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## Chelated zinc leaf application on nutrients concentration and yield of Mombasa grass

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#### ABSTRACT

The intensification of livestock yield requires advances in the management and fertilization of forage crops. This study was conducted to determine the dry weight yield (DWY) along with the concentration and amount of nutrients of *Megathyrsus maximum* cv. Mombasa. The treatments were arranged in a factorial scheme  $6 \times 2$ , six leaf rates of Zn chelate and two soil types (Entisols and Ultisol) with low and medium Zn content and four replicates. Entisols with low Zn content and Ultisol with average Zn content were used plus a control treatment with Zn sulfate. The Zn chelate and Zn sulfate foliar application did not affect the DWY. The tillering and DWY were higher in Ultisol than in Entisol. The Zn content in DWY was directly proportional to rate chelated Zn. The root dry weight yield of Mombasa grass reduced with the chelated Zn foliar application in Entisol and had no effect on Ultisol.

#### ARTICLE HISTORY

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#### KEYWORDS

Pasture; forage; nutritional status; zinc fertilization

#### Introduction

The cattle industry is one in which productivity, efficiency, profitability, and the sustainability of production processes are aspects of great importance. As a result, there is currently an intensification of production, with fertilizers being used to increase both the productivity and quality of pastures. This should result in a greater demand for micronutrients in the system (Manoj et al. 2016). In addition, the study of micronutrients in pastures is important for mineral nutrition of plants and the nutrition of animals that feed on the forage.

Pastures are the main food source for herds of ruminants, which constitute the support base of Brazilian livestock. *Megathyrsus maximum* (syn. *Panicum maximum*), varieties Tanzania and Mombasa, are among the innovative forage responsible for the technological leap which have increased productivity per area, as well as the nutritional value of the pastures' production system (Moreno et al. 2014; Heinrichs et al. 2016). To complement the soil fertilization, foliar fertilization is also used. This is traditionally intended for correction of nutrient deficiencies. It is also a good alternative to using small rates and reducing common losses in soil fertilization, such as leaching and immobilization (Boonchuay et al. 2013).

Micronutrients are indispensable for the growth and development of plants. However, their use in tropical grass pasture fertilization programs does not always result in an increase of forage

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yield. This lack of response may occur because the soils with low pH, with no liming or low fertilization with macronutrients and micronutrients application in the soil may also lead to a lack of response (Guimarães et al. 2016).

The primary cause of nutritional deficiency is the low concentration of Zn in soil, compounded by the intensification of the removal of the micronutrient crops and in some situations, the excessive increase in soil pH by liming. At high pH levels, the availability of Zn for plants decreases due to reduced solubility and redistribution of micronutrients of exchangeable fraction for oxidic fractions (Fe oxides and crystalline and amorphous Al) less available to plants (Jha et al. 2015).

The objective of this study was to evaluate the SDW, root dry weight, nutrients concentration and amount of the forage *Megathyrsus maximum* cv. Mombasa fertilized with Zn chelated leaf application, in two soils (Entisols and Ultisol) with different Zn availabilities.

