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# Efficiency of urea with urease and nitrification inhibitors in the production of bermuda grass fodder

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kg ha-1 of N) applied after each growth period. Then, the study proceeded to estimate forage dry mass and N concentrations in dry mass for each of the growth cycles and their sum, as well as the apparent N recovery. N critical levels were set when relative production reached 90%. The use of urease (NBPT) or nitrification (DMPP) inhibitors did not improve the efficiency of urea regarding growth and production of Tifton 85 Bermuda grass. The appropriate dose of nitrogen for Tifton 85 Bermuda grass in a hay production system is 40 kg per ton of dry mass produced in each growth period.

#### RESUMEN

#### Palavras-chaves

*Cynodon* spp nitrógeno fertilizantes estabilizados

Eficiencia de la urea con inhibidores de ureasa y nitrificación en la producción de forraje de pasto bermuda La adopción de estrategias destinadas a un uso más eficiente del nitrógeno (N) y la obtención de altos rendimientos son importantes para satisfacer la demanda de alimentos para los animales y seres humanos. El pasto bermuda Tifton 85 (Cynodon spp.) es un cultivo con un alto potencial productivo, que puede utilizarse para la producción de heno, pero es un cultivo que requiere altas dosis de N. En este sentido, el objetivo de este experimento fue evaluar en condiciones de campo, el crecimiento y la producción del pasto bermuda Tifton 85 en función de las dosis de nitrógeno con urea que contiene ureasa e inhibidor de la nitrificación durante dos años agrícolas, totalizando ocho ciclos de crecimiento de forraje. Los tratamientos fueron evaluados en un diseño de bloques completos al azar con tres repeticiones en un esquema factorial de 3 x 5. Los tratamientos consistiéron en: I) urea; II) urea + triamida N-(n-butil)tiofosfórica (NBPT), inhibidor de la ureasa y III) urea + inhibidor de la nitrificación (DMPP - 3,4-dimetilpirazol fosfato) y dosis de N (0, 40, 80, 120 y 160 kg ha-1 de N aplicado después de cada período de crecimiento). Posteriormente, se estimo la mass seca de forraje y las concentraciones de N en masa seca (para cada ciclo de crecimiento y suma de los ciclos de crecimiento), así como la recuperación aparente de N. El nivel crítico de N se estableció cuando la producción relativa fue del 90%. El uso del inhibidor de la ureasa (NBPT) o la nitrificación (DMPP) no mejoró la eficiencia de la urea con respecto al crecimiento y la producción de pasto bermuda Tifton 85. La dosis adecuada de nitrógeno para el Tifton 85 en un sistema de producción de heno es de 40 kg por tonelada de masa seca producida en cada período de crecimiento.

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## INTRODUCTION

L reality for over 50 years, food demand will continue to grow globally in the coming decades, with an intense increase in the need for animal products, especially in underdeveloped countries (Bodirsky et al., 2015). Besides the rise in population numbers and in power consumption, energy production through biomass is another factor contributing to pressure in agricultural systems, which requires from them a higher efficiency (Cicera & Masset, 2010).

The adoption of strategies aiming to enhance the use of nutrients such as nitrogen (N) and to obtain high yield is important to meet such demand (Tilman et al., 2011). The Tifton 85 Bermuda grass (*Cynodon* spp.) is a crop with high productive potential and may be used to create hay, but its culture requires elevated doses of nitrogen (Mandebvu et al., 1999; Muir et al. 2010; Evers et al., 2014).

Urea is the main form of nitrogen fertilizer used in agriculture throughout the world (Espíndula et al., 2013). This fertilizer — after being distributed onto the soil — is susceptible to ammonia losses by volatilization through hydrolysis (Malhi et al., 2001; Raij, 2017). In addition, other processes result in N losses to the environment. After the incorporation of urea into the soil, nitrification will take place, transforming NH4+ into NO3- because of microorganisms. After this process, the nitrate is susceptible to leaching and there may be loss by denitrification when NO3- is converted into N2 (NO3-  $\rightarrow$  NO2-  $\rightarrow$  NO  $\rightarrow$  N2O  $\rightarrow$  N2), and N2O is emitted into the atmosphere (Subbarao et al., 2006).

Generally, the recovery efficiency of applied N rarely exceeds 50% (Abbasi et al., 2001). The loss by volatilization may reach 78% of the N applied. This process can be intensified when urea is applied on crop residues, which increase urease activity in soils (Cabezas et. al, 1997).

