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Nitrogen recovery, use efficiency, dry matter yield, and chemical composition of palisade grass fertilized with nitrogen sources in the Cerrado biome

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Abstract

The high cost and the low efficiency of fertilizers, especially nitrogen (N), are of major concerns in agriculture. This study aimed to evaluate the effect of N fertilizers sources in *Urochloa brizantha* cv. Marandu (palisade grass). The study was conducted in Cerrado of Brazil to evaluate dry-matter yield (DMY), recovery of applied N (RAN), N use efficiency (NUE), and chemical composition of palisade grass in response to sources of N (ammonium nitrate, ammonium sulfate, ammonium sulfate-nitrate, urea, urea with urease inhibitor, polymer-coated urea, and control) in seven harvests (100 kg ha^{-1} N were applied after each harvest). The N fertilization increased DMY and growth of palisade grass compared to control (without N fertilization). However, there was not any difference in DMY due to N sources. Application of ammonium sulfate-nitrate increased RAN. The urea, urea with urease inhibitor, and polymer-coated urea improved NUE. Concentration of neutral detergent fiber (NDF) was decreased, while concentrations of acid detergent fiber (ADF) and cellulose were not affected by the fertilizers. Crude protein (CP) content was increased with N supply, but CP fractions A and B were not changed, except in the fifth period of growth. The results indicated protected fertilizers (ammonium sulfate-nitrate, urea with urease inhibitor, and polymer-coated urea) are more recommended for the cultivation of palisade grass in the Cerrado biome of Brazil.

Keywords: cell wall; crude protein fractionation; lignin; protected fertilizers; *Urochloa brizantha*.
Abbreviations: ADF_acid detergent fiber; ADIN_acid detergent insoluble nitrogen; CP_crude protein; DMY_dry matter yield; N_nitrogen; NDF_ neutral detergent fiber; NDIN_neutral detergent insoluble nitrogen; NUE_nitrogen use efficiency; RAN_recovery of applied nitrogen; TN_total nitrogen.

Introduction

Approximately 196,000,000 ha (22%) of the Brazilian territory is covered by grasslands, where the genus *Urochloa* (*Brachiaria* Syn.) stands out for occupying about 48% of this area (De Bona and Monteiro, 2010a; FAO, 2011). However, it is estimated that forty million hectares of *Urochloa* are undergoing a degradation process (Bonfim-da-Silva and Monteiro, 2006). Improper management of pastures, low natural soil fertility, and low fertilization rates are the main factors that result in pasture degradation (Bennett et al., 2008; Costa et al., 2008, 2010). Therefore, the application of fertilizers, mainly N, is crucial to the recover the pastures, since the N from the mineralization of the soil organic matter may not be sufficient to meet the nutrient requirements of high-yield grasses such as the genus *Urochloa* (Boddey et al., 2004; Costa et al., 2009; De Bona and Monteiro, 2010a; Dupas et al., 2010; Silva et al., 2011, 2013). Among the nutrients, N is the one that most influences DMY and forage quality (Primavesi et al., 2005; Bonfim-da-Silva and Monteiro, 2006; Bennett et al., 2008; Oliveira et al., 2010). Additionally, the constant technological innovation in the manufacturing process of N fertilizers interferes with the available form of N in the soil solution and with processes related to N losses, such as volatilization of ammonia (N-NH_3), among other factors (Cantarella et al., 2008; Sanz-Cobena et al., 2008). These changes in the amount of N available in the system and in the ratio of nitrate (N-NO_3^-) to ammonium (N-NH_4^+) in the soil solution affects the recovery of applied nitrogen (RAN), nitrogen use efficiency (NUE), dry matter yield (DMY), and the chemical composition of pastures (Peyraud and Astigarraga, 1998; Primavesi et al., 2005; Santos et al., 2013; Silveira et al., 2013). Thus, some studies have been and are being conducted to evaluate the effect of N fertilizers on the above-mentioned parameters (Gilbert et al., 1958; Kresge and Younts, 1962; Martha Junior et al., 2004, 2009; Primavesi et al., 2004, 2005; Costa et al., 2008, 2009, 2010; Silva et al., 2011, 2013; Silveira et al., 2007, 2013). Bernardi et al. (2014) evaluated the effect of different Nitrogen sources (urea, urea with inhibitor urease, urea with zeolite and ammonium nitrate) in the growth of Italian ryegrass (*Lolium multiflorum* Lam.), and reported that RAN increased under nitrate ammonium supply and NUE was higher with urea with the application of urease inhibitor (NBPT). Costa et al. (2010) studied sources (ammonium sulfate and urea) and rates (0, 200, 400 and 600 kg ha^{-1}) of N for xaraes grass (*U. brizantha* Jacq. cv. Xaraés), and reported that the source ammonium sulfate was more effecient to increase the DMY of xaraes grass in the seasons evaluated.

