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SOIL SCIENCE

Brachiaria enrichment with selenium-coated urea

Letícia de Abreu Faria[1*] Marcos Canto Machado[2] Felippe Hoffmann Silva Karp[3] Marcos Yassuo Kamogawa[3] Adibe Luiz Abdalla Filho[2] Ricardo Antunes de Azevedo[3] Adibe Luiz Abdalla[2]

[1]Universidade Federal Rural da Amazônia (UFRA), Paragominas, PA, Brasil. E-mail: leticiadeabreufaria@gmail.com. *Corresponding author.
[2]Universidade de São Paulo (USP), Centro de Energia Nuclear na Agricultura (CENA/USP), Piracicaba, SP, Brasil.
[3]Universidade de São Paulo (USP), Escola Superior de Agricultura Luiz de Queiroz (ESALQ/USP), Piracicaba, SP, Brasil.

ABSTRACT: *Selenium (Se) fertilization in grazing to biofortification of animal products have been carried out in low Se soils. The objective of this study was to increase the Se content in the biomass of Brachiaria spp.with urea coated with Se. The experiment was performed in a typical Hapludox soil under greenhouse conditions. A completely randomized block design with four replicates in a factorial structure with two cuts and six Se doses (0, 10, 20, 40, 80, and 160gha^{-1}) was used. The Brachiaria brizantha demonstrated the absence of changes in higher harmful doses without modifying the activity of glutathione peroxidase enzyme. We concluded that enrichment of Brachiaria brizantha with coated urea is obtained with 34.5gha^{-1}of Se.*
Key words: *coated fertilizer, pasture fertilization, sodium selenate.*

Enriquecimento de Brachiaria com ureia revestida com selênio

RESUMO: *A adubação com Selênio (Se) em pastagem para a biofortificação de alimentos de origem animal tem sido realizada em solos com baixo Se. O objetivo foi avaliar o aumento no conteúdo de Se na biomassa de Brachiaria spp. com aplicação de ureia revestida com Se. O experimento foi conduzido em casa de vegetação em Latossolo Amarelo. O delineamento foi em blocos casualizados com quatro repetições em esquema fatorial com dois cortes e seis doses de Se (0, 10, 20, 40, 80 e 160gha^{-1}). A Brachiaria brizantha demonstrou ausência de mudanças em altas e perigosas doses de Se sem alterar a atividade da enzima antioxidante glutationa peroxidase. Com isso, concluiu-se que seu enriquecimento com ureia revestida é obtida com 34,5gha^{-1} de Se.*
Palavras-chave: *fertilizante revestido, adubação de pasto, selenato de sódio.*

INTRODUCTION

Selenium (Se) is essential in animal nutrition; however, several countries, including Brazil, have levels of intake of Se for animal below the requirements (VALLE et al., 2007). A potential explanation is due to low Se bioavailability and concentration in the soil. Selenium fertilization in these areas could improve concentration of Se in the soil and consequently improving the metabolism and quality of plants (RIOS et al., 2010).There are complex paths associated with enrichment of Se by fertilization, which include the existence of different Se forms, difficulties to reach desirable levels in leaves or edible parts of plants (LI et al., 2008) and at the same time take care to avoid yield damages and/or toxicity (MARTINEZ et al., 2009). However, there are cereals and forages that are able to uptake high levels of Se and do not develop phytotoxicity (OLIVEIRA et al., 2007) that could result in animal intoxication, since requirement of Se by cattle in a dry matter base is between 0.1 to 0.3mg kg^{-1} (NRC, 2001).

Selenium availability in soil changes depending on the chemical form, soil acidity, content of oxides and hydroxides forms of iron and aluminum, and levels of clay and organic matter in the soil. However, the higher solubility and mobility of selenate in the soil make this form a potential candidate to fertilization (RIOS

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et al., 2010). Low amount of Se required for biofortification in grazing areas could allow Se application via urea coating. There are few studies (RAMOS et al., 2012) investigating the use of Se as a fertilizer on forage quality in tropical countries.

