

Single and split nitrogen application on the forage yield, morphogenesis, chemical composition, and tissue nitrogen fractions of *Cynodon nlemfuensis* under mob grazing

Aplicação de nitrogênio em dose única ou parcelada na produção de forragem, morfogênese, composição química e frações de nitrogênio tecidual de *Cynodon nlemfuensis* sob pastejo

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Highlights

Nitrogen fertilizer in single application increased forage yield production.

The leaf appearance rate and total green blade increased in single application.

Climatic conditions and vegetative phase alter forage morphology.

Split the nitrogen fertilizer does not change the chemical composition.

Abstract

The aim of this study was to evaluate the forage production, nutritional value (including protein fractions), and morphogenic characteristics of African star grass on nitrogen fertilizer in a single application and split applications of two and four, under mob grazing. The study was conducted on a 1.5 ha area with well-established African star grass (*Cynodon nlemfuensis*). The area was divided into 18 paddocks of 400 m². for three treatments and six replications that were assigned through a randomized block design. The treatments were: Nap1 =Single N application; Nap2= split into two N applications; and Nap4: split into four N applications. Fertilizer application was 200 kg ha⁻¹ of N in topdressing. Pasture was monitored weekly by sward height. Grazing began when the forage reached between 25 and 30 cm in height and ceased at 10 cm. In almost

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all months evaluated, Nap1 recorded the highest forage production, daily accumulation rate and available forage mass. Some morphogenic characteristics changed between the fertilization strategies, particularly in the months with greater water availability. The chemical composition did not fluctuate between treatments. African star grass can be fertilized with a single application of nitrogen at 200 kg ha⁻¹ to attain greater forage mass and proportion of leaves, without any changes to the chemical composition and nitrogen fractions. These components were modified by the vegetative phase of the forage.

Key words: African star grass. Tropical grasses. Topdressing fertilization. Forage. Protein fractions.

Resumo

O objetivo deste estudo foi avaliar a produção de forragem, o valor nutricional, frações protéicas e as características morfogênicas da grama estrela africana estabelecida (*Cynodon nlemfuensis*) sob adubação nitrogenada parcelada em duas, quatro ou em uma única aplicação. O estudo foi conduzido em uma área de 1,5 ha, que foi dividida em 18 piquetes de 400 m². O experimento foi delineado estatisticamente em blocos ao acaso, constando de três tratamentos e seis repetições. Os tratamentos corresponderam a aplicação de 200 kg ha⁻¹ de N em cobertura na forma de ureia: Nap1 = aplicação única; Nap2 = parcelado em duas aplicações; e Nap4: parcelado em quatro aplicações. A pastagem foi monitorada semanalmente a partir da medição da altura do pasto. O rebaixamento da pastagem foi realizado por novilhas leiteiras da raça holandesa com 12 meses de idade. Os animais entravam nas parcelas quando a pastagem apresentava altura de 25 a 30 cm e saíam com 10 cm. Em quase todos os meses avaliados, Nap1 apresentou a maior produção de forragem, taxa de acúmulo diário e massa de forragem disponível. Algumas características morfogênicas mudaram entre as estratégias de fertilização, principalmente nos meses de maior disponibilidade hídrica. A composição química não oscilou entre os tratamentos. O capim-estrela africano pode ser fertilizado com uma única aplicação de nitrogênio de 200 kg ha⁻¹ para obter maior quantidade de forragem e proporção de folhas, sem alteração da composição química e das frações do nitrogênio. Esses componentes foram modificados apenas pelo ciclo vegetativo da forragem foram modificados apenas pelo ciclo vegetativo da forragem.

Palavras-chave: Grama estrela africana. Gramíneas tropicais. Fertilização em cobertura. Forragem. Fracionamento de proteína.

Introduction

Different methods of N application have been developed to increase the productivity of pastures and in turn the recycling of nutrients. Developing strategies for the conscious use of available resources is therefore essential to increase the productivity of the pastoral system and to reduce the loss of nutrients through leaching. A common recommendation is to split the N application, which according to Assmann, Sonogo, Assmann, Adami and Cuzzi (2018), can increase the synchronization

between the rate of N supply and N uptake by plants, thereby reducing the potential loss of N. However, production costs can be minimized by using a single application of N, especially if doing so does not affect the crop yield.

The amount of N can affect the biomass yield, as well as the morphogenesis and chemical composition of grass (Delevatti et al., 2019; Paciullo, Gomide, Castro, Fernandes, & Morenz, 2017). The addition of N to the system also causes a change in the C:N ratio of the soil, which is usually high in grass residues

(Cardoso, Bento, Moreski, & Gasparotto, 2014). This change can increase nutrient cycling by increasing the degradation of organic matter by soil microorganisms and in turn the availability of soil nutrients (Sartor et al., 2011). The aim of this study was to evaluate, throughout the pasture production cycle and with mob grazing, the forage production, nutritional value (including protein fractions), and morphogenic characteristics of African star grass treated with a single N application and split N applications of two and four.