Regarding the chemical composition (forage quality), the concentrations of fibrous components and protein are among the most important factors affecting forage intake by ruminants (Moore et al., 1999; Huhtanen et al., 2002; Detmann et al., 2003). Hence, it is important to determine the inter-relationship between the concentrations of fibrous components and protein (N compounds), especially in countries, where animal production is carried out on pastures, since N fertilization affects the growth of forage plants, changing the composition of the cell wall and the biosynthesis of N compounds (Sniffen et al., 1992; Van Soest, 1994; Barcellos et al., 2008). Kawakami et al. (2013) found that the N uptake and the protein content in cotton plants (*Gossypium hirsutum* L.) was not affected by sources of N [urea, urea with urease inhibitor (NBPT), and urea with nitrification inhibitors (dicyandiamide-DCD)]. However, Costa et al. (2013) reported that the protein content in xaraes grass supplied with ammonium sulfate in the autumn was higher compared to the grass supplied with urea, but the cellulose + hemicellulose + lignin content of xaraes grass supplied with urea was higher. The results cited show that the evaluation of new N sources is extremely important to increase the efficiency use of this nutrient and improve forage quality, especially in the Brazilian Cerrado, which is the second largest biome in South America, where most of the Brazilian pastures concentrated (Barcellos et al., 2008; MMA, 2016). In addition, there are few studies demonstrating the effect of new N sources (*eg*, urea with urease inhibitor and polymer-coated urea) in DMY and chemical composition of the forage grasses in Brazilian Cerrado. Therefore, this study aimed to evaluate the effect of N fertilizers on DMY, RAN, NUE, and chemical composition (composition of the cell wall and CP fractionation) of *U. brizantha* cv. Marandu (palisade grass) established in Brazilian Cerrado.

Results

Effect of N sources in dry matter yield, recovery and use efficiency of N by palisade grass

Application of ammonium sulfate resulted in the highest DMY of palisade grass in the first period of growth, 50.67% higher than that of control treatment. Nitrogen sources did not affect the DMY of palisade grass in the second, third (except control treatment), and seventh periods. The dry matter yield of palisade grass fertilized with urea with urease inhibitor in the fourth period of growth was 156% higher than that obtained in the control treatment. The highest DMY of palisade grass were obtained with the application of ammonium nitrate (+139%), ammonium sulfate (+121%), ammonium sulfate-nitrate (+117%), and urea with urease inhibitor (+103%) in the fifth period and ammonium sulfate-nitrate (+118%) and polymer-coated urea (+116%) in the sixth period of growth as compared with control (Table 1).

Although there was a statistically significant difference ($P \leq 0.05$) in the DMY of palisade grass in the first, third, fourth, fifth, and sixth periods of growth as a function of N sources (Table 1), there was no significant difference in total DMY (except control) (Table 2). Regarding the RAN, the best results were obtained with ammonium sulfate in the first period; ammonium nitrate, ammonium sulfate-nitrate, urea and polymer-coated urea in the third period; and ammonium sulfate-nitrate in the fifth period of growth of palisade grass. There was no significant difference ($P > 0.05$) in RAN as a function of N sources in the second, fourth, sixth, and seventh periods of growth (Table 1). Considering the average RAN at the end of the study, ammonium sulfate-nitrate application resulted in the highest value, 34.8% higher than that obtained with the application of urea, which provided the lowest RAN (Table 2). The nitrogen use efficiency by palisade grass in the first, second, third, and fourth periods of growth was higher ($P \leq 0.05$) in the control treatment than in the treatments with N application. In the fourth period of growth, the NUE of the grass cultivated with polymer-coated urea was higher in relation to the other sources of N. In general, the NUE of palisade grass in the fifth, sixth, and seventh periods of growth were higher when there was no N application (control) (Table 1). Analyzing the average NUE of the grass, the highest value was found in control treatment, followed by the application of conventional and protected urea (NBPT and polymers), ammonium sulfate, ammonium nitrate, and ammonium sulfate-nitrate, respectively (Table 2).

Effect of N sources in cell wall composition and fractionation of protein in palisade grass

Nitrogen sources did not significantly change ($P > 0.05$) the NDF concentration in the palisade grass on the first, third, and seventh periods of growth. However, in the second, fifth, and sixth periods of growth, the application of urea decreased the NDF concentration by 6.1, 10.0, and 6.7%, compared with the control treatment, respectively. The use of ammonium sulfate decreased the NDF content in the fourth (-8.3%) and fifth (-9.4%) forage growth periods, compared to control treatment. However, N sources did not significantly change ($P > 0.05$) the ADF concentration of palisade grass in any growth period (Table 3). The effect of N sources on the concentration of fibrous components is determined with greater accuracy when the NDF and ADF fractions are separated. The cellulose concentration of the grass was not significantly affected ($P > 0.05$) by the N sources during the growth periods. However, hemicellulose concentration decreased with applications of ammonium sulfate (-7.3%), ammonium sulfate-nitrate (-5.0%), and polymer-coated urea (-5.0%) in the fourth period; ammonium sulfate (-12.9%) in the fifth period; and urea (-11.2%) in the sixth growth period, as compared with control treatment. Lignin concentration in the palisade grass was significantly changed only in the third period of growth, whereas the application of urea increased the lignin concentration by 27%, compared with control (Table 4). Nitrogen sources increased CP concentration in palisade grass in relation to control treatment, in the second and third periods of growth. Ammonium nitrate increased the CP concentration of the grass in the fourth growth period (+65%), and so ammonium sulfate (+49%) and urea (+42%) in the fifth period, compared to control treatment. Application of ammonium nitrate (+45%), ammonium sulfate (+38%), and urea with urease inhibitor (+33%) also increased

Table 1. Dry matter yield, recovery of applied nitrogen, and nitrogen use efficiency of palisade grass fertilized with N sources in the Cerrado.