#### **Materials and methods**

#### Forage plant and soil

The experiment was conducted in a greenhouse at São Paulo State University,  $(21^{\circ}29' \text{ LS} \text{ and } 51^{\circ}32' \text{ LW}$ ; 396 m altitude), Dracena county, Brazil, using the forage grass *Megathyrsus maximum* cv. Mombasa. Two soils were used (Embrapa 2013): i) Entisol: dystrophic, with Cerrado vegetation, with the following chemical attributes: pH 4.0 [calcium chloride (CaCl<sub>2</sub> 0.1 mol L<sup>-1</sup>)], 7.0 g kg<sup>-1</sup> organic matter (OM), 3.0 mg kg<sup>-1</sup> phosphorus (P) (resin-extracted), 7.0 mg kg<sup>-1</sup> sulfur (S)-sulfate (SO<sub>4</sub><sup>2-</sup>), 0.1 mmol<sub>c</sub> kg<sup>-1</sup> potassium (K<sup>+</sup>), 3.0 mmol<sub>c</sub> kg<sup>-1</sup> calcium (Ca<sup>2+</sup>), 2.0 mmol<sub>c</sub> kg<sup>-1</sup> magnesium (Mg<sup>2+</sup>), 3.0 mmol<sub>c</sub> kg<sup>-1</sup> aluminum (Al<sup>3+</sup>), 25.0 mmol<sub>c</sub> kg<sup>-1</sup> potential acidity (H<sup>+</sup>+Al<sup>3+</sup>), 30.0 mmol<sub>c</sub> kg<sup>-1</sup> cation exchange capacity (CEC), 17% base saturation (V), 0.3 mg kg<sup>-1</sup> boron (B), 0.5 mg kg<sup>-1</sup> copper (Cu), 73 mg kg<sup>-1</sup> iron (Fe), 1.1 mg kg<sup>-1</sup> manganese (Mn), and 0.3 mg kg<sup>-1</sup> Zn. b) Ultisol, dystrophic, with pH 5.2 (CaCl<sub>2</sub> 0.1 mol L<sup>-1</sup>), 5.0 g kg<sup>-1</sup> OM; 5.0 mg kg<sup>-1</sup> Mg<sup>2+</sup>, 0.1 mmol<sub>c</sub> kg<sup>-1</sup> Al<sup>3+</sup>; 18.0 mmol<sub>c</sub> kg<sup>-1</sup> H<sup>+</sup>+Al<sup>3+</sup>; 33.0 mmol<sub>c</sub> kg<sup>-1</sup> cation exchange capacity (CEC), 46% V, 0.3 mg kg<sup>-1</sup> B, 1.6 mg kg<sup>-1</sup> Cu; 10 mg kg<sup>-1</sup> Fe, 4.2 mg kg<sup>-1</sup>Mn, and 0.8 mg kg<sup>-1</sup> Zn (Raij et al. 2001). In this experiment, soils were sampled from a 0–20 cm depth for a composite sample; the soil was crumbled, air-dried and then sieved (4.0 mm).

#### Experimental design and treatments

The experiment was arranged in a completely randomized block design with four replicates. The  $6 \times 2$  factorial treatments consisted of six foliar zinc chelates (EDTA) rates 0.0, 43.5, 87.0, 174.0, 348.0, and 696.0 g applied with 200 L ha<sup>-1</sup> of H<sub>2</sub>O, in two soil conditions [low (Entisol) and high zinc content (Ultisol)], plus a control treatment with zinc sulfate (PA reagent, with 355.6 g 200 L ha<sup>-1</sup> of Zn).

#### Experimental conditions and plant growth

The base saturation of soils was increased to 70% (Raij et al. 1996) by adding  $CaCO_3$  and magnesium carbonate (MgCO<sub>3</sub>), P.A. reagents at a ratio of 3:1. The soils plus carbonate salts were then incubated for 30 days in the pots for the reaction, with moisture maintained at 80% field capacity. At the end of the incubation period, the soils were air-dried for seven days. The soils of each pot (4.0 kg with soil density of 1.3 g cm<sup>-3</sup>) were transferred to plastic trays where the treatments and fertilizers were applied: ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>), with 300.0 mg kg<sup>-1</sup> N; Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>, with

Soils	First cut (tiller pot <sup>-1</sup> )	Second cut (tiller $pot^{-1}$ )	Third cut (tiller $pot^{-1}$ )
Entisol	28.9 B	33.2 B	37.7 B
Ultisol	33.2 A	39.6 A	44.1 A
DMS	2.4*	3.2*	3.8*
CV %	13.2	14.9	16.0
Chelate Zn <sup>ns</sup>	31.0 (±4.6) <sup>a</sup>	36.4 (±6.5)	40.9 (±7.8)
ZnSO <sub>4</sub>	35.8 (±4.0)	36.4 (±3.6)	37.9 (±6.6)

Table 1. Tillering per pot of Mombasa grass grown in three cuts in Entisol and Ultisol.

<sup>a</sup>Standard deviation.

\*Significant at 5% by the Tukey test.