Material and Methods

Local and experimental design

This study was carried out in 25°44'S and 53°04'W, elev. 520 m a.s.l.. The soil of

the region is classified as dystroferric Red Latosol and has an argillaceous texture. Before starting the trail, soil samples were collected at a depth of 20 cm and the acidity correction was performed, together with base fertilization, as recommended by Comissão de Química e Fertilidade do Solo [CQFS], (2004), in the amount of 200 kg/ha of a fertilizer, formulated with 5:20:10 NPK. The soil analysis to average were: organic matter=42.55 g dm⁻³; P=5.25 mg dm⁻³; K=0.39 cmol dm⁻³; Ca=4.5 cmol dm⁻³; Mg=1.9 cmol/dm³; bases=7.12 cmol dm⁻³; Al³⁺=2.85%, and pH(CaCl)=4.8. The climate was classified as humid subtropical mesothermal (Cfa) according to the criteria of Köppen. The climatological data of the evaluation period were recorded and obtained through an automatic surface weather station, from UTFPR-DV, located about 100 meters from the experimental area (Figure 1).

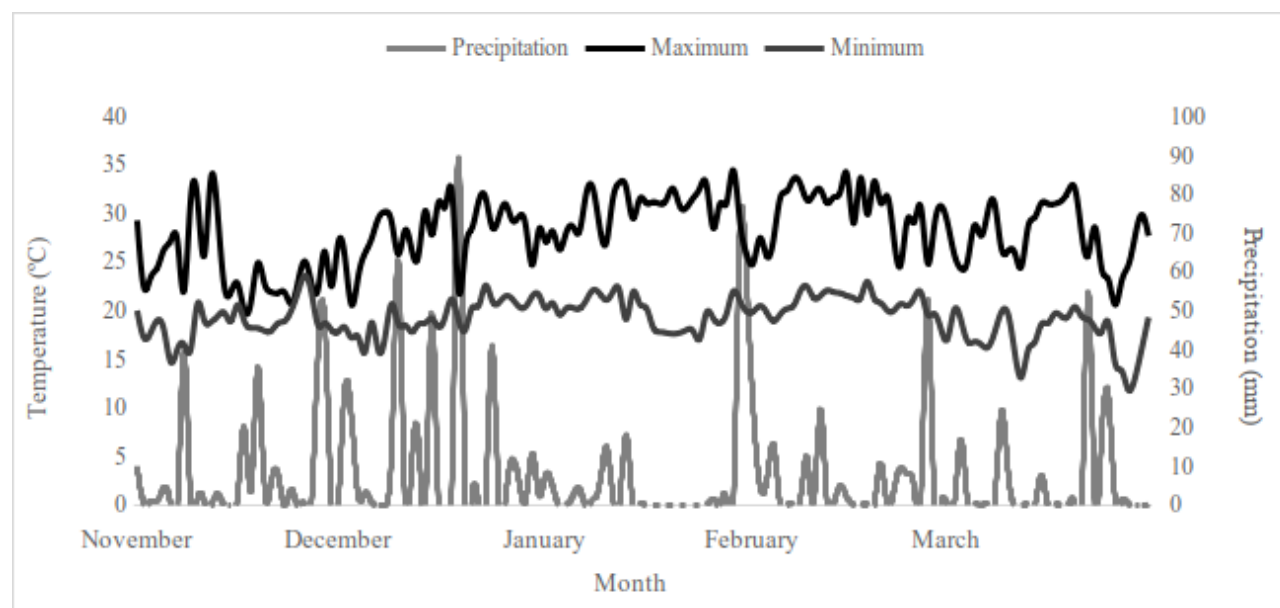


Figure 1. Precipitation, maximum and minimum temperature for the experimental months. Dois Vizinhos, PR, 2016.

The total area was 1.5 ha and was implanted with African star grass (*Cynodon nlemfuensis*) in 2014. The 0.72 ha was divided into 18 paddocks of 400 m², consisting of three treatments and six replications that were assigned using a randomized block design. The treatments were: Nap1 = Single N application; Nap2= split into two N applications; and Nap4: split into four N applications. The nitrogen fertilizer applied was 200 kg ha⁻¹ of urea (46% of N) in topdressing. The first application was in November, at the start of the experimental period, and the subsequent applications for Nap2 and Nap4 were each 30 days.