N source	1st period	2nd period	3rd period	4th period	5th period	6th period	7th period
	Dry matter yield (t ha^{-1})						
Control treatment	2.21 d	2.48	0.81 b	1.25 d	0.79 b	0.98 b	1.02
Ammonium nitrate	2.53 bcd	2.81	2.13 a	2.29 c	1.89 a	1.93 ab	1.13
Ammonium sulfate	3.33 a	2.55	1.90 a	2.26 c	1.75 a	1.62 ab	1.54
Ammonium sulfate-nitrate	2.72 bc	2.41	2.22 a	2.89 ab	1.72 a	2.14 a	1.62
Urea	2.70 bc	2.54	2.23 a	2.50 bc	1.35 ab	1.65 ab	1.37
Urea treated with urease inhibitor	2.89 b	2.68	1.65 a	3.20 a	1.61 a	1.86 ab	1.63
Polymer-coated urea	2.46 cd	3.01	1.99 a	2.85 ab	1.40 ab	2.12 a	1.71
$LSD_{0.05}$	0.39	0.69	0.77	0.49	0.63	1.08	0.78
P-value	<0.0001	0.12	<0.0001	0.005	<0.0001	0.009	0.38
	Recovery of applied nitrogen (%)						
Control treatment	-	-	-	-	-	-	-
Ammonium nitrate	28.63 ab	25.70	36.10 a	4.54 a	26.22 ab	37.53	14.04
Ammonium sulfate	41.80 a	17.46	30.82 ab	7.70 a	27.87 ab	18.73	25.76
Ammonium sulfate-nitrate	32.31 ab	16.45	37.76 a	17.81 a	32.24 a	28.10	27.45
Urea	26.19 b	15.34	35.86 a	7.45 a	20.78 bc	17.68	19.14
Urea treated with urease inhibitor	25.62 b	20.70	22.34 b	22.04 a	30.15 ab	22.38	23.08
Polymer-coated urea	24.38 b	27.55	36.46 a	0.77 a	12.92 c	33.67	22.09
$LSD_{0.05}$	14.67	21.29	11.49	23.14	9.81	27.67	20.49
P-value	<0.0012	0.12	<0.0001	0.005	<0.0001	0.81	0.38
	Nitrogen use efficiency (kg kg^{-1})						
Control treatment	56.50 a	51.94 a	50.40 a	59.26 a	45.61 a	74.62 a	53.59 a
Ammonium nitrate	37.39 b	38.20 b	37.29 b	39.79 cd	40.93 ab	46.50 b	36.73 b
Ammonium sulfate	41.30 b	38.83 b	39.68 b	37.19 cd	35.03 c	50.06 b	37.23 b
Ammonium sulfate-nitrate	38.58 b	37.44 b	39.03 b	35.58 d	34.50 c	45.91 b	37.40 b
Urea	41.56 b	40.07 b	40.31 b	41.29 c	34.76 c	59.79 ab	38.46 b
Urea treated with urease inhibitor	44.83 b	39.99 b	39.10 b	42.64 c	33.85 c	52.31 b	41.72 ab
Polymer-coated urea	39.00 b	40.25 b	38.90 b	53.30 b	38.69 bc	50.35 b	40.93 ab
$LSD_{0.05}$	7.79	6.91	7.26	5.44	5.69	17.12	13.30
P-value	<0.0001	0.002	<0.0001	0.009	<0.0001	0.009	0.005

[a-d]Means followed by different letters in the column differ at $P \leq 0.05$ by Tukey's test.

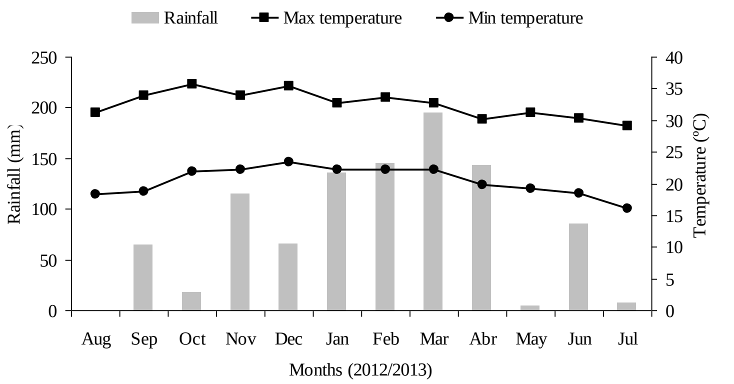

Fig 1. Monthly accumulated rainfall and mean maximum and minimum temperatures during the experimental period (August 2012 to July 2013) in Ilha Solteira - SP, Brazil.

the concentration of CP in the grass compared with control in the sixth period of growth. In the seventh period, the highest CP concentration in relation to control treatment were observed with ammonium nitrate (+71%), ammonium sulfate (+69%), and ammonium sulfate-nitrate (+80%) (Table 3).

Regarding the fractionation of CP in the palisade grass, there was no significant difference among the N sources ($P > 0.05$) in the first, second, third, fourth, sixth, and seventh periods of growth. However, CP fractions A and B differed significantly ($P \leq 0.05$) as a function of treatments in the fifth period of growth. The application of urea increased the concentration of CP fraction A by 48% as compared with the application of ammonium sulfate-nitrate, while the concentration of fraction B in palisade grass increased by 15% with the application of ammonium sulfate-nitrate compared to the application of urea (Table 5).

Discussion

Nitrogen fertilization is crucial to increase the DMY of forage plants (Table 2), especially in longer periods, but there was no difference in DMY among the N sources, as reported by Silveira et al. (2013). However, factors intrinsic to the fertilizer and the environment can alter the response of plants

Table 2. Total dry matter yield (TDMY), average of recovery of applied nitrogen (RAN), and average nitrogen use efficiency (NUE) by palisade grass fertilized with N sources in the Cerrado.