180.0 mg kg<sup>-1</sup> P; dipotassium sulfate (K<sub>2</sub>SO<sub>4</sub>), with 150.0 mg kg<sup>-1</sup> K; boric acid (H<sub>3</sub>BO<sub>3</sub>), with 0.5 mg kg<sup>-1</sup> B; copper sulfate (CuSO<sub>4</sub>), with 1.0 mg kg<sup>-1</sup> Cu; iron sulfate (FeSO<sub>4</sub>), with 10.0 mg kg<sup>-1</sup> Fe; H<sub>2</sub>MoO<sub>4</sub>, with 0.1 mg kg<sup>-1</sup> Mo; manganese sulfate (MnSO<sub>4</sub>), with 3.0 mg kg<sup>-1</sup>Mn. 4.0 kg of the treated soils were then transferred to the treatment labeled pots, and after one day Mombasa grass was sown and the seeds were evenly covered with a thin soil depth. After 2 weeks, the plants were thinned to four plants per pot. Soil moisture was maintained close to 80% field capacity (FC) by daily irrigation with deionized water. The amount of water to be added was determined by weighing the five pots.

The water volume for the application of foliar nutrients was  $200 \text{ L} \text{ ha}^{-1} \text{ of } \text{H}_2\text{O}$ , and 10 sprays of foliar fertilizer per pot were administered, which represented about  $5.0 \text{ mL pot}^{-1}$ . During this application, the surface of the pot was protected with tissue paper and cotton was put around the stems to prevent nutrient contact with the soil. Zn was applied 17 days after the germination and one week after each cut.

The plants were cut 5.0 cm above the soil surface and three cuts were made. The first cut was 34 days after sowing. Then the second and the third cuts were carried out after 28 and 32 days of growth, respectively. Plant material was dried at  $65 \,^{\circ}$ C at constant weight to determine the SDW. The number of tillers was counted at cutting. The dried plant material was ground in a Willey mill and the total content of Zn, K, Ca, and Mg were determined (Malavolta, Vitti, and Oliveira 1997) in the first and third cut.

#### Statistical analysis

Data were tested for normality and homogeneity of variance errors and statistical analysis were performed using SAS (Statistical Analysis System, version 8.2; SAS Inc, Cary, NC). The results were submitted to ANOVA, F test and Tukey's multiple comparison of averages, the 5% of probability. Regression analysis was performed according to the doses of zinc (Pimentel-Gomes and Garcia 2002).

#### **Results and discussion**

#### Tillers, shoot dry weight yield, and morphological composition

Tillering results showed no significant effect for Zn rates via leaf fertilizer (Table 1). The higher yield of tillers e dry weight with Ultisol is possibly associated with greater natural fertility in relation to Entisol (Table 1). The lack of tillering response has been observed in other studies carried out with Marandu grass (Beraldo et al. 2012; Martins, Monteiro, and Pedreira 2014). Thus, further studies are needed with Zn foliar application, since the absence of responses in the production of tillers and dry weight of Mombasa grass can be connected to experimental conditions with potted content  $4.0 \, \text{dm}^{-3}$  of soil.

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Soils	First cut (g pot <sup>-1</sup> )	Second cut (g $pot^{-1}$ )	Third cut (g $pot^{-1}$ )	TDW (g pot <sup>-1</sup> )
Entisol	14.3 A	28.1 B	36.5 B	78.9 B
Ultisol	13.2 B	30.2 A	39.1A	82.5 A
DMS	0.9*	1.3*	1.8*	2.6*
CV %	11.0	7.8	8.1	5.6
Chelate Zn <sup>ns</sup>	13.7 (±1.5) <sup>a</sup>	29.2 (±2.4)	37.8 (±3.4)	80.7 (±4.7)
ZnSO <sub>4</sub>	14.3 (±2.1)	30.0 (±1.0)	37.9 (±3.4)	82.2 (±5.3)

Table 2. Shoot dry weight of Mombasa grass grown in three cuts and total dry weight (TDW) in Entisol and Ultisol.

<sup>a</sup>Standard deviation.

\*Significant at 5% by the Tukey test.

Table 3. Morphological composition [leaf blade/(stem + sheaths)] in three cuts by Mombasa grass grown in Entisol and Ultisol fertilized with Zn leaf rates.

