA allowed the monitoring of some not routine elements that may be under observation to avoid potential plant nutritional disorders in production systems with high limestone and fertilizer use.

Introduction

Forage grass undergoes to a constant loss of vigor, productivity and quality in the pasture degradation process, on account of inadequate nutrient management, besides of high stocking rate. Thus, to restore or to maintain good tropical pastures the knowledge of adequate fertilizer supply and of limestone use to overcome soil acidity is needed. In most of the extensively managed tropical pasturelands, rotation with an annual fertilized cash crop is performed. However, when intensification of land use is needed, direct fertilizer use to grasses is recommended. Studies have shown that use of fertilizer, mainly nitrogen, on soil surface is sufficient to recover the so called “degraded” tropical pastures, with an exception when the introduction of a new grass species is needed.

The performance of phosphorus and of nitrogen to establish, to restore or to maintain pastures with forages in tropical climate is pointed out in literature.^{1–3} But great amounts of nitrogen, around 250 to 400 kg/ha/year spread 4 to 5 times, to improve forage yield in 3 to 5 times, will sharply reduce tropical soil pH, switch of pH-dependent charges and will allow cation losses in soil depth.

Acidity correction occurs with burying of limestone into the soil by means of plow and harrow, in conventional agricultural systems, to provide larger contact among limestone particles and soil colloids.⁴ However, according to CAIRES,⁵ surface liming of soils in no-tillage system would have the advantage to keep the chemical attributes and the soil structure with larger economical control of erosion.

In present work, instrumental neutron activation analysis (INAA)^{7–9} followed by gamma-ray spectrometry was applied to estimate the concentrations of Ba, Br, Ca, Cl, Co, Cr, Cs, Eu, Fe, K, La, Mg, Mn, Mo, Rb, Sc, Se, Sm, Th, V and Zn in the aboveground part of *Brachiaria decumbens*. This forage grass was grown-up on a degraded pastureland, with the goal to study the effects of limestone, buried or not buried in the soil, and the use of fertilizer on mineral content and forage yield of the pasture under restoration.

Experimental

Sampling protocol, collection and sample treatment

The field trial was performed at the experimental farm of Southeast Embrapa 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.

The experimental design was a random block, with 6 replications and 4 treatments. The 100 m² blocks were established in the pasture. Each block received the following treatment: (a) 0 t/ha of limestone with NK, tagged as T0; (b) 4 t/ha of limestone applied on soil surface with NK, (T4); (c) 4 t/ha of limestone buried in the soil with NK, (T4i); and (d) 4 t/ha of limestone applied on soil surface without NK, (T4WF). NK, fertilizing were the use of 100 kg N as ammonium sulphate and 100 kg K₂O as KCl, after each cutting (4 to 5 times a year in the rainy season).

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The 33 days old aboveground part of the plants, composed by leaves and slender stems, was considered in this work. Samples were collected 14 cm above the soil surface, from continuous 40 m²/plot, 3 years after limestone spreading. Total number of samples collected was 24. Each fresh sample was oven dried at 60 °C during 72 hours under forced air circulation, and divided into 2 parts, one part to determine the dry matter yield, and the other to perform mineral analysis. 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 dilute HNO₃ (1:5).

Preparation of standards

Standard solutions of Ba, Br, Ca, Cl, Co, Cr, Cs, Eu, Fe, K, La, Mg, Mn, Mo, Rb, Sc, Se, Sm, Th, V and Zn (Spex Certiprep) were used to prepare the standards. Aliquots (50–100 µl) were pipetted on small sheets of analytical filter paper (Whatman No. 42) for irradiation. After drying, these filter papers were placed into polyethylene bags. Standards contained: Ba (245 µg), Br (24.5 µg), Ca (991 µg), Cl (246 µg), Co (2.5 µg), Cr (2.5 µg), Cs (24.7 µg), Eu (2.4 µg), Fe (245 µg), K (988 µg), La (2.5 µg), Mg (495 µg), Mn (4.8 µg), Mo (24.5 µg), Rb (24.4 µg), Sc (2.5 µg), Se (24.6 µg), Sm (2.5 µg), Th (5.0 µg), V (2.5 µg) and Zn (24.5 µg).