N source	TDMY (t ha^{-1})	RAN (%)	NUE (kg kg^{-1})
Control treatment	13.57 b	-	57.60 a
Ammonium nitrate	16.32 a	24.68 ab	38.89 bc
Ammonium sulfate	16.22 a	24.31 ab	39.97 bc
Ammonium sulfate-nitrate	16.38 a	27.44 a	38.26 c
Urea	16.14 a	20.35 b	42.67 b
Urea treated with urease inhibitor	16.96 a	23.76 ab	42.30 b
Polymer-coated urea	17.05 a	22.52 ab	43.30 b
$LSD_{0.05}$	1.71	6.33	3.50
P-value	<0.0001	0.002	<0.0001

[a-c]Means followed by different letters in the column differ at $P \leq 0.05$ by Tukey's test.

Table 3. Neutral detergent fiber, acid detergent fiber, and crude protein contents of palisade grass fertilized with N sources in the Cerrado.

N sources	1st period	2nd period	3rd period	4th period	5th period	6th period	7th period
	Neutral detergent fiber (g kg^{-1} of DM)						
Control treatment	702.1	709.3 a	687.6	725.3 a	698.5 a	656.5 a	683.9
Ammonium nitrate	696.6	671.8 ab	694.4	677.3 ab	689.9 ab	622.6 ab	657.3
Ammonium sulfate	679.4	678.5 ab	701.1	664.6 b	632.8 c	637.7 ab	662.8
Ammonium sulfate-nitrate	690.0	668.8 ab	686.7	685.1 ab	684.9 ab	623.3 ab	651.1
Urea	687.8	665.8 b	685.0	717.4 a	628.9 c	612.2 b	656.0
Urea treated with urease inhibitor	673.9	678.7 ab	681.1	700.6 ab	658.2 bc	628.4 ab	662.9
Polymer-coated urea	676.0	672.4 ab	685.2	706.1 ab	670.0 ab	622.2 ab	651.4
$LSD_{0.05}$	44.77	42.17	48.30	52.42	32.18	7.54	51.36
P-value	0.44	0.05	0.85	0.02	0.0001	0.02	0.43
	Acid detergent fiber (g kg^{-1} of DM)						
Control treatment	343.3	361.5	348.7	377.9	382.0	329.4	335.1
Ammonium nitrate	339.5	331.7	356.0	335.4	346.7	315.1	338.3
Ammonium sulfate	339.3	340.0	360.4	342.7	364.5	324.6	330.5
Ammonium sulfate-nitrate	345.0	329.6	349.4	355.3	376.1	322.7	338.4
Urea	333.3	329.7	348.3	369.8	357.8	321.7	335.6
Urea treated with urease inhibitor	331.9	342.1	341.3	362.0	329.5	326.4	340.5
Polymer coated urea	300.5	329.0	348.4	373.8	368.5	311.3	333.9
$LSD_{0.05}$	61.95	34.14	37.39	56.47	63.90	29.37	34.99
P-value	0.30	0.06	0.74	0.07	0.09	0.44	0.97
	Crude protein (g kg^{-1} of DM)						
Control treatment	134.6	92.0 b	92.6 b	94.6 b	112.2 b	94.0 b	115.7 c
Ammonium nitrate	157.4	143.2 a	134.2 a	156.1 a	138.3 ab	136.4 a	197.9 a
Ammonium sulfate	143.4	140.3 a	132.8 a	155.4 ab	168.1 a	129.4 a	196.0 a
Ammonium sulfate-nitrate	143.0	131.4 a	136.8 a	142.3 ab	136.4 ab	121.7 ab	208.7 a
Urea	145.2	125.7 a	124.4 a	139.9 ab	159.2 a	113.9 ab	168.7 b
Urea treated with urease inhibitor	138.6	120.1 a	127.8 a	119.9 ab	148.9 ab	125.4 a	166.8 b
Polymer-coated urea	137.0	125.7 a	124.5 a	118.1 ab	143.5 ab	117.1 ab	162.1 b
$LSD_{0.05}$	43.74	27.97	24.96	60.98	36.92	30.39	22.22
P-value	0.69	0.0003	0.0003	0.02	0.04	0.007	<0.0001

[a-c]Means followed by different letters in the column differ at $P \leq 0.05$ by Tukey's test.