The use of urease and nitrification inhibitors are technologies that have been tested to increase the efficiency of nitrogen fertilization. The first reduces the chances of N loss by volatilization due to a delay in the action of the urease enzyme; the second inhibits the action of the bacteria Nitrosomonas (Bodirsky et al., 2015), promoting delay in the conversion of ammonium to nitrate in such a way that the plant is able to reach its highest growth stage and is more capable of absorbing the nutrient, avoiding greater losses. However, there is little research in Brazil with such technologies, especially in forage production.

Nitropyrene and dicyandiamide (DCD) are the most extensively tested nitrification inhibitors in the world, with 3,4-dimethylpyrazole phosphate (DMPP) being added to this list recently (Calderon et al., 2015; Gilsanz et al., 2016). The latter, according to Malla et al. (2005), is one of the inhibitors with the highest potential to reduce N losses in the soil.

In view of the above, this experiment sought to evaluate, in field conditions, the growth and production of Tifton 85 Bermuda grass according to nitrogen doses with urea containing urease and nitrification inhibitors.

# MATERIALS AND METHODS

### Location, time period and soil features

The study was carried out in an already established Tifton 85 hayfield, at the São Paulo State University (UNESP/FCAV), Campus of Jaboticabal, São Paulo, Brazil. The soil is classified as a Typic Haplustox, according to the criteria defined by Embrapa (2018). The field experiments were conducted from November through March of the agricultural years 2013/2014 and 2014/2015. Both experiments were implemented on November 15.

A sample from the topsoil (0 to 0.10 m deep) was collected and its fertility was analyzed according to the methodology described by Raij et al. (2001). The chemical attributes were: pH (CaCl2) = 4.8; organic matter = 29 g dm-3; P (resin) = 10 mg dm-3; K = 4.4 mmolc dm-3; Ca = 25 mmolc dm-3; Mg = 14 mmolc dm-3; H + Al = 52 mmolc dm-3; CEC = 95.4 mmolc dm-3; S-SO4 = 4 mg dm-3; base saturation (V%) = 45; B (hot water) = 0.30 mg dm-3; Cu = 3.3 mg dm-3; Fe = 13 mg dm-3; Mn = 33.6 mg dm-3 and Zn (diethylenetriaminepentaacetic acid, DTPA extractor) = 1.3 mg dm-3. Granulometry identified clay (579 g kg-1), silt (139 g kg-1) and sand (282 g kg-1).

Forty days prior to the beginning of the experiment, limestone (CaO: 45%, MgO: 20%, PRNT = 90%) was applied at a rate required to raise the base saturation to 60%. Grass was cut at 0.07 m from ground level to standardize it. The daily values of precipitation and mean air temperature related to the experimental period are presented in Figure 1.



Figure 1 - Daily rainfall and average temperature at the experimental area during 2013 to 2014 and 2014 to 2015

#### **Experiment design and treatments**

The treatments were laid out in a randomized complete block design with three replications in a 3x5 factorial scheme. Treatments were: I) urea; II) urea + urease inhibitor N-(n-Butyl) triphosphoric Triamide (NBPT); and III) urea + nitrification inhibitor 3,4-Dimethylpyrazole phosphate (DMPP) with N rates (0, 40, 80, 120 and 160 kg ha-1 of N) applied after each growth period. Each lot had an area of 9 m<sup>2</sup>.

#### **Experiment conduction and evaluations**

Four cuts were made each year at intervals of 30 days. The lots were then fertilized with 100 kg ha-1 of P2O5 (single superphosphate). N doses and 120 kg ha-1 of K2O (potassium chloride) were applied together.

Forage cutting was done by a mower in a pattern of 1.6 m wide and 0.07 tall. After cutting, the fresh

forage mass was weighed, and a sample of 0.5 kg was taken to the laboratory, where it was washed with water and detergent, running water, HCl solution (0.1 mol L-1) and distilled water. The material was then put to dry in a forced ventilation oven at 65 °C until the mass was constant. N concentration readings were performed according to the methodology described by Bataglia et al. (1983). Afterwards, the apparent N recovery (ANR) rate was calculated according to Moll et al. (1982), through the following equation:

$$ARN~(\%) = \frac{(NAcf - NAsf)}{N~rate} \times 100$$

Where:

ANR: apparent N recovery (%);

Acf: N accumulation with N fertilizer (kg ha-1) NAsf: N accumulation without fertilizer (kg ha-1) N rate: N rate applied at plot (kg ha-1)

#### Statistical analysis

The data were submitted to analysis of variance for main and interaction effects, and to polynomial regression analysis when a significant effect for the N rate factor could be verified. The Tukey's test (p <0.05) was used to compare the means when a significant effect was identified for the fertilizer factor, through the AGROESTAT program (BARBOSA; MALDONADO JÚNIOR, 2015). N critical levels were set when relative production reached 90%. The linear plateau model was performed to determine N critical levels in shoots of Tifton 85 Bermuda grass, which were defined by the intersection of the two lines (Alvarez et al., 1994).