This study aimed to evaluate if doses of Se-coated urea can promote enrichment of *Brachiaria brizantha* cv. Marandu. Soil samples were collected at the 0–0.2m top layer of a sandy loam soil classified as Typical Hapludox at Piracicaba-SP, Brazil with low Se level (0.5mg dm^{-3}). Soil characterization was: 180g kg^{-1} of clay, 20g kg^{-1} of silt, and 800g kg^{-1} of sand; pH 4.6 ($CaCl_2$); 5mg dm^{-3} of S-SO_4^{2-}, 12mg dm^{-3} of P; 0.3$mmol_c$ dm^{-3} of K; 10$mmol_c$ dm^{-3} of Ca; 8$mmol_c$ dm^{-3} of Mg; 38$mmol_c$ dm^{-3} of H+Al; 0$mmol_c$ dm^{-3} of Al. Cation-exchange capacity was 56.3$mmol_c$ dm^{-3} and base saturation 33%. Other results were 0.24mg dm^{-3} of B, 0.6mg dm^{-3} of Cu, 97mg dm^{-3} of Fe, 7.9mg dm^{-3} of Mn, 1.4mg dm^{-3} of Zn, and < 4mg dm^{-3} of Na. Liming was performed to raise the saturation base up to 70% using calcium carbonate followed 15 days incubation with soil moisture maintained at 60% of water holding capacity. Germination of commercial seeds of *Brachiaria brizantha* cv. Marandu was done in vermiculite and seedlings were transplanted to experimental pots after 15 days. Experimental pots containing 7kg of dry soil and 7 seedlings were daily distribution of deionized water to keep humidity at the 60% of water holding capacity. Application of nutrient solutions were done based on pure reagents to raise soil contents up to 40mg dm^{-3} of P and 3$mmol_c$ dm^{-3} of K (KH_2PO_4 and KCl, respectively) at the time of seedlings transplant. After harvesting the plants for the first time, we performed an application of N (urea) and B (boric acid) at the respective rates of 25mg dm^{-3} and 0.2mg dm^{-3}.

The experimental design was a completely randomized block with four replicates in a bifactorial of 2 (cuts) x 6 (Se doses). Harvesting plants were standardized by height of 5cm and application of fertilizer treatments after 16 days post transplantation and biomass was harvested on days 30 and 60 after Se application. Roots were collected on after the second harvest. Biomass samples were separated and dried (60°C in a forced air oven for 72h) into leaves, stem + sheath, and roots for Se analysis.

Coating was prepared with a mixture of boric acid (0.4g kg^{-1} of boron) and copper (Cu) sulfate (0.14g kg^{-1} of Cu) (FARIA et al., 2013). Selenium treatments were added to the coating mixture as sodium selenate from Sigma Aldrich® (21.4g kg^{-1} of Se) to achieve 0, 0.035, 0.07, 0.14, 0.28 and 0.56mg dm^{-3} in equivalence to 0, 10, 20, 40, 80, and 160g ha^{-1} of Se, respectively, before coating urea granules. Urea amount (46.6% of N) was applied 28mg dm^{-3} in equivalency to pot surface to application of 100kg ha^{-1} of N.

The extraction of total Se was performed by digestion. Briefly, material was placed in microwave oven (Milestone ETHOS ONE) containing 250mg of dry and milled (1mm) samples with 3mL of HNO_3 and 2.5mL of H_2O_2. Deionized water was added (20mL) to extract and followed by a pre-reduction process. At this stage, 1mL of H_2O_2 and 5mL of HCl were added to 10mL of plant extract. Incubation was done in a hot water bath (80°C) for 60min, with 20mL of deionized water added before performing the final analyses. Content of Se in plant extracts was quantified by using a combination of hydride generation technique and Atomic Absorption Spectroscopy (HG-AAS) using sodium borohydride (0.35% mv^{-1}), NaOH (0.2% mv^{1}), 10mol L^{-1} of HCl, and deionized water.

Concentrations of reduced (GSH) and oxidized glutathione (GSSG) were measured at the third fully expanded leaf from the top in both periods of cuts. Briefly, fresh matter (0.2g) was homogenized in 2.0mL of 1% sulfosalicylic acid with pre-cooled mortar and pestle to maintain cold temperature. The homogenate was centrifuged at 10000g for 30min. Supernatant was used for GSH and GSSG measurement as described by ANDERSON (1985) with modification. The amount of GSSG was calculated by subtracting GSH from total glutathione concentrations. A standard curve was prepared using reduced glutathione concentrations.

Data obtained were subjected to statistical analysis using SAS® v.9.2 (Statistical Analysis System Institute, Cary NC, USA). The normality of the data was verified by the Shapiro-Wilk test. Regression analysis for Se doses was performed using PROC GLM with linear adjustment, evaluating significance levels of 1% and 5%.

There was no effect or interaction between Se doses and cuts for dry matter production, tiller density and root (Table 1). Selenium doses had no overall effect on total glutathione, GSH, GSSG, and ratio of GSH/GSSG on diagnostic leaf; however, was observed cuts effects (Table 1).There was interaction between Se doses and cuts for Se enrichment in biomass. Doses of Se in fertilizer promoted a linear increasing content of Se in biomass and roots. Content of Se in leaves and stem+sheath increased according to Se doses from fertilizers reaching up 11mg kg^{-1} of Se in leaves 30 days after

Table 1 - Dry matter of *B. brizantha* reduced (GSH) and oxidized glutathione (GSSG) contents in fractions of *B. brizantha* under selenium (Se) doses fertilization.