to N fertilization in shorter periods (Sun et al., 2008). The low availability of water associated with temperatures below 30 °C (Fig 1) limits the DMY of grasses fertilized with N (Table 1, second and seventh periods of growth), mainly in tropical areas (Sun et al., 2008; Alencar et al., 2009). Nutritional factors also limit the response of forage plants to N supply (Sun et al., 2008). Plants supplied with N require more sulfur for growth (De Bona and Miller, 2010a, b; De Bona et al, 2013; Arthur and Miller, 2014), as seen in the first and sixth periods of forage growth, where higher DMY occurred when ammonium sulfate and ammonium sulfate-nitrate were applied, respectively (Table 1). The fertilizer ammonium sulfate-nitrate has a nitrification inhibitor that reduces N losses by volatilization when more water is available and increases DMY when compared with ammonium sulfate (Table 1, sixth growing period) (Arrobas et al., 2009; Teixeira Filho et al., 2009). The loss of N by leaching and volatilization in tropical regions may be higher due to high temperatures and rainfall, limiting the DMY of forage plants (Primavesi et al., 2005, 2006; Silveira et al., 2007, 2013). This fosters the use of N sources that minimizes these losses (Arrobas et al., 2009). Application of urea with urease inhibitor and polymer-coated urea increased the DMY of palisade grass by 156 and 116% compared with control in the fourth and sixth periods of growth, respectively (Table 1). This result indicates that the use of protected N fertilizers in tropical regions is promising (Bennett et al., 2008; Arrobas et al., 2009; Silveira et al., 2013). The highest RAN by palisade grass was obtained with the use of ammonium sulfate in the first forage growth period and with ammonium sulfate-nitrate in the fifth period (Table 1) and by the end of the experiment (Table 2). Overall, the fertilizer containing N-NH_4^+ showed higher RAN than the urea-based fertilizers [$CO(NH_2)_2$], with the exception of the third forage growth period (Table 1), because the N loss by volatilization of ammonium salts is less than urea (Martha Junior et al., 2004; Primavesi et al., 2006; Silva et al., 2011). The urease enzyme present in the soil transforms urea into ammonium carbonate, which can be converted to N-NH_3, increasing N losses by volatilization, particularly when the fertilizer is not incorporated into the soil in environments with high precipitation, soil temperatures exceeding 5 °C, and elevated soil pH values

Table 4. Cellulose, hemicellulose, and lignin contents of palisade grass fertilized with N sources in the Cerrado.

N sources	1st period	2nd period	3rd period	4th period	5th period	6th period	7th period
	Cellulose (g kg^{-1} of DM)						
Control treatment	296.8	312.2	304.2	241.9	312.9	282.6	293.0
Ammonium nitrate	290.4	284.7	313.3	296.7	313.7	274.5	295.2
Ammonium sulfate	288.8	290.8	321.5	303.0	276.6	277.8	289.1
Ammonium sulfate-nitrate	295.1	286.2	308.0	311.8	318.1	271.5	295.9
Urea	287.0	286.0	303.4	329.2	277.0	278.5	294.6
Urea treated with urease inhibitor	284.1	293.5	299.6	317.7	295.5	281.9	299.6
Polymer-coated urea	259.6	285.6	303.4	328.4	302.6	267.7	290.7
$LSD_{0.05}$	54.24	28.68	29.74	117.11	47.19	29.16	28.84
P-value	0.34	0.06	0.27	0.65	0.06	0.61	0.92
	Hemicellulose (g kg^{-1} of DM)						
Control treatment	358.8	347.8	338.9	347.4 a	340.8 a	327.1 a	348.8 a
Ammonium nitrate	347.1	340.1	338.4	341.9 a	334.8 a	307.5 ab	319.0 b
Ammonium sulfate	340.0	338.5	340.7	321.9 c	296.9 b	313.1 ab	332.2 ab
Ammonium sulfate-nitrate	345.0	339.2	337.2	329.8 bc	312.9 ab	300.6 ab	312.7 b
Urea	354.4	336.1	336.7	347.6 a	316.8 ab	290.5 b	320.4 b
Urea treated with urease inhibitor	342.0	336.7	339.8	338.7 ab	318.8 ab	302.0 ab	322.3 b
Polymer-coated urea	323.0	343.4	336.8	329.8 bc	321.7 ab	310.9 ab	317.5 b
$LSD_{0.05}$	52.16	18.87	17.92	9.96	33.88	31.30	23.83
P-value	0.41	0.44	0.98	<0.0001	0.01	0.04	0.002
	Lignin (g kg^{-1} of DM)						
Control treatment	30.2	29.1	22.0 b	35.5	27.3	33.5	28.0
Ammonium nitrate	34.6	32.0	28.4 ab	27.0	29.2	28.5	32.9
Ammonium sulfate	33.7	33.1	27.8 ab	33.8	27.4	36.3	31.6
Ammonium sulfate-nitrate	32.7	35.9	28.0 ab	33.3	39.6	41.1	33.9
Urea	37.0	30.4	28.9 a	31.1	22.8	31.7	29.5
Urea treated with urease inhibitor	32.9	32.6	26.0 ab	33.5	29.6	33.0	30.3
Polymer-coated urea	30.7	30.4	27.5 ab	36.2	32.0	31.3	34.0
$LSD_{0.05}$	11.10	11.92	6.55	20.03	19.92	18.37	7.00
P-value	0.49	0.61	0.04	0.64	0.22	0.41	0.07

[a-c]Means followed by different letters in the column differ at $P \leq 0.05$ by Tukey's test.

Table 5. Fractions A (non-protein nitrogen), B (variable degradation), and C (indigestible) of palisade grass fertilized with N sources in the Cerrado.