#### RESULTS

In both agricultural years, nitrogen fertilization significantly increased (p < 0.01) the dry mass amount obtained in each growth period (Figures 2 and 3) and in the whole production (sum of all four growth periods) (Figure 4).



Figure 2 - Dry mass production of Tifton 85 Bermuda grass according to doses and sources of N in four consecutive growth periods in the agricultural year 2013/2014\*Significant at (p < 0.05), \*\*Significant at (p < 0.01)



Figure 3 - Dry mass production of Tifton 85 Bermuda grass according to doses and sources of N in four consecutive growths in the agricultural year 2014/2015. \*Significant at (p < 0.05), \*\*Significant at (p < 0.01)



Figure 4 - Dry mass production of Tifton 85 Bermuda grass according to doses and sources of N in four consecutive growths in the agricultural years 2013/2014 and 2014/2015. \*Significant at (p < 0.05), \*\*Significant at (p < 0.01)

For N content in shoots of Tifton 85 Bermuda grass (Figures 5 and 6), linear increases (p < 0.01)

were observed in both agricultural years because of nitrogen doses.



Figure 5 - Nitrogen content in shoots of Tifton 85 Bermuda grass according to doses and sources of N in four consecutive growth periods in the agricultural year 2013/2014. \*Significant at (p < 0.05), \*\*Significant at (p < 0.01)



Figure 6 - Nitrogen content in shoots of Tifton 85 Bermuda grass according to doses and sources of N in four consecutive growth periods in the agricultural year 2014/2015. (to be continued)



Figure 6 - Nitrogen content in shoots of Tifton 85 Bermuda grass according to doses and sources of N in four consecutive growth periods in the agricultural year 2014/2015. (continuation) \*Significant at (p < 0.05), \*\*Significant at (p < 0.01)

Data indicate there was significant influence (p<0.05) by fertilizer type only in the second and fourth growth periods of the second agricultural year regarding N content in the plant. In such cases, a higher N content was observed in the urea +

NBPT treatment if compared to the one where only urea was applied (Table 1). There was no significant interaction between doses and N sources (p > 0.05) regarding N content in shoots or in dry mass production.

Table 1 - Apparent nitrogen recovery in Tifton 85 Bermuda grass according to doses and sources of N in the second third and fourth growth periods of the agricultural year 2014/2015

N content in shoots (g kg <sup>-1</sup> )		Apparent N recovery (%)		
	2nd growth stage	4th growth stage	2nd growth stage	4th growth
Urea	16,54 b	20,51 b	35 b	62 b
Urea + NBPT	18,34 a	22,87 a	42 a	75 a
Urea + DMPP	17,19 ab	21,74 ab	34 b	62 b

Regarding ANR, there was neither significant effect (p> 0.05) for the factors studied nor for the interaction between the two groups in any of the four growth periods of the agricultural year 2013/14, with general averages of 71%, 63%, 30%,

and 81% respectively. Effects (p <0.01) of the nitrogen dose factor were only verified in the third and fourth growth periods of the second agricultural year (Figure 7).



Figure 7 - Apparent nitrogen recovery in Tifton 85 Bermuda grass according to doses and sources of N in the third and fourth growth periods in the agricultural year 2014/2015. \*Significant at (p < 0.05), \*\*Significant at (p < 0.01)

The source influenced (p < 0.01) the ANR in the second and fourth growth stages of the second year, where urea + NBPT surpassed the others (Table 1). There was no significant interaction between doses and sources in any of the growth periods in the two agricultural years. In the first growth stage of the

second agricultural year, the overall average for apparent nitrogen recovery was 45%. Critical levels of N in the shoots of Tifton 85 Bermuda grass associated with 90% of the relative production were 22.5 g kg-1 (Figure 8).