Irradiation and counting

Two types of irradiation were carried out at the IEA-R1 nuclear research reactor. In one case, the sample and standards (Ca, Cl, K, Mg, Mn and V) were irradiated together in a nylon container for 2.5 minutes, and after a decay time of 2 minutes the ⁴⁹Ca (at 3083 keV), ³⁸Cl (at 1642 keV), ²⁷Mg (at 1014 keV) and ⁵²V (at 1434 keV) were measured in the sample and standards, consecutively. The ⁴²K (at 1524 keV) and ⁵⁶Mn (at 846 keV) were measured after 90 minutes of decay. In the second irradiation, sample and standards (Ba, Br, Co, Cr, Cs, Eu, Fe, La, Mo, Rb, Sc, Se, Sm, Th and Zn) were irradiated together in an aluminum container for 8 hours. The ⁹⁹Mo (at 140 keV), ¹³¹Ba (at 496 keV), ⁸²Br (at 776 keV) and ¹⁵³Sm (at 103 keV) were measured after 3 days of decay, while ⁶⁰Co (at 1332 keV), ⁵¹Cr (at 320 keV), ¹³⁴Cs (at 604 keV), ¹⁵²Eu (at 1408 keV), ⁵⁹Fe (at 1099 keV), ¹⁴⁰La (at 1596 keV), ⁸⁶Rb (at 1077 keV), ⁴⁶Sc (at 889 keV), ⁷⁵Se (at 264 keV), ²³³Pa (at 312 keV) and ⁶⁵Zn (at 1115 keV) were measured after, at least, 10 days of decay time. The thermal neutron flux utilized ranged from 5·10¹¹ to 3·10¹² n·cm⁻²·s⁻¹.

The equipment used to measure the gamma-radiation was a Canberra Model GX2020 hyperpure Ge detector coupled to a 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 ⁵⁷Co and 1.9 keV for 1332 keV gamma-ray of ⁶⁰Co.

Variance analysis (*F*-test) was applied to the results, with Tukey test, to verify significant differences (SMD) among treatment means concerning to forage yield and element concentrations in *Brachiaria* tissue, as affected by liming and fertilizer, using the statistical analysis system – SAS.⁶

Results and discussion

Certified reference materials NIST 1515 Apple Leaves and NIST 2710 Montana Soil were analyzed for quality control. The results showed a good agreement with the certified values, in most of the cases (Table 1).

Arithmetic mean values and concentration ranges of Ba, Br, Ca, Cl, Co, Cr, Cs, Eu, Fe, K, La, Mg, Mn, Mo, Rb, Sc, Se, Sm, Th, V and Zn contained in the 33 days old *Brachiaria decumbens* forage of 6 blocks are presented in Table 2. Results of forage dry matter yield, 3 years of limestone treatment, are shown in Table 3.

Table 1. Concentrations of Ba, Br, Ca, Cl, Cr, Cs, Co, Eu, Fe, K, La, Mg, Mn, Mo, Rb, Sc, Se, Sm, Th, V and Zn obtained in certified reference materials by INAA

