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Hay Tifton 85 grass under nitrogen doses in different days of regrowth

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ABSTRACT. The objective of this study was to evaluate the yield, nutritional value and potential ruminal degradation of Tifton 85 hay (*Cynodon* spp.) harvested at four regrowth ages (28, 35, 42 and 49 days) and fertilized with two nitrogen doses (N; 100 and 300 kg N ha⁻¹). The experimental design was a randomized block design, arranged in a 4 x 2 factorial scheme, with six replications each. Nitrogen fertilization increased ($p < 0.05$) dry matter yield and digestible dry matter production of hay and also lignin and crude protein contents. Nitrogen fertilization increased ($p < 0.01$) the dry matter yield and the digestible dry matter production of hay, as well as lignin ($p = 0.01$) and crude protein ($p = 0.01$). The neutral detergent fiber corrected for ash and protein ($p = 0.01$), acid detergent fiber ($p = 0.01$) and cellulose ($p = 0.02$) adjusted to the quadratic function regression model of the regrowth ages. For potential rumen degradability hay, it was observed quadratic effect ($p = 0.01$) with maximum value of 73.1% at 37 days of regrowth. Tifton 85 hay fertilized with 300 kg ha⁻¹ of N and harvested up to 37 days of regrowth results in higher hay dry matter yield, higher crude protein content, and better dry matter rumen degradability.

Keywords: fertilizing; *Cynodon* spp.; digestibility; neutral detergent fiber; lignin.

Feno do capim-tifton 85 sob doses de nitrogênio em diferentes dias de rebrota

RESUMO. Objetivou-se avaliar a produção, o valor nutritivo e a degradação ruminal potencial do feno de capim-Tifton 85 (*Cynodon* spp.) colhido em quatro idades de rebrota (28, 35, 42 e 49 dias) e adubado com duas doses de nitrogênio (N; 100 e 300 kg N ha⁻¹). O delineamento foi o em blocos ao acaso, arranjado em esquema fatorial 4 x 2, com seis repetições. A área experimental foi o critério de blocagem. A adubação nitrogenada incrementou ($p < 0,01$) a produção de matéria seca e a produção de matéria seca digestível do feno e também os teores de lignina ($p = 0,01$) e proteína bruta ($p = 0,01$). Os teores de fibra em detergente neutro corrigida para cinzas e proteína ($p = 0,01$), fibra em detergente ácido ($p = 0,01$) e celulose ($p = 0,02$) ajustaram-se ao modelo quadrático de regressão em função das idades de rebrota. Para degradabilidade ruminal potencial do feno foi observado efeito quadrático ($p = 0,01$), com valor máximo de 73,1% aos 37 dias de rebrota. O feno de capim-Tifton 85 adubado com 300 kg ha⁻¹ de N e colhido até os 37 dias de rebrota resulta em maior produção de matéria seca de feno, maiores teores de proteína bruta, melhor degradabilidade ruminal potencial da matéria seca.

Palavras-chave: adubação, *Cynodon* spp., degradabilidade, fibra em detergente neutro, lignina.

Introduction

The seasonality in forage production is characterized by instability in production and in the roughage nutritive value, which are caused by climate changes throughout the year (Oliveira et al., 2016).

In semi-arid regions, besides the low rainfall associated with its irregular distribution, high temperatures and prolonged periods of summer are also factors that contribute to oscillations in the production and supply of roughage for animal feed throughout the year. Thus, maximizing forage

production strategically in the rainy season is important for storing food and maintaining pasture longevity throughout the year (Monção et al., 2016). The technique of haymaking stands out as an option for the conservation of forages to be used by the animals during periods of forage shortage, and it contributes to maintain animal production throughout the year (Oliveira et al., 2014b).

The forages with high productive potential and nutritional value, such as those of the genus *Cynodon*, can be used for hay production (Oliveira, Monção, Gabriel, Lempp, & Moura, 2014a, Oliveira

et al., 2014b; Sanches, Gomes, Rickli, & Friske, 2016) and, within this genus, the Tifton 85 grass (*Cynodon dactylon* cv. Tifton 85), mainly due to its morphological and structural characteristics favorable to rapid dehydration such as thin stems and high leaf/stem ratio (Sanches et al., 2016; Taffarel et al., 2014).

The physiological maturity of the plant is broadly related to the nutritional value and yield of forage matter (Monção et al., 2016), being that in young plants there are bigger components of the cellular content and better digestibility, however, lower production of dry matter (Oliveira et al., 2016). Therefore, the equilibrium between mass production and forage quality should be sought.

The nitrogen fertilization (N) is an alternative to intensify mass production and the nutritional quality of forage grasses (Sales et al., 2014). According to Silva et al. (2012), the successful use of N in pasture depends not only on the availability of nutrients or the choice of the forage species, but also on the understanding of its morphophysiological mechanisms and their interaction with the environment and management. Thus, it is necessary to understand the forage growth in different management conditions, such as different regrowth ages and application rates of the input (Gomes et al., 2015). These authors observed linear increase in mass production Tifton 85 grass under increasing doses of N.