Pasture management

Pasture was monitored weekly by sward height, using a graduated ruler. Mob grazing began when the forage in the paddock reached a height between 25 and 30 cm and stopped at a height of 10 cm, after approximately 36 h (4 or 5 animals per paddock). Sixteen Holstein heifers with a live weight of 200 kg were used. At the end of grazing, the animals were sent to an area with similar pasture, then reintroduced when foraged returned to between 25-30 cm. Thirty height measurements were made and the cut for sample was obtained in a representative area of each paddock (in terms of average forage height), a 0.25 m²

Three cuts were made at ground level for each paddock (pre and post-grazing). The samples were separated into leaf blade, stems + sheath, and dead material. For each grazing cycle, the daily rate of forage accumulation was calculated as the difference between the forage mass after the preceding grazing and the forage mass in the current pre-grazing, divided by the number of days in the corresponding rest period.

Chemical analysis

Specifically, samples for the chemical composition analysis were obtained by an observer who evaluated the height and the structural part of the plant being ingested by an animal and manually collected a similar portion of the plant consumed (Campos et al., 2016). This samples were pre-dried in a 55 °C forced-air oven for 72 h, then ground to pass a 1 mm sieve of a Wiley-type mill™. Chemical analysis included: dry matter (DM; Method 967.03; AOAC, (Association of Official Analytical Chemists [AOAC], 2016); crude protein, assayed indirectly by N content using the Kjeldahl method (CP; Method 984.13; AOAC, (AOAC, 2016); neutral detergent fiber (NDF) and acid detergent fiber (ADF) determined using the method described in Van Soest, Robertson and Lewis (1991), with 16 µm polyester bags and an autoclave set to 110 °C for 40 min; and acid detergent lignin (ADL), determined by 12 M sulfuric acid treatment on ADF residue (AOAC, 2016). Nitrogen fractionation was conducted according to the method in Licitra, Hernandez and Van Soest (1996). Estimation of DM in vitro digestibility (IVDDM) followed methodology adapted from Tilley and Terry (1963), using polyester bags. After incubation, samples were treated in neutral detergent solution for 40 min at 105 °C in an autoclave (Goering & Van Soest, 1970). Total digestible nutrients (TDN) was estimated following the method of Kunkle and Bates (1998).

Tissue emission

To evaluate the morphogenic variables, the "marked tillers" technique was used (Carrere, Louault, & Soussana 1997). In

each paddock, a three meter transect was marked and then in each of these 10 tillers were marked. Evaluations were made once a month during the rest periods, starting three to four days after the animals departed and ending when the animals entered for grazing. New tillers were marked in each paddock in each month to maintain representativeness. The length of the green portion of the fully expanded leaf blades, in expansion and senescence, was measured with a graduated ruler to obtain the variable of total green blades (including expanding leaves).

The leaf appearance rate (leaf day⁻¹) was determined by dividing the number of leaves in the evaluation period by the number of days in that period. The phyllochron (degree-days), leaf senescence rate (degree-days), number of live leaves (by tiller), total green blade (cm) and leaf life time (degree-days) were also calculated. The accumulated daily thermal sum was calculated using the equation: $[(T^{\circ} Mx + T^{\circ} Mn) / 2] - 10$; where: $T^{\circ} Mx$ = maximum temperature, $T^{\circ} Mn$ = minimum temperature.

Statistical analysis

The morphological and chemical results were analyzed using the generalized linear mixed models with the SAS Glimmix procedure (Statistical Analysis System Institute [SAS Institute], 2013). The choice of which distribution would best fit the data was

made using the corrected Akaike value (AICc). For variables that followed normal distribution, the SAS Mixed procedure (SAS Institute, 2013) was used with the restricted maximum likelihood method (REML), and choice of the variance and covariance matrix that best fit the data was determined using the corrected Akaike value (AICc). The analysis of variance, in both procedures, followed the mathematical model:

$$Y_{ij} = \mu + \alpha_i + m_j + (\alpha^*m)_{ij} + \varepsilon_{ij};$$

where: Y_{ij} is the observation concerning the i -th treatment (α_i) in the j -th month (m_j). Treatments, the month and our interaction (α^*m) were considered as fixed effects. The data were submitted to analysis of variance and the means were compared using the Tukey-Kramer test ($P = 0.05$).

Results and Discussion

Fertilization and productive response

In all months evaluated, excluding January, single-dose fertilization (Nap1) yielded a greater forage production, daily accumulation rate, available forage mass and available leaf mass than the other strategies (Nap2 and Nap4). In January, these variables did not differ statistically between Nap1 and Nap2 (Table 1). The highest stem mass in all months was also recorded from Nap1, except in February when it had the lowest stem mass among the three strategies.