Element, unit	This work (mean ± SD) ^a	Certified values
Ba, µg·g ⁻¹	50 ± 5	49 ± 2 ^b
Br, µg·g ⁻¹	1.8 ± 0.1	(1.8) ^b
Ca, mg·g ⁻¹	14.3 ± 0.9	15.26 ± 0.15 ^b
Cl, µg·g ⁻¹	560 ± 55	579 ± 23 ^b
Cr, µg·g ⁻¹	36 ± 3	(39) ^c
Cs, µg·g ⁻¹	112 ± 6	(107) ^c
Co, µg·g ⁻¹	0.096 ± 0.006	(0.09) ^b
Eu, µg·g ⁻¹	0.23 ± 0.03	(0.2) ^b
Fe, µg·g ⁻¹	79 ± 2	83 ± 5 ^b
K, mg·g ⁻¹	15.6 ± 0.3	16.1 ± 0.2 ^b
La, µg·g ⁻¹	19.8 ± 0.9	(20) ^b
Mg, mg·g ⁻¹	2.62 ± 0.2	2.71 ± 0.08 ^b
Mn, µg·g ⁻¹	53 ± 4	54 ± 3 ^b
Mo, µg·g ⁻¹	0.14 ± 0.04	0.094 ± 0.013 ^b
Rb, µg·g ⁻¹	9.5 ± 0.9	10.2 ± 1.5 ^b
Sc, µg·g ⁻¹	0.031 ± 0.003	(0.03) ^b
Se, µg·g ⁻¹	0.09 ± 0.03	0.050 ± 0.009 ^b
Sm, µg·g ⁻¹	3.0 ± 0.3	(3) ^b
Th, µg·g ⁻¹	0.028 ± 0.003	(0.03) ^b
V, µg·g ⁻¹	0.25 ± 0.05	0.26 ± 0.03 ^b
Zn, µg·g ⁻¹	12.1 ± 0.6	12.5 ± 0.3 ^b

^a Mean and standard deviation from 4 individual determinations.

^b NIST 1515 Apple Leaves.

^c NIST 2710 Montana Soil.

Numbers in parentheses are information values.

Table 2. Mean values and concentrations ranges of elements in *Brachiaria decumbens* forage for different ways of limestone application

Element	Treatment					Statistics	
	T0	T4	T4i	T4WF	F-test	SMD	CV, %
Ba, $\mu\text{g}\cdot\text{g}^{-1}$	11 (7-14)	16 (14-19)	16 (12-23)	18 (11-26)	*	6	20
Br, $\mu\text{g}\cdot\text{g}^{-1}$	8.7 (7.5-10.6)	10.2 (9.0-11.5)	9.9 (8.9-12.5)	5.1 (3.0-7.7)	**	2.1	17
Ca, $\text{mg}\cdot\text{g}^{-1}$	1.3 (0.8-1.9)	2.9 (2.3-3.6)	2.1 (1.7-2.5)	3.9 (3.5-4.2)	*	0.4	11
Cl, $\text{mg}\cdot\text{g}^{-1}$	8.4 (7.4-9.6)	10.3 (9.2-12.5)	10.5 (7.8-16.3)	3.1 (2.0-4.0)	*	1.7	15
Co, $\mu\text{g}\cdot\text{kg}^{-1}$	59 (48-81)	47 (37-60)	52 (34-69)	72 (29-140)	NS		39
Cr, $\mu\text{g}\cdot\text{kg}^{-1}$	353 (216-495)	352 (173-516)	365 (265-469)	641 (278-1325)	NS	111	56
Cs, $\mu\text{g}\cdot\text{kg}^{-1}$	151 (104-268)	151 (117-196)	151 (71-186)	303 (143-559)	*		41
Eu, $\mu\text{g}\cdot\text{kg}^{-1}$	7 (3-9)	9 (4-14)	8 (2-14)	13 (5-28)	NS		47
Fe, $\mu\text{g}\cdot\text{g}^{-1}$	100 (77-123)	108 (91-134)	109 (79-125)	277 (75-603)	*	156	73
K, $\text{mg}\cdot\text{g}^{-1}$	34.8 (28.8-44.2)	35.6 (30.3-41.6)	35.6 (28.4-42.8)	15.8 (11.8-21.5)	**	5.2	12
La, $\mu\text{g}\cdot\text{kg}^{-1}$	547 (222-813)	743 (311-1065)	645 (112-940)	698 (292-1099)	*	170	17
Mg, $\text{mg}\cdot\text{g}^{-1}$	1.8 (1.4-2.2)	3.3 (2.6-3.8)	3.0 (2.2-3.9)	3.2 (2.6-4.0)	**	0.6	16
Mn, $\mu\text{g}\cdot\text{g}^{-1}$	174 (146-218)	171 (138-210)	183 (138-229)	106 (84-126)	**	33	14
Mo, $\mu\text{g}\cdot\text{kg}^{-1}$	499 (429-629)	458 (277-687)	393 (208-623)	500 (334-736)	NS		18
Rb, $\mu\text{g}\cdot\text{g}^{-1}$	21 (18-26)	24 (13-31)	24 (13-32)	31 (23-40)	**	5	13
Sc, $\mu\text{g}\cdot\text{kg}^{-1}$	15 (11-22)	19 (12-28)	18 (16-23)	78 (22-149)	*	42	90
Se, $\mu\text{g}\cdot\text{kg}^{-1}$	136 (100-177)	112 (85-151)	122 (76-191)	73 (33-116)	**	41	25
Sm, $\mu\text{g}\cdot\text{kg}^{-1}$	27 (13-40)	36 (18-51)	31 (9-45)	50 (21-105)	*	18	34
Th, $\mu\text{g}\cdot\text{kg}^{-1}$	10 (7-12)	13 (10-22)	12 (11-15)	51 (16-96)	*	27	83
V, $\mu\text{g}\cdot\text{kg}^{-1}$	168 (117-232)	185 (142-227)	184 (133-235)	779 (191-1863)	NS		96
Zn, $\mu\text{g}\cdot\text{g}^{-1}$	26.7 (30-36)	27.5 (18-36)	29.2 (18-38)	18.1 (6-23)	NS		28