Se doses (g ha1)	Roots	Dry matter production	Tiller	GSH	Total Glutathione	GSSG
	--------------------------g pot-1	------------------------------		--------------------------mM g-1 MF	------------------------	
0	47.3	21.7	31.1	2.96	4.26	1.29
10	42.4	21.8	32.8	3.46	4.74	1.41
20	41.2	22.4	31.4	3.32	5.03	1.70
40	43.9	19.6	30.9	3.46	4.75	1.31
80	31.6	24.5	31.1	2.50	3.82	1.31
160	36.9	21.0	29.9	3.34	5.44	2.09
P	ns	ns	ns	ns	ns	ns
---------------------	---Cuts (days)					----------------------
30 days	-	22.7 a	24.8 a	2.77	3.51	0.78
60 days	-	20.9 a	37.6 a	3.66	6.10	2.44
P	-	ns	ns	*	*	*
Se doses x cuts	ns	ns	ns	ns	ns	ns
C.V. (%)	32.7	18	9.9	29.7	27.1	42.7

treatments application; however, these values decreased at the time of second harvest at 60 days after treatments application (Table 2).

Selenium is involved in plant defense by influence in glutathione peroxidase activity (RIOS et al., 2010), but molecules GSH and GSSG were not affected by the Se doses or interaction with cuts. However, we observed non-stressful conditions in GSH/GSSG ratios of 1.6 to 2.6 for harvesting day 30 and 60, respectively, since stressful conditions affects reduction and oxidation rates and consequently is observed ratio of GSH/GSSG lower than 0.9 (NOCTOR et al., 1998). In the first harvest, we observed that doses of Se that were greater than 90g ha^{-1} promoted the concentration of Se in the plant biomass to exceed 5mg kg^{-1} of Se in dry matter, that is maximum recommendation of Se to avoid toxicity problems in cattle (NRC, 2005). Even though, no toxic effects were observed in plants, high intakes of forage fertilized with Se doses above 40g ha^{-1} in the first 30 days of this study could result in livestock intoxication.

Our results showed that a recommendation of 34.5g ha^{-1} of Se fertilization is necessary to ensure a production of biomass containing Se

Table 2 - Selenium (mg kg^{-1} DM) content in fractions of *B. brizantha* under Se doses fertilization.

Se doses (gha-1)	-------------------Leaves---------------		----------------Stem + sheath--------		Roots	Se recovery by plant (%)
	30 days	60 days	30 days	60 days	60 days	
0	0.037	0.002	0.001	0.019	0.25	0
10	0.436	0.106	0.171	0.044	0.27	27.0
20	1.179	0.202	0.544	0.134	0.25	35.7
40	2.549	0.421	1.031	0.303	0.29	35.1
80	6.102	1.244	3.521	0.669	0.79	50.1
160	11.09	1.694	6.234	0.753	1.20	41.50
	Y=0.0712x-0.1121	Y=0.0112x+0.0316	Y=0.0408x-0.1935	Y=0.005x+0.0634	Y=0.0065x+0.1743	Y=0.0869x+32.492
R^2	0.9956	0.9478	0.9861	0.8754	0.9500	0.3832
P	*		*		*	*
C.V. (%)	33		28		26	15

* = P<0.01; C.V. = Coefficient of variation.

at the concentration of 0.3mg kg^{-1}of DM, which is required by cattle (NRC, 2001). At this rate, a desirable content of Se in biomass is present in the forage for at least two harvesting periods (up to 60 days). This estimated rate might ensure Se at a concentration lower than 5mg kg^{-1} of dry matter during the first harvest and the residual effect ensures that cattle requirement is met during the second harvest. Low content of Se in biomass (Table 1) was an evidence of a low residual effect of the fertilizer in the second cut. Research carried out on *P*aspalum notatum reached acceptable Se levels in forage (> 0.1mg kg^{-1} of Se) by using 10 to 20g ha-1of Se slow releasing fertilizer; however, Se content reduced to deficient levels after 8 and 12 weeks (VALLE et al., 2007).

Increasing doses of Se in urea lead to greater accumulation of this element in the biomass. The low accumulation of Se in the biomass when lower doses of Se were applied was probably consequence of availability losses due Se adsorption by the soil (ROVIRA et al., 2008). Soil capacity to accumulate remaining Se after fertilizations becomes a concern once the high levels of Se may become available in a possible change of the environment in the soil, such as liming or mineralization of the organic matter. Content of Se in roots were increased mainly by the application of Se doses above 80g kg^{-1}. Absorption of Se was mobilized to leaves and stem + sheath until 60 days after treatments were applied (Table 1). Selenate form has been shown to be highly mobile in the xylem transport system (RAMOS et al., 2012).

Agronomic Se enrichment for *Brachiaria brizantha* cv. Marandu in weathered tropical soil through urea coating fertilizer can be obtained at an application rate of 34.5g ha^{-1} of Se.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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