Table 1
Forage yield, forage mass and sward characteristics of African star grass under single or split Nitrogen application

Treatments	Month				
	November	December	January	February	March
Forage Production [§]					
Nap1	4176.9 ^{Ab}	5855.5 ^{Aa}	5128.8 ^{Aa}	3229.7 ^{Ac}	5174.2 ^{Aa}
Nap2	3586.5 ^{Bc}	5402.0 ^{Ba}	5000.7 ^{Aa}	2594.2 ^{Bc}	3463.5 ^{Bc}
Nap4	3330.4 ^{Cc}	4631.9 ^{Ca}	4138.7 ^{Bb}	2419.3 ^{Cc}	3051.0 ^{Cc}
SEM= 163.97; CV= 22.7					
Daily Accumulation Rate [§]					
Nap1	199.6 ^{Aa}	189.5 ^{Aa}	166.0 ^{Ab}	111.7 ^{Ac}	167.5 ^{Ab}
Nap2	171.4 ^{Ba}	174.8 ^{Ba}	161.85 ^{Ab}	89.7 ^{Bc}	112.1 ^{Bc}
Nap4	159.1 ^{Ca}	149.9 ^{Ca}	133.9 ^{Bb}	83.7 ^{Cc}	98.8 ^{Cc}
SEM= 196.95; CV= 33.3					
Instantaneous Forage Mass [§] (pre-grazing)					
Nap1	5092.9 ^{Ab}	6690.2 ^{Aa}	6495.0 ^{Aa}	5232.9 ^{Ab}	4743.2 ^{Ac}
Nap2	4593.9 ^{Bc}	4872.2 ^{Bb}	6108.2 ^{Ba}	5166.2 ^{Ab}	4522.5 ^{Bc}
Nap4	4607.4 ^{Bb}	4577.4 ^{Cb}	5301.2 ^{Ca}	4319.9 ^{Bc}	4298.8 ^{Cc}
SEM= 577.98; CV= 14.8					
Leaves Mass [§] (pre-grazing)					
Nap1	2460.2 ^{Ab}	3199.0 ^{Aa}	2723.5 ^{Aa}	2018.5 ^{Ab}	1442.7 ^{Ac}
Nap2	2106.1 ^{Bb}	2071.0 ^{Bb}	3024.8 ^{Aa}	1332.3 ^{Bc}	1428.2 ^{Ac}
Nap4	1975.4 ^{Bb}	1906.6 ^{Bb}	2152.9 ^{Ba}	1263.3 ^{Bc}	1395.9 ^{Bc}
SEM= 374.70; CV= 30.1					
Stems Mass [§] (pre-grazing)					
Nap1	2514.8 ^{Ab}	3045.9 ^{Aa}	3155.4 ^{Aa}	2299.4 ^{Cc}	2634.9 ^{Ab}
Nap2	2259.9 ^{Bb}	2603.6 ^{Ba}	2374.3 ^{Ca}	3010.2 ^{Aa}	2326.2 ^{Bb}
Nap4	2307.8 ^{Bb}	2353.3 ^{Cb}	2775.4 ^{Ba}	2540.1 ^{Ba}	2372.9 ^{Bb}
SEM= 86.15; CV= 11.3					
Dead Material [§] (pre-grazing)					
Nap1	119.3 ^{Cc}	454.9 ^{Ac}	626.7 ^{Bb}	928.3 ^{Aa}	676.7 ^{Bb}
Nap2	230.4 ^{Bb}	201.6 ^{Cb}	717.9 ^{Aa}	818.8 ^{Ba}	778.7 ^{Aa}
Nap4	324.3 ^{Ab}	319.4 ^{Bb}	369.7 ^{Cb}	521.1 ^{Ca}	538.8 ^{Ca}
SEM = 60.56; CV = 48.4					

§kg DM ha⁻¹; Nap1 =Single N application; Nap2= split into two N applications; and Nap4: split into four N applications; Similar letters, lowercase in line and uppercase in column, do not differ by Tukey-Kramer test (P = 0.05); SEM: Standard error of the mean; CV: Coefficient of variation.

Nap1 produced the highest daily rate of forage accumulation and the highest biomass accumulation (forage production, leaf mass, and stems) (Table 1), indicating they may be related to the nutrient response efficiency of the grass to the application strategy used. Dubeux and Sollenberger (2020) reported that most nutrients consumed return to the pasture through the deposition of excrement. Apolinário et al. (2013) found increased grazing pressure resulted in a higher proportion of N being recycled by animals through the increased deposition of urine and feces. The splitting of N application (smaller dose per application) may not produce the expected result in terms of N use by soil microorganisms to balance the C:N ratio because of the limitation of the nutrient, arising primarily from its assimilation by the rhizosphere (Mooshammer et al., 2014). Hence, the single fertilization strategy increases the amount of N available, supplying the needs of the plants and contributing to reducing the C:N ratio. A reduced C:N ratio would accelerate the decomposition of organic matter as the litter of tropical grass pastures is hard to decompose owing to its high C:N ratio, lignin:N ratio, and acid detergent insoluble N (Dubeux & Sollenberger, 2020). Pastures presented the same N (crude protein) content in their chemical composition, which supports the idea that the greater availability of N provided by Nap1 was central to producing the greater accumulation of pasture biomass.