N source	1st period	2nd period	3rd period	4th period	5th period	6th period	7th period
	Fraction A (g kg^{-1} of CP)						
Control treatment	374.6	354.0	362.7	262.6	244.2 bc	417.4	354.9
Ammonium nitrate	352.6	375.1	372.5	202.4	265.6 abc	391.7	413.2
Ammonium sulfate	309.0	386.3	315.2	272.2	309.8 ab	326.1	357.6
Ammonium sulfate-nitrate	332.6	367.6	333.3	266.8	222.1 c	378.2	389.4
Urea	255.1	391.2	365.7	345.1	329.0 a	418.6	370.4
Urea treated with urease inhibitor	249.8	366.3	350.7	260.4	278.9 abc	398.0	356.8
Polymer-coated urea	318.7	407.4	342.1	232.1	291.9 abc	393.6	428.4
$LSD_{0.05}$	192.05	108.92	95.59	160.49	82.19	109.40	119.14
P-value	0.31	0.73	0.47	0.55	0.02	0.62	0.44
	Fraction B (g kg^{-1} of CP)						
Control treatment	538.6	533.2	523.2	648.1	690.2 ab	515.2	584.1
Ammonium nitrate	549.4	525.7	554.8	723.9	673.6 ab	548.7	540.7
Ammonium sulfate	555.4	505.1	548.7	654.5	635.8 ab	613.0	582.3
Ammonium sulfate-nitrate	626.1	531.7	547.8	660.0	712.3 a	556.5	553.5
Urea	645.1	531.5	570.5	592.8	615.8 b	519.6	570.5
Urea treated with urease inhibitor	653.1	522.1	508.3	659.9	654.4 ab	547.6	590.3
Polymer-coated urea	601.5	506.3	530.5	685.7	650.2 ab	551.1	517.8
$LSD_{0.05}$	174.67	113.85	121.03	131.42	79.39	105.11	103.57
P-value	0.45	0.68	0.13	0.21	0.02	0.45	0.97
	Fraction C (g kg^{-1} of CP)						
Control treatment	86.8 b	141.1	114.2	89.3	65.6	67.4	61.0
Ammonium nitrate	98.0 b	99.2	126.7	73.7	60.9	59.6	46.0
Ammonium sulfate	93.6 b	103.8	136.1	73.3	54.4	60.9	60.1
Ammonium sulfate-nitrate	90.5 b	101.2	118.9	73.3	65.6	65.3	57.0
Urea	144.5 a	103.1	104.7	62.1	55.2	61.8	59.2
Urea treated with urease inhibitor	97.1 b	111.6	141.0	79.8	66.6	54.4	52.9
Polymer-coated urea	91.2 b	86.3	127.4	82.2	57.9	55.3	53.8
$LSD_{0.05}$	46.42	68.09	55.56	62.10	43.16	24.64	24.39
P-value	0.01	0.28	0.39	0.69	0.84	0.57	0.65

[a-c]Means followed by different letters in the column differ at $P \leq 0.05$ by Tukey's test.

(which was the case of this study) (O'Connor and Hendrickson, 1987; Bremner, 1995). The coating of urea with urease inhibitors or polymers reduces the losses of N through volatilization and increases RAN, compared with the conventional urea (Table 2) (Gans et al., 2006; Cantarella et al., 2008; Martha Junior et al., 2009; Tasca et al., 2011). Ammonium sulfate-nitrate provided the highest RAN during the study, but the NUE of palisade grass with this fertilizer was the lowest among the treatments (Table 2). Palisade grass supplied with urea, urea with urease inhibitor, and polymer-coated urea showed better NUE than ammonium sulfate-nitrate in the fourth growth period (Table 1) and by the end of the study (Table 2). This result is possibly associated with the increase of $N\text{-}NH_4^+$ uptake rather than $N\text{-}NO_3^-$ by plants fertilized with urea, urea and urease inhibitor, and polymer-coated urea, since the relationship between these ions changes the DMY (Masclaux-Daubresse et al., 2010; Santos et al., 2013). In the soil, urea is hydrolyzed by the urease enzyme, resulting in the formation of ammonium carbonate, which rapidly decomposes yielding $N\text{-}NH_4^+$, which is taken up and assimilated without energy expenditure (Rochette et al., 2009; Taiz and Zeiger, 2009). The coating of urea with NBPT or polymers decreases the hydrolysis rate, which allows the $N\text{-}NH_4^+$ to be taken up for a longer time (Clay et al., 1990; Sanz-Cobena et al., 2008). This result allows the plant to save energy in the reduction of $N\text{-}NO_3^-$ improving the NUE (Masclaux-Daubresse et al., 2010).

Besides increasing the DMY, N supply decreases NDF concentration (Bennett et al., 2008; Dupas et al., 2010; Oliveira et al., 2010; Silveira et al., 2013), as occurred with urea supply in the second (-6.1%), fifth (-10.0%), and sixth (-6.7%) growth periods, while ammonium sulfate supply decreased NDF in the fourth (-8.3%) and fifth (-9.4%) growth periods, compared with control (Table 3). This fact is a result of the increased CP content (Table 3) that accumulates in the cell and causes the dilution of the cell wall (Van Soest, 1994; and Peyraud and Astigarraga, 1998). Although urea and ammonium sulfate supply decreased the NDF concentration of palisade grass (compared to control treatment), values above 60% are correlated negatively with forage intake (Van Soest, 1965; Detmann et al., 2003). In other studies, no changes in NDF concentration due to N sources were reported (Bennett et al., 2008; Costa et al., 2010, 2013).

The ADF concentration of palisade grass was not reduced with the use of N and or affected by N sources (Table 3), because there were no differences in cellulose and lignin concentrations (except for the third growth period) of the grass (Table 4), which are known to form the fraction named ADF (Van Soest, 1994; Casali et al., 2009). Similar results were reported by Viana et al. (2011). However, the hemicellulose concentration of palisade grass decreased with the use of N. The application of ammonium sulfate (-7.3%), polymer-coated urea (-5.0%), and ammonium sulfate-nitrate (-5.0%) in the fourth period; ammonium sulfate (-12.9%) in the fifth period; and urea (-11.2%) in the sixth period of growth decreased the hemicellulose concentration of the grass, compared to control (Table 4). This has been shown in other studies (Campos et al., 2013; Ames et al., 2014). This result is due to the increase in CP concentration (Table 3), which caused the plant to utilize the carbon chains for protein synthesis and production of energy required for the reduction $N\text{-}NO_3^-$ to $N\text{-}NH_4^+$ for protein synthesis rather than the use of the carbon chains for synthesis of structural carbohydrates such as hemicellulose (Peyraud and Astigarraga, 1998; Taiz and Zeiger, 2009). It should be noted that much of the N from the fertilizer is in $N\text{-}NO_3^-$ form in the soil solution due to the marked nitrification in tropical regions, even with the use of technologies to minimize or delay the nitrification process (Primavesi et al., 2004; Cantarella et al., 2008).

In general, the N sources increased the CP concentration in palisade grass compared with treatment control, except for the first period of growth (Table 3). The greatest increase was observed with the addition of ammonium nitrate in the fourth period (+65%); ammonium sulfate (+49%) and urea (+42%) in the fifth period; ammonium nitrate (+45%), ammonium sulfate (+38%), and urea with urease inhibitor (+33%) in the sixth period; and ammonium nitrate (+71%), ammonium sulfate (+69%) and ammonium sulfate-nitrate (+80%) in the seventh period of growth of palisade grass. The increase in the concentration of CP in the grass fertilized with N occurs due to increase in the synthesis of amino acids and proteins (Bennett et al., 2008; Oliveira et al., 2010; Silveira et al., 2013). Crude protein concentrations during all the periods of growth of palisade grass (including control treatment) were above the 7% recommended for ruminant animals for an effective rumen microbial fermentation (Van Soest, 1994). The concentrations of fibrous components and CP are among the most important factors affecting forage intake by ruminant animals, and there is a close relationship among these factors (Sniffen et al., 1992; Moore et al., 1999; Huhtanen et al., 2002; Detmann et al., 2003). Therefore, it is important to fractionate the forage protein by the Cornell Net Carbohydrate and Protein System (CNCPS) to more precisely ascertain the relationship between carbohydrate and N compounds in N-fertilized pastures (Sniffen et al., 1992).

In the fifth period of growth of palisade grass, the application of urea increased the concentration of fraction A by 48% in relation to the application of ammonium sulfate-nitrate, whereas the concentration of fraction B increased by 15% with the application of ammonium sulfate-nitrate, compared to application of urea (Table 5). This fact can be attributed to the greater accumulation of N (data not shown) by palisade grass fertilized with ammonium sulfate-nitrate, allowing a higher protein N synthesis (fraction B of CP) compared with non-protein N (fraction A of CP) (Cazzato et al., 2011). When there is less N uptake, plants can prioritize the allocation of this nutrient in non-protein compounds such as allantoin and allantoic acid, working as N reserve (Marschner, 1995; Masclaux-Daubresse et al., 2010). In addition, in the fifth period it was the greatest variation in leaf/stem ratio (data not shown), which alters the protein fractionation of the plant (Silva et al., 2009). In other periods of growth, the NDF (Table 3) and leaf/stem ratio (data not shown) variation was lesser, which reduces the changes of the protein fractions (except the fraction C in the first period of growth) (Silva et al., 2009; Velásquez et al, 2010). In the first period of growth of palisade grass the urea supply increased the fraction C in relation to other treatments, but it should be noted that the concentration of fraction C was lower than the concentrations of fractions A and B (Table 5). This result is highly desirable, since fraction C corresponds to the unavailable N, where proteins and N compounds are associated with lignin, tannin-protein complexes and Maillard products, which are highly resistant to attack by enzymes of microbial origin (Sniffen et al., 1992; Tylutki et al., 2008).

Materials and Methods

Location, climatic conditions and characteristics of soil

The experiment was implemented in the Experimental Station of Universidade Estadual Paulista "Julio de Mesquita Filho", located in Ilha Solteira - SP, Brazil, at an altitude of 226 m (20°21'S and 51°22'W). The climate is characterized as humid subtropical type (rainy in the summers and dry in the winters), according to the Köppen classification (Köppen and Geiger, 1928). Climatic conditions recorded during the study period (August 2012 to July 2013) are shown in Fig 1. The soil of the area was classified as Dark Red Alfisol (EMBRAPA, 2013) of sandy texture, with the following chemical characterization in the 0-20 cm layer (Raij et al., 2001): pH ($CaCl_2$) = 5.5; resin P = 21 mg dm^{-3}; K^+ = 3.7

$mmol_c\ dm^{-3}$; Ca^{2+} = 11 $mmol_c\ dm^{-3}$; Mg^{2+} = 7 $mmol_c\ dm^{-3}$; Al^{3+} = 0 $mmol_c\ dm^{-3}$; H + Al = 18 $mmol_c\ dm^{-3}$; base saturation = 55; and organic matter = 20 g dm^{-3}.