Based on the above, the objective was to evaluate the yield and nutritive value of Tifton 85 hay harvested at four regrowth ages and fertilized with two doses of N.

Material and methods

The experiment was conducted at the Experimental Farm of the State University of Montes Claros – UNIMONTES, in the municipality of Janaúba, Minas Gerais, during the period from 10/29/2015 to 05/12/2016.

The municipality of Janaúba is located in the northern region of Minas Gerais, at 15° 47' south latitude, 43° 18' west longitude and 516 m altitude. The climate of the region, according to the classification of Köppen and Geiger (1928), is Aw type with summer rains and dry periods well defined in winter (Antunes, 1986). The average annual rainfall is 876 mm, with an annual average temperature of 24°C. The climate is tropical mesothermal, almost megahermic, due to altitude, sub humid and semi-arid, with irregular rains, causing long periods of drought (Figure 1).

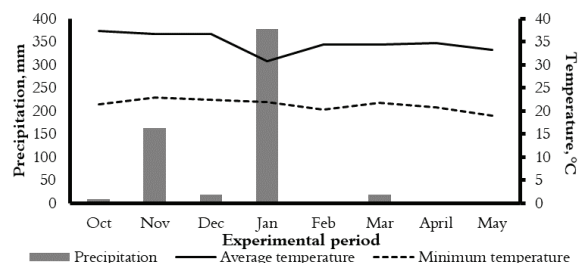


Figure 1. Climatic data of the experimental period.

Source: Instituto Nacional de Meteorologia [INMET] (2016).

The experiment was carried out in a flat area with Tifton 85 grass (*Cynodon dactylon* cv. Tifton 85), already established since 2007, on dystrophic red-yellow soil with clay texture with the following chemical characteristics: pH in water, 6.0, P (Mehlich): 6.0 mg dm⁻³; K (Mehlich): 68 mg dm⁻³; Ca²⁺ (KCl 1 mol L⁻¹): 3.7 cmolc dm⁻³; Mg²⁺ (KCl 1 mol L⁻¹): 1.0 cmolc dm⁻³; Al³⁺ + (KCl 1 mol L⁻¹) 0.1 cmolc dm⁻³ H + Al (calcium acetate 0.5 mol L⁻¹): 2.6 cmolc dm⁻³ base sum: 4.9 cmolc dm⁻³; cation exchange capacity: 7.4 cmolc dm⁻³ V: 65%.

The experimental design was a randomized block in a factorial 4 x 2, with four regrowth ages (28, 35, 42 and 49 days) and two levels of nitrogen (100 to 300 kg ha⁻¹) in six replicates, totaling 48 experimental units, of 25 m² (5x5m) each. The blocking criterion was the variation in the experimental area. At regrowth ages (28, 35, 42 and 49 days), the mean heights were 21, 22, 28 and 30 cm, respectively. The regrowth ages were chosen because of the high growth of grass *Cynodon*, which reach physiological maturity early, as shown in previous researches of Velásquez et al. (2010), Oliveira et al. (2014b) and Sanches et al. (2016).

On October 29, 2015, a standardization cut was carried out on Tifton 85 grass close to the soil, using costal brush cutters. Then, the plots were fertilized with 100 kg of P₂O₅ and 100 kg of K₂O in the single superphosphate and potassium chloride forms, respectively. Nitrogen fertilization was done in split-plot three times after the cut of uniformity and in the two subsequent cuts. Urea (45% N) was used as the source of N and the doses tested were calculated equivalent to the area of each plot. During the experimental period the Tifton 85 grass was irrigated once a week as recommended by Mota et al. (2010). The irrigation remained during the whole experimental period, being activated in the absence of rain.

The haymaking process of Tifton 85 grass was done manually, with the aid of a costal brush, in the morning after dew drying. During the dehydration of the forage, the material was stirred and turned daily between 10:00 and 15:00 hours until it reached

the point of phenation (85% dry matter, Evangelista and Lima (2013)). After haymaking, the dehydrated grass was weighed to determine hay production in natural matter, and then stored in airy sacks and transported to a ventilated shed and stored on wooden decking.

For calculation of dry matter production of hay (DMP), it was used a total of 6, 5, 4 and 4 cuts regrowth ages 28, 35, 42 and 49 days, respectively. The DMP was calculated by the sum of the cuts made for each cutting age over the experimental period. Dry matter yields were determined from the dry matter results at 105°C.

A sample of hay was removed from each cut at the different cutting ages, homogenized, milled in a Willey mill, using a 1 and 2 mm sieve and packed in polyethylene bottles, fitted with lids and previously identified. The hay was analyzed for dry matter (DM, method 934.01), crude protein (CP