The treatments had no effect on sward height in all months (Table 2). Some of the morphogenic variables differed between the fertilization strategies. The senescence rate was statistically different between the fertilization strategies in November. The lowest number of live leaves and highest rate of senescence were recorded in this month, both by Np1 (Table 2).

The largest leaf and stem mass was frequently recorded for Nap1 and appears to be due to the assimilation of N by the plant. The monthly availability of leaves was higher in January. In February and March, the quantities of leaves decreased, likely due to reduced photosynthesis from the combined decrease in temperature nearness fall. Quaresma et al. (2011) found a linear increase in leaf mass with increasing applications of N and greater proportions of leaf blades in the forage. This suggests that fertilization alters the morphological characteristics on the plants (Paciullo et al., 2017). The single application strategy (Nap1) led to a higher leaf appearance rate and total green blades. The shorter phyllochron ($p > 0.05$) contributed to higher number of live leaves on Nap1, compared to the other strategies ($p > 0.05$) (Table 2). These results may have subsequently contributed to the greater leaf mass of Nap1 (Table 1). The reduced rate of leaf appearance in March may be related to the lower thermal accumulation in that month.

Table 2
Morphological characteristics of African star grass under single or split Nitrogen application

Treatments	Month				
	November	December	January	February	March
Sward Height [§] (pre-grazing)					
Nap1	28.49	28.71	29.12	28.74	27.77
Nap2	28.42	27.24	28.42	28.71	28.74
Nap4	28.99	29.88	29.54	29.54	28.70
SEM= 2.18; CV= 7.6					
Number of live leaves					
Nap1	7.09 ^b	9.83 ^a	9.50 ^a	9.36 ^a	9.30 ^a
Nap2	7.41	7.81	7.90	8.39	7.85
Nap4	8.63	8.26	7.76	8.13	7.29
SEM= 0.09; CV= 22.1					
Senescence Rate ^{§§}					
Nap1	0.031 ^A	0.016	0.016	0.016 ^B	0.017
Nap2	0.015 ^{Bb}	0.012 ^b	0.019 ^{ab}	0.022 ^{Aab}	0.027 ^a
Nap4	0.034 ^{Aa}	0.018 ^b	0.016 ^b	0.027 ^{Aa}	0.026 ^{ab}
SEM= 0.00; CV= 58.8					
Leaf Appearance Rate ^{**}					
Nap1	0.584 ^{Aab}	0.667 ^{Aa}	0.549 ^b	0.650 ^{Aa}	0.406 ^c
Nap2	0.419 ^{Bab}	0.489 ^{Ba}	0.453 ^a	0.484 ^{Ba}	0.348 ^b
Nap4	0.557 ^{Aa}	0.493 ^{Ba}	0.464 ^a	0.503 ^{Ba}	0.318 ^b
SEM= 0.00; CV= 32.0					
Total Green Blade [§]					
Nap1	87.37 ^b	100.72 ^{Aa}	93.74 ^{Aab}	85.75 ^{Ab}	96.77 ^{Aab}
Nap2	72.78	68.10 ^B	65.52 ^B	65.84 ^B	75.27 ^{AB}
Nap4	87.81 ^{ab}	96.11 ^{Aa}	69.94 ^{Bbc}	66.56 ^{Bbc}	61.34 ^{Bc}
SEM=4.85; CV=8.9					
Phyllochron *					
Nap1	36.37 ^b	43.33 ^{ab}	44.09 ^{ab}	35.96 ^b	52.94 ^a
Nap2	55.68	50.88	56.37	51.60	62.99
Nap4	35.80 ^c	29.50 ^c	54.13 ^b	49.62 ^b	78.44 ^a
SEM= 1.01; CV= 42.9					
Leave Life Time*					
Nap1	249.12 ^b	416.46 ^{Aa}	417.39 ^a	331.66 ^{Bb}	484.10 ^a
Nap2	364.42	330.89 ^{AB}	387.68	361.37 ^{AB}	437.41
Nap4	340.59 ^{bc}	274.61 ^{Bc}	424.35 ^{ab}	410.94 ^{Aab}	567.91 ^a
SEM= 6.77; CV= 32.0					

§cm; §§cm day⁻¹; *Degrees day; Nap1 =Single N application; Nap2= split into two N applications; and Nap4: split into four N applications; Similar letters, lowercase in line and uppercase in column, do not differ by Tukey-Kramer test (P = 0.05); SEM: Standard error of the mean; CV: Coefficient of variation (%).