* ($P<0.05$).** ($P<0.01$).

NS: No significant.

SMD: Significant minimum difference.

CV: Coefficient of variation.

T0: Check plot.

T4: 4 t/ha limestone.

i: Buried in.

WF: Without fertilizer.

T0-T4-T4i: With fertilizer.

Table 3. Treatment versus forage dry matter yield, and soil pH

Treatment	Dry matter yield, kg/ha	Soil pH in CaCl ₂ , for the depths of		
		2.5 cm	5.0 cm	10 cm
T0	3235	4.7	4.3	4.3
T4	4148	5.8	4.9	4.4
T4i	3901	5.8	5.8	5.8
T4WF	234	6.1	5.1	4.6
F test	**			
SMD	543			
CV, %	13.2			

SMD: Significant minimum difference.

** ($P < 0.01$).

Differences in dry matter yield and of elemental absorption by *Brachiaria decumbens* for different ways of limestone and fertilizer use were evaluated by variance analysis (F -test, $P < 0.01$) and by Tukey test (SMD, $P < 0.05$) applied to the means (Tables 2 and 3).

Calcium absorption by forage was greater on plot without NK fertilizer (T4WF) and low dry matter yield (Table 3), perhaps due to the concentration effect of slow growing plants, although soil pH was higher, than at NK fertilized plot without limestone (T0) and the lowest pH at surface layer. No difference was observed between on surface (T4) and buried in (T4i) limestone. Absorption of Ba, Ca, Cs, Fe, La, Mg, Rb, Sc, Sm, and Th by *Brachiaria decumbens* forage was higher for limestone treatment without NK fertilizer (T4WF) while the inverse occurred for Br, Cl, K, Mn and Se. Concentration of Co, Cr, Eu, La, Mo, V and Zn in forage were not affected by the treatments. At any treatment, the nutritional restoration process of pasture by means of limestone and fertilizer did not cause changes of elemental concentration that could negatively affect metabolism of livestock, considering toxicity levels of essential nutrients.^{10,11} Coefficient of variation values showed the great variance for some essential elements, although the use of 6 plot replications, for plant nutrition (Fe), animal nutrition (Co), and some other elements that may be considered in future (Cr, V, Cs, Sc, Sm, Th and Eu), pointing also to the need of a more confident analysis method, that could be the INAA.

INAA reinforced the potential to be an important tool to manage high input production systems, without nutritional troubles, by allowing also the monitoring of no routine elements, mainly in experimental areas.

Dry matter yield was affected positively (Table 3) in both ways of limestone application when compared with control treatment (T0). However, the effect of limestone treatments was greater with the use of NK fertilizer. These results agree with those presented in the literature¹⁻³ about fertilizer use for pasture restoration in tropical climate. It seems that limestone is not a key input to restore degraded tropical pastureland, grown on acid soils, without nitrogen in the system, when comparing dry matter yield of treatment T4WF against T4, T4i and also T0.

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