	F	irst cut	Sec	cond cut	Thirdcut		
Treatments	Leaf blade (%)	Stem + sheaths (%)	Leaf blade (%)	Stem + sheaths (%)	Leaf blade (%)	Stem + sheaths (%)	
Entisol <sup>ns</sup>	74.4 (±2.3) <sup>a</sup>	25.6 (±2.3)	76.8 (±1.0)	23.2 (±1.0)	75.1 (±0.9)	24.9 (±0.9)	
Ultisol	77.9 (±2.1)	22.1 (±2.1)	75.8 (±1.0)	24.3 (±0.9)	76.1 (±0.7)	23.9 (±0.7)	
Chelate Zn <sup>ns</sup>	76.2 (±2.2)	23.8 (±2.2)	76.3 (±0.9)	23.7 (±0.9)	75.6 (±1.0)	24.4 (±1.0)	
ZnSO4 <sup>ns</sup>	75.7 (±1.6)	24.4 (±1.6)	75.9 (±0.6)	24.1 (±0.6)	75.7 (±1.0)	24.3 (±1.0)	

ns: Zn rates was not significant at  $p \leq .05$ .

<sup>a</sup>Standard deviation.

However, differential responses between the soils were observed, with higher tillering in Ultisol. The SDW followed the same trend of tillering with higher yield (Table 2). These results were a consequence of greater tillering. In the morphological separation [(blade leaf/ (stem + sheath)] variation was not observed due to the foliar fertilization and soil (Table 3).

#### Root dry weight

There was a significant interaction with the Zn rates applied and the types of soil used for the roots dry weight (RDW) yield. Overall, Entisol had the highest average ranging from 150.6 to  $56.6 \text{ g} \text{ pot}^{-1}$  at 87 and 696 g rates of Zn chelate, respectively. While averages Ultisol were lower, ranging from 42.8 to 29.9 g pot<sup>-1</sup> at 174.0 and 348.0 g 200 L<sup>-1</sup> rates of Zn chelate, respectively. Treatment with ZnSO<sub>4</sub>, RDW yield was 34.6 and 207.5 g pot<sup>-1</sup> for Ultisol and Entisol, respectively (Figure 1A,B).

In Ultisol, there was no effect of Zn rates in RDW yield, while in Entisol a reduction in the RDW occurred in the larger leaf Zn levels (Figure 1). This confined the roots to a small space with greater contact root-soil and provided greater nutrients utilization from the soil or plant. As a result, it could not express its maximum yield potential and, hence, the nutrient content in the soil was sufficient. It is possible that the Ultisol with an average Zn content in the soil had enough of this micronutrient to meet the physiological needs of plants, maintaining the same RDW yield, independent to the rate applied to the leaves. On the other hand, in Entisol low Zn content in the soil and the presence of the micronutrients foliar application reduced RDW yield, which may be associated with less need for uptake via the roots (Christophersen and Lyons 2012).

#### Zinc and potassium concentration

The chelated Zn application linearly increases micronutrient concentrations in the shoots of Mombasa grass in the first cut, with no significant difference between the soils. The levels ranged from 38.8 to 130.0 mg kg<sup>-1</sup> at 43.5 and 696.0 g in 200 L ha<sup>-1</sup> rates of Zn chelate, respectively. In





Figure 1. Root dry weight yield of *Megathyrsus maximum* cv. Mombasa with chelated Zn rates. a: Cultivated in Ultisol. b: Cultivated in Entisol.

the treatments with  $ZnSO_4$ , the concentration of 88.8 mg kg<sup>-1</sup> Zn at 355.0 g 200.0 L ha<sup>-1</sup> rate was obtained (Figure 2A).

In the third cut, the Zn content in the plant showed a significant interaction between the dose of chelated Zn and soil, ranging from 12.5 to 138.5 mg kg<sup>-1</sup> in Entisol and 39.2 to 262.3 mg kg<sup>-1</sup> in Ultisol in the dose range from 0.0 (control) and 696 g 200 L ha<sup>-1</sup> of Zn chelate, respectively (Figure 2B,C).