Table 3
Nutritive Value of African star grass in pre-grazing, under single or split Nitrogen application

Treatments	Month				
	November	December	January	February	March
Crude Protein [§]					
Nap1	21.42 ^a	21.41 ^a	18.97 ^b	18.98 ^b	19.56 ^b
Nap2	21.15 ^a	21.38 ^a	19.73 ^b	19.13 ^b	19.24 ^b
Nap4	21.14 ^a	21.76 ^a	19.09 ^b	19.06 ^b	19.51 ^b
SEM= 1.22; CV= 5.4					
In vitro Digestibility of Dry Matter [§]					
Nap1	67.99 ^a	67.26 ^a	63.15 ^b	62.18 ^b	61.11 ^b
Nap2	67.69 ^a	68.12 ^a	63.96 ^b	62.43 ^b	61.33 ^b
Nap4	67.90 ^a	67.64 ^a	63.43 ^b	62.01 ^b	61.44 ^b
SEM= 8.18; CV= 4.4					
Neutral Detergent Fiber [§]					
Nap1	71.67 ^a	69.82 ^b	68.51 ^b	72.72 ^a	72.91 ^a
Nap2	72.11 ^a	70.01 ^b	69.23 ^b	73.46 ^a	72.64 ^a
Nap4	71.51 ^a	69.74 ^b	69.77 ^b	73.15 ^a	72.70 ^a
SEM= 2.71; CV= 2.3					
Acid Detergent Fiber [§]					
Nap1	33.32 ^c	35.73 ^b	36.99 ^b	40.19 ^a	38.71 ^a
Nap2	33.64 ^c	36.87 ^b	36.9 ^b	40.53 ^a	39.05 ^a
Nap4	34.46 ^c	36.59 ^b	37.6 ^b	40.44 ^a	38.87 ^a
SEM= 5.47; CV= 6.2					
Total Digestible Nutrients [§]					
Nap1	63.19 ^a	63.42 ^a	62.51 ^b	60.74 ^b	60.42 ^b
Nap2	63.11 ^a	63.43 ^a	61.75 ^b	62.56 ^b	60.21 ^b
Nap4	63.26 ^a	63.13 ^a	61.31 ^b	60.46 ^b	59.90 ^b
SEM= 1.86; CV= 2.2					

§% of Dry Matter; Nap1 =Single N application; Nap2= split into two N applications; and Nap4: split into four N applications; Similar letters, lowercase in line and uppercase in column, do not differ by Tukey-Kramer test (P = 0.05); SEM: Standard error of the mean; CV: Coefficient of variation (%).

Nutrient content

The chemical composition did not vary between treatments (Table 3). Protein content decreased and indigestible protein (Fraction C) increased as the vegetative phase of the forage progressed (Table 4).

The chemical composition of the grass varied with the progression of the forage cycle due to the greater availability of green blades at the start of the experimental period. However, the findings of the present study did not support this because further application of N did not change the N content of the plant tissue that was collected in the grazing simulation. T. S. Assmann et al. (2018) showed that dividing small N rates (less than 200 kg ha⁻¹) reduces the N concentration in forages in the initial growth phase, which can impair performance of plant growth and biomass accumulation. This was evidenced in this study by the greater amount of leaves present in the forage mass (Table 1).

The digestibility of biomass (IVDDM) also fluctuated during the forage cycle and can be attributed to increased lignification and a higher proportion of cell wall mass in the leaves. According to Ziech et al. (2015), the decrease in IVDDM over the seasons is related to a possible deficit of N in pastures. However, variations in chemical composition related to the climate can also occur. The decreased rainfall in January was coupled with lower temperatures in the following months, which may have reduced the rate of tissue deposition and also decreased the decomposition of soil organic matter. This material degrades more easily after senescence, adding N back into the soil (J. M. Assmann et al., 2015). The stocking rate of moderate to intense grazing also affects biomass losses due to trampling and increases the stocks of C and N particulate in the soil (J. M. Assmann et al., 2014). This can contribute to an increase in organic matter in the soil, thereby decreasing the leaching potential.