Figure 8 - Relationship between plant N concentration and relative production of Tifton 85 grass in the 2013/14 and 2014/15 growing years

### DISCUSSION

The lower production observed in the third growth period of the first agricultural year (Figure 2) is due to low precipitation (76 mm). From the eighth to the 28th day, it rained only 14.1 mm and 13 days within this period passed without precipitation. In the agricultural year of 2014/2015, in the second growth period, the drought also productivity. Figure undermined crop demonstrates that precipitation (110.2 mm) in the fourth growth cycle of the first agricultural year was similar to the second growth period (121.1 mm) of the following year. However, in this cycle, high productivity was observed (Figure 3). During this period, there was a better distribution of precipitation, while, in the second growth cycle of 2014/2015, it rained mostly in the first days of plant growth. Also, from the ninth to the 27th day, it rained only 19.1 mm.

It is necessary to emphasize how capable this type of grass is in recovering the applied nitrogen

because it reveals a degree of energy efficiency. Primavesi et al. (2004) highlight the importance of knowing how forages extract nutrients, especially in intensive systems with high doses of fertilizers. In this study, it is verified that, most of the time, regardless of the dose, the forage also responded to the recovery of N applied.

According to Terman (1980), when urea is applied to the soil surface, 10 to 20 mm of rainfall is sufficient to drastically reduce NH3 losses by volatilization. In 2013/2014, it rained 13.4 mm in the first three days of the first growth period; and in the fourth growth period, the amount was 6.6 mm in the first two days. These precipitations appear to have been sufficient to incorporate urea into the soil, with no significant losses of N, since the ANR in the first and fourth growth periods were 71 and 81% respectively. However, in the second growth period, only a relevant precipitation occurred on the seventh day after the application, which probably resulted in a lower apparent recovery of N (63%).

In the study performed by Soares et al. (2012),

the highest losses of ammonia by volatilization were observed until the sixth day after applying urea. In field conditions, Dominghetti et al. (2016) and Chagas et al. (2016) found that these losses were concentrated in the first seven days after application procedures. In the agricultural year 2014/15, in the second and fourth growth cycles, precipitations took place only on the sixth day after fertilizer was applied. Thus, the action of the urease inhibitor probably resulted in lower N losses and, consequently, in higher N concentrations and higher ANR in the treatment lot where urea and NBPT were combined (Table 1).

As seen in this study, Fontoura and Bayer (2010) and Mota et al. (2015) also did not observe a significant increase in production comparing the use of urea + NBPT and common urea.

The Tifton 85 grass stands out from other grasses not only because of the quality of fodder produced, but also for its development and, consequently, for the greater efficiency of its root system (Hancock, 2012). In the case of *Cynodon* hybrids, the roots can go 2.43 m deep (Acuña, 2014) — thus, lower nitrate leaching may occur in the soil profile (Hancock, 2012). Taking this into account, it is possible to understand better the high apparent recovery of N observed in this work, as well as the reason why the use of urea + DMPP did not result in higher readings if compared to common urea. Even if a higher nitrate leaching rate was supposed to happen due to the use of common urea, the efficiency of the root system allowed absorption at deeper distances.

According to Hu et al. (2014), when plants can make better use of the nutrient applied, there may be no significant differences between the yields obtained with or without the use of inhibitors. These authors analyzed several studies conducted in Germany in the last 15 years and concluded that nitrification inhibitors did not significantly influenced the production of several crops, such as winter wheat, winter barley, maize and potatoes.

When evaluating studies conducted in the field, Gilsanz et al. (2016) and Yang et al. (2016) demonstrated that DMPP did not significantly increase crop productivity.

The nitrogen fertilization recommendation for grasses in hay production systems for the state of São Paulo (Werner et al., 1997) is based on the N extraction by the dry mass of the plant, which is defined as 20 kg of N per ton of dry mass produced. However, the authors did not present the amount extracted by Tifton 85 Bermuda grass, presenting only the amount of N extracted from another grass of the genus *Cynodon* (coast-cross) — 16 kg of N per ton of dry mass.

In this study, considering the concentrations that are equivalent to 90% of the relative production, one could infer that the Tifton 85 grass extracted 22.5 kg of N per ton of dry mass produced. However, it should be noted that the recovery efficiency of N applied, or the apparent recovery of N is not 100%. For example, the average (total of eight growths) for apparent N recovery, in the present study, was 55%. Taking into account this potential loss of N, it would be necessary to add 16.5 kg of N to the amount extracted by the plant, resulting in 39 kg of N per ton of dry mass produced. In view of this, the appropriate dose for Tifton 85 Bermuda grass should be 40 kg of N per ton of dry mass produced.

# CONCLUSIONS

The use of the urease (NBPT) or nitrification (DMPP) inhibitors does not improve the efficiency of urea in the growth and production of Tifton 85 Bermuda grass. The appropriate dose of nitrogen for this species in a hay production system is 40 kg per ton of dry mass produced in each growth period.

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