The 43.5 g 200 L ha<sup>-1</sup> application of leaf Zn were enough to reach the lower limit of the level considered appropriate for Zn in the shoots of Mombasa grass, ranging from 20 to 50 mg kg<sup>-1</sup>, as well, when grown in Entisol with micronutrient deficiency ( $\leq 0.3 \text{ mg dm}^{-3}$ ). The results show



**Figure 2.** Zn and K concentration in *Megathysus maximum* cv. Mombasa due to foliar rate of chelated Zn grown in Ultisol and Entisol. a: Zn in the first cut to the average of the two soil types; b: Zn in the third cut the forage grown in Ultisol; c: Zn in the third cut the forage grown in Entisol; d: K in the third cut the forage grown in Entisol.

Table 4.	K, Ca	a, and	Mg	concentration	in	Mombasa	grass	in	first	and	third	cutting	in	Entisol	and	Ultisc	۶I.

Soils	K (g kg <sup>-1</sup> )	Ca (g kg <sup>-1</sup> )	Mg (g kg <sup>-1</sup> )	
First cutting				
Entisol	22.8 B	6.8 A	7.7	
Ultisol	30.3 A	5.8 B	8.0	
DMS	1.7*	0.7*	0.4 <sup>ns</sup>	
CV %	11.1	18.2	8.6	
Chelate Zn <sup>ns</sup>	26.5 (±1.9) <sup>a</sup>	6.3 (±0.8)	7.8 (±0.6)	
ZnSO <sub>4</sub>	25.0 (±5.3)	7.4 (±1.3)	7.7 (±0.5)	
Third cutting				
Entisol	16.3 B	7.4	2.0 B	
Ultisol	18.8 A	7.4	2.2 A	
DMS	1.2*	0.4 <sup>ns</sup>	0.1*	
CV%	11.8	9.4	10.4	
Chelate Zn <sup>ns</sup>	_	7.3 (±0.5) <sup>a</sup>	2.1 (±0.2)	
ZnSO <sub>4</sub>	-	6.3 (±0.9)	2.0 (±0.2)	

<sup>a</sup>Standard deviation.

\*Significant at 5% by the Tukey test.

that the leaf fertilization with Zn can contribute to the nutrition of Mombasa grass (Martins, Monteiro, and Pedreira 2015) and contribute to the forage yield of better quality minerals for animals. In the Zn analysis results in the control treatment (0 Zn), in the first and third cut it is possible to verify the reduction of the micronutrients concentration in the SDW as a function of successive cuts (Figure 2A–C), suggesting the need for replacement of the nutrient in the system to maintain mineral forage quality.



Figure 3. Zn and K uptake by *Megathysus maximum* cv. Mombasa due to foliar rate of chelated Zn grown in Ultisol and Entisol: a: Zn in the first cut to the average of the two soils; b: Zn in the third cut the forage grown in Ultisol; c: Zn in the third cut the forage grown in Entisol.

The 696.0 g 200 L ha<sup>-1</sup> rate of Zn is considered high and achieved very high levels in the plant (Figure 2A–C). However, toxicity symptoms were not observed. No references were found of Zn levels that would be toxic to the Mombasa grass, but forage grasses adequate levels are usually cited up to 50.0 mg kg<sup>-1</sup> (Raij et al. 1996). It is possible that Zn, when retained in vacuoles and complexed with organic acids, reduces or prevents its toxicity (Shanti, Dietz, and Mimura 2016).

Soils	K (g pot <sup>-1</sup> )	Ca (g pot <sup>-1</sup> )	Mg (g pot <sup>-1</sup> )		
First cutting					
Entisol	0.32 B	0.09 A	0.11		
Ultisol	0.39 A	0.07 B	0.10		
DMS	0.02*	0.01*	0.01 <sup>ns</sup>		
CV%	11.7	21.03	15.06		
Chelate Zn <sup>ns</sup>	26.54 (±1.91) <sup>a</sup>	6.28 (±0.78)	7.84 (±0.58)		
ZnSO <sub>4</sub>	25.00 (±5.29)	7.43 (±1.31)	7.68 (±0.51)		
Third cutting					
Entisol	0.63 B	0.26 B	0.07 B		
Ultisol	0.71 A	0.28 A	0.08 A		
DMS	0.04*	0.01*	0.005*		
CV%	13.65	10.27	10.70		
Chelate Zn <sup>ns</sup>	0.69 (±0.15) <sup>a</sup>	0.27 (±0.18)	0.07 (±0.19)		
ZnSO <sub>4</sub>	0.66 (±0.04)	0.26 (±0.36)	0.07 (±0.03)		

<sup>a</sup>Standard deviation.