Table 4
Nitrogen fractionation of the tissue of African star grass in pre-grazing under single or split Nitrogen application

Treatments	Month				
	November	December	January	February	March
Fraction A [§]					
Nap1	18.97 ^c	20.42 ^b	27.32 ^a	27.07 ^a	26.77 ^a
Nap2	16.62 ^c	21.23 ^b	26.24 ^a	27.76 ^a	26.29 ^a
Nap4	14.93 ^c	23.85 ^b	26.90 ^a	26.87 ^a	26.84 ^a
SEM= 18.53; CV= 18.0					
Fraction B1 [§]					
Nap1	7.16 ^a	5.01 ^b	7.24 ^a	7.00 ^a	7.19 ^a
Nap2	6.36 ^a	4.98 ^b	6.39 ^a	7.52 ^a	6.92 ^a
Nap4	5.49 ^a	5.03 ^b	6.67 ^a	7.41 ^a	7.43 ^a
SEM= 0.88; CV= 14.4					
Fraction B2 [§]					
Nap1	13.95 ^b	18.06 ^a	10.83 ^b	8.24 ^c	12.67 ^b
Nap2	15.20 ^a	18.86 ^a	11.93 ^b	6.91 ^c	11.19 ^b
Nap4	17.38 ^a	18.73 ^a	9.70 ^b	7.76 ^c	12.03 ^b
SEM= 16.13; CV= 31.1					
Fraction B3 [§]					
Nap1	39.67 ^a	32.11 ^b	28.22 ^b	29.72 ^b	27.56 ^b
Nap2	41.07 ^a	32.01 ^b	28.48 ^b	29.76 ^b	28.25 ^b
Nap4	40.42 ^a	27.56 ^b	29.14 ^b	29.92 ^b	27.70 ^b
SEM= 23.49; CV= 15.4					
Fraction C [§]					
Nap1	20.24 ^b	24.41 ^b	26.81 ^a	28.21 ^a	25.83 ^a
Nap2	20.74 ^b	23.83 ^b	26.96 ^a	28.07 ^a	27.37 ^a
Nap4	21.77 ^b	24.85 ^b	27.40 ^a	28.05 ^a	26.01 ^a
SEM= 7.15; CV= 10.5					

§% of Crude protein; Fractions: A = Nonprotein nitrogen; B1 = True soluble protein; B2 = Insoluble protein (neutral-detergent soluble protein); B3 = Acid-detergent soluble protein; C = Indigestible protein. Nap1 =Single N application; Nap2= split into two N applications; and Nap4: split into four N applications; Similar letters, lowercase in line and uppercase in column, do not differ by Tukey-Kramer test (P = 0.05); SEM: Standard error of the mean; CV: Coefficient of variation.

Conclusion

The use of 200 kg of nitrogen in a single application, as opposed to split applications, increases both the production of the African Star and the proportion of leaves in the pre-grazing forage mass.

African star grass can be fertilized with 200 kg of nitrogen in a single application, without causing changes to the chemical composition and nitrogen fractions. These components are modified by the vegetative stage of the plant.

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Ethics approval

Committee on Animal Research and Experimentation - Universidade Tecnológica Federal do Paraná - protocol number: 2016-002.

References

Association of Official Analytical Chemists (2016). Official methods of analysis of AOAC International. In G. W. Latimer Jr. (Ed.), *Official methods of analysis of AOAC International* (20nd ed., 3172p). Rockville, Maryland, USA: AOAC.

Apolinário, V. X. O., Dubeux, J. C. B., Jr., Mello, A. C. L., Vendramini, J. M. B., Lira, M. A., Santos, M. V. F., & Muir, J. P. (2013). Deposition and decomposition of signal grass pasture litter under varying nitrogen fertilizer and stocking rates. *Agronomy Journal*, 105(4), 999-1004. doi: 10.2134/agronj2012.0433

Assmann, J. M., Anghinoni, I., Martins, A. P., Costa, E. V. G. A., Cecagno, D., Carlos, F. S., & Carvalho, P. C. F. (2014). Soil carbon and nitrogen stocks and fractions in a long-term integrated crop-livestock system under no-tillage in southern Brazil. *Agriculture, Ecosystem & Environment*, 190(1), 52-59. doi: 10.1016/j.agee.2013.12.003

Assmann, J. M., Anghinoni, I., Martins, A. P., Costa, S. E. V. G. de A., Kunrath, T. R., Bayer, C., & Franzluebbers, A. J. (2015). Carbon and nitrogen cycling in an integrated soybean-beef cattle production system under different grazing intensities. *Pesquisa Agropecuária Brasileira*, 50(10), 967-978. doi: 10.1590/S0100-204X2015001000013

Assmann, T. S., Sonogo, E. T., Assmann, A. L., Adami, P. F. & Cuzzi, C. (2018). Effect of splitting nitrogen fertilization on Tifton 85: Yield, nitrogen use efficiency, and nitrogen nutritional status of plants and soil. *African Journal Agricultural Research*, 13(22), 1154-1162. doi: 10.5897/AJAR 2017.12869

Campos, F., P., Nicácio, D. R. O., Sarmiento, P., Cruz, M. C. P., Santos, T. M., Faria, A. V. F.,... Lima, C. G. (2016). Chemical composition and in vitro ruminal digestibility of hand-plucked samples of Xaraes palisade grass fertilized with incremental levels of nitrogen. *Animal Feed Science*