Plant material and experimental conditions

Treatments were represented by the cultivation of *Urochloa brizantha* cv. Marandu (Syn. *Brachiaria brizantha*) fertilized with the following nitrogen sources: ammonium nitrate (32% N), ammonium sulfate (20% N and 23% S), ammonium sulfate-nitrate (26% N and 12% S, Entec®), urea (45% N), urea with urease inhibitor (45% N, NBPT, Super N®), mineral additive- and polymer-coated urea (41% N, Kimcoat N®), and a control treatment (without application of nitrogen), in measures repeated over time (seven periods of growth of palisade grass shoots). A levelling cut was made after the initial growth period (without application of nitrogen), and then 100 kg ha^{-1} N were applied in each one of the periods of growth of palisade grass shoots (28, 34, 29, 43, 29, 49, and 42 days of growth in the first, second, third, fourth, fifth, sixth, and seventh periods, respectively). The experimental design was in randomized blocks, with four replicates and three plots with an area of 9 m^2 (3 × 3 m) each, spaced 2 m apart. The palisade grass was sown by a mechanized operation with a seeder/fertilizer into the rows on 08/30/2012 using 12 kg ha^{-1} of pure, viable seeds, followed by fertilization with 100 kg ha^{-1} P_2O_5 (superphosphate: 18% P_2O_5, 16% Ca, and 8% S), also applied by same mechanical seeding operation. After the forage plant was sown, the area was irrigated until 10/29/2012 (plot-leveling cut) by a fixed sprinkler system with nozzles spaced 12 × 12 m apart, average precipitation of 3.6 mm h^{-1}, and average Christiansen's uniformity coefficient of 85%. The irrigation schedule adopted was every three days, with reference evapotranspiration (ET0) replacement estimated by Penman-Monteith (Allen et al., 1998) and crop coefficient (Kc) of 1.0. Nitrogen applications began immediately after the plot-leveling cut (10 to 15 cm above the soil surface), according to the treatments. Nitrogen was applied as topdressing the day after each cut of the forage shoots. In addition to N fertilization, 20 kg ha^{-1} P_2O_5 (superphosphate) and 30 kg ha^{-1} K_2O (potassium chloride: 60% K_2O) were applied in the fourth and seventh periods of growth, based on the soil analyses carried out in the third and sixth periods of forage growth (data not shown). This fertilization was performed aiming to increase the soil phosphorus and potassium so as not to limit the response of the palisade grass to N application (Werner et al., 1997). At the end of each growth period, shoot samples were collected for analyses using a 2 m^2 metal frame thrown randomly, and a reel mower. After completion of harvests and sample collection, the border was cut off and the plant material from the area was removed.

Analyses of DMY, RAN, NUE, and chemical composition

Plant shoots collected in each growth period were weighed on an analytical scale to determine fresh matter yield and subsequently packed in paper bags for drying in a forced-air oven at 60 °C for 72 h to obtain the dry matter yield. Dry matter yield results were extrapolated to one hectare, considering the area of the metal frame used in the collection of the shoots material. Next, the NUE of the palisade grass was calculated according to the equation described by Delogu et al. (1998). After drying, samples were ground through a Wiley mill with a 1-mm mesh sieve and analyzed for the cell wall composition and protein fractions.

Neutral detergent fiber and ADF concentrations were determined using 5 × 10 cm non-woven fabric bags (grammage 80) (Casali et al., 2009), after being immersed in a jar containing 50 mL of a neutral detergent solution for determination of NDF, and 50 mL of acid detergent solution for determining the ADF, with autoclave digestion at 105 °C for 60 min (Pell and Schofield, 1993). Lignin concentration was determined by adding 720 g L^{-1} sulfuric acid to the insoluble residue from ADF determination (Van Soest and Wine, 1968). Cellulose and hemicellulose concentrations were estimated by difference (Casali et al., 2009). Total nitrogen (TN) concentration was determined following the method described by the Association of Official Analytical Chemists (AOAC, 1990), and the crude protein (CP) concentration was obtained as the TN content multiplied by the factor 6.25. With the DMY and TN concentration data, RAN was calculated as described by Fageria (1998). After the determination of the neutral (NDIN) and acid (ADIN) detergent insoluble N (Licitra et al., 1996; Krishnamoorthy et al., 1982), the CP of the samples was fractionated according to the method described by Licitra et al. (1996). Fraction A was determined after treating the sample with trichloroacetic acid (TCA) at 100 g L^{-1}. Fraction B3 was obtained as the difference between fraction A and NDIN + ADIN, while the true protein (fractions B1 + B2) was obtained as the difference between fraction A and NDIN. Fraction C was considered equal to ADIN. Subsequently, fractions B1 + B2 and B3 were added to obtain fraction B.

Statistical analysis

All results were subjected to analysis of variance (ANOVA) and, depending on the significance level of the F test. The mean values were compared by Tukey's test at 5% significance within each period of growth of the palisade grass, using the Statistical Analysis System (SAS, 2004).

Conclusion

The dry matter yield of palisade grass increased with N application, but there was no difference among N fertilizers in long term comparisons. On the other hand, the comparison made in each growth period demonstrates that factors intrinsic to the environment, the fertilizer, and the plant interfere with the response of the palisade grass to fertilizers in a unique manner. The results obtained for RAN and NUE show that the use of N fertilizers that minimize N loss, such as ammonium sulfate-nitrate, urea with urease inhibitor, and polymer-coated urea, is very promising, especially for minimizing the environmental impact of N fertilization. Overall, the chemical composition of the palisade grass shoots improved when N fertilization was applied, and urea and ammonium sulfate applications decreased NDF concentration. Nitrogen fertilization also decreased hemicellulose content. Although N fertilization decreased the concentration of fibrous components of the palisade grass, the observed NDF concentrations may be limiting to the intake of ruminants and seem to be more closely related to characteristics of the forage grass than to nutritional disorders. Nitrogen fertilization also increased the CP concentration of palisade grass, regardless of the fertilizer used. The obtained CP concentrations do not limit the feeding of ruminants.

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