\*Significant at 5 % by the Tukey test.

In relation of the K, was observed lower concentration with higher Zn rate in Entisol (Figure 2D), may be associated with similar effects observed in the Mg to Zn inhibition (Moreira et al. 2003, 2005). There also may be some inhibition of the K uptake due to high Zn rates. However, more studies are necessary on ionic competition, especially involving micronutrients (Marschner 2012).

In the first cut, the K content in the shoots was higher when cultivated in Ultisol ( $30.3 \text{ g kg}^{-1}$ ) compared to Entisol ( $22.8 \text{ g kg}^{-1}$ ). In the third cut, the Ultisol continued with the highest average ( $18.8 \text{ g kg}^{-1}$ ), 15% higher than the Entisol (Table 4). The difference between the soils can be attributed to the higher natural fertility of Ultisol. In Entisol, the K content in the shoots of Mombasa grass of the third cut was significant. The minimum point of  $14.8 \text{ g kg}^{-1}$  K at 288 g 200 L ha<sup>-1</sup> rate of Zn chelate was observed (Figure 2D).

The Ca content in both soils and cuts is within the desired range. Meanwhile, Mg showed high concentration in the first cut and reduction in the subsequent cut (Table 4). These results may suggest the need for replacement of the nutrients in crops with high technology, because the amount provided via liming cannot meet the demand of the plant. Although there was a difference between the soil in Ca and Mg, in all cases they are within the same range of interpretation. Regarding Zn rates, no effect on the Ca and Mg concentration in forage was observed. The results were very close to those seen with the ZnSO<sub>4</sub> application (Table 4).

#### Uptake of zinc and potassium

The Zn uptake during the first growth cycle of Mombasa grass showed a linear response as a function of foliar fertilization of 696 g 200 L ha<sup>-1</sup> Zn chelate and no difference between the soils (Figure 3A). In turn, the third cut produced a differential response as a function of the soil, with higher absorption in Ultisol compared to Entisol, although both showed a linear response. As described above, the highest values found in Ultisol should be related to the higher contribution of the nutrients in the soil (Figure 3B,C). Regarding the additional treatment with ZnSO<sub>4</sub>, the content found in plants was very similar to that observed with the chelated Zn application.

Regarding the cationic elements K, Ca, and Mg, results showed that the largest amounts were absorbed in Ultisol with the exception of the first cut, when the Ca values were higher in Entisol and Mg showed no difference between soils (Table 5). According to NRC (1996) the values  $30.0 \text{ mg kg}^{-1}$  Zn,  $6.0 \text{ g kg}^{-1}$  K<sup>-1</sup>,  $1.2 \text{ g kg}^{-1}$  Ca, and  $1.0 \text{ g kg}^{-1}$  Mg supply the daily necessities for a calf of 350 kg. As a result, it is possible to note that the fertilizer used provided the growth and forage production with a nutrient concentration that would ensure proper animal nutrition,

particularly in relation to Zn. In the absence of foliar fertilization, the levels were well below the reference value previously mentioned. Overall, the results of increased nutrients uptake in Ultisol may be associated with different physicochemical characteristics of the soil, such as clay content, presence of oxides and original nutrient content in the soil, among others (Melo et al. 2016).

#### Conclusions

The zinc chelate and zinc sulfate foliar application did not affect the shoot dry weight (SDW) yield of Mombasa grass. The tillering and SDW was higher in Ultisol than in Entisol. The Zn content in the dry weight of Mombasa grass was directly proportional to the rate chelated Zn foliar, contributing to the yield of better quality forage. The root dry weight of Mombasa grass was reduced with the chelated Zn foliar application in Entisol and had no effect on Ultisol. Further research is needed to evaluate the ionic competition of cationic nutrients, especially involving Zn and foliar fertilization.

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