- Technology*, 215(1), 1-12. doi: 10.1016/j.anifeedsci.2015.12.013
- Cardoso, R. A., Bento, A. S., Moreski, H. M., & Gasparotto, F. (2014). Influência da adubação verde nas propriedades físicas e biológicas do solo e na produtividade da cultura de soja. *Semina: Ciências Biológicas e da Saúde*, 35(2), 51-60. doi: 10.5433/1679-0367.2014v35n2p51
- Carrere, P., Louault, F., & Soussana, J. F. (1997). Tissue turnover within grass-clover mixed swards grazed by sheep. methodology for calculating growth, senescence and intake fluxes. *Journal Applied Ecology*, 34(2), 333-348. doi: 10.2307/2404880.
- Comissão de Química e Fertilidade do Solo - RS/SC. (2004). Manual de adubação e calagem para os Estados do Rio Grande do Sul e Santa Catarina. (10nd ed.), Porto Alegre, RS, Brasil.
- Delevatti, L. M., Cardoso, A. S., Barbero, R. P., Leite, R. G., Romanzini, E. P., Ruggieri, A. C., & Reis, R. A. (2019). Effect of nitrogen application rate on yield, forage quality, and animal performance in a tropical pasture. *Scientific Reports*, 9(1), 1-9. doi: 10.1038/s41598-019-44138-x
- Dubeux, J. C. B., & Sollenberger, L. E. (2020). Nutrient cycling in grazed pastures. In M. Rouquete, & G. E. Aiken (Eds.), *Management strategies for sustainable cattle production in southern pastures*, (pp. 59-75). London, UK: Academic Press.
- Goering, H. K., & Van Soest, P. J. (1970). *Forage fiber analysis. Apparatus, reagents, procedures and some applications*. Washington, USA: Agricultural Handbook.
- Kunkle, W. E., & Bates, D. B. (1998). Evaluating feed purchasing options: energy, protein, and mineral supplements. *Proceeding of the Annual Florida Beef Cattle Short Course*, Gainesville, Florida, USA, 47.
- Licitra, G., Hernandez, T. M., & Van Soest, P. J. (1996). Standardization of procedures for nitrogen fractionation of ruminant feeds. *Animal Feed Science Technology*, 57(4), 347-358. doi: 10.1016/0377-8401(95)00837-3
- Mooshammer, M., Wanek, W., Hämmerle, I., Fuchslueger, L., Hofhansl, F., Knoltsch, A., Richter, A. (2014). Adjustment of microbial nitrogen use efficiency to carbon:nitrogen imbalances regulates soil nitrogen cycling. *Nature Communications*, 5(3694), 3694. doi: 10.1038/ncomms4694
- Paciullo, D. S. C., Gomide, C. A. M., Castro, C. R. T., Fernandes, P. B., & Morenz, M. J. F. (2017). Morphogenesis, biomass and nutritive value of *Panicum maximum* under different shade levels and fertilizer nitrogen rates. *Grassland Forage Science*, 72(3), 590-600. doi: 10.1111/gfs.12264
- Quaresma, J. P. S., Almeida, R. G., Abreu, J. G., Cabral, L. S., Oliveira, M. A., & Carvalho, D. M. G. (2011). Produção e composição bromatológica do capim-tifton 85 (*Cynodon* spp.) submetido a doses de nitrogênio. *Acta Scientiarum Animal Science*, 33(2), 145-150. doi: 10.4025/actascianimsci.v33i2.9261
- Sartor, L. R., Assmann, T. S., Soares, A. B., Adami, P. F., Assmann, A. L., & Pitta, C. S. R. (2011). Eficiência de uso da adubação nitrogenada, recuperação e lixiviação do nitrogênio em pastagem de capim-papuã. *Revista Brasileira de Ciência do Solo*, 35(3), 899-906. doi: 10.1590/S0100-06832011000300024

- Statistical Analysis System Institute (2013). SAS/STAT® 13.1 *User's guide*. Cary, North Carolina, USA. SAS Institute Inc.
- Tilley, J. M. A. A., & Terry, R. A. (1963). A two-stage technique for the in vitro digestion of forage crops. *Grassland Forage Science*, 18(2), 104-111. doi: 10.1111/j.1365-2494.1963.tb00335.x
- Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). A two-stage technique for the in vitro digestion of forage crops. *Journal Dairy Science*, 74(10), 3583-3597. doi: 10.3168/jds.S0022-0302(91)78551-2
- Ziech, M. F., Olivo, C. J., Ziech, A. R. D., Paris, W., Agnolin, C. A., & Meinerz, G. R. (2015). Nutritive value of pastures of *Cynodon* mixed with forage peanut in southwestern Paraná State. *Acta Scientiarum Animal Science*, 37(7), 243. doi: 10.4025/actascianimsci.v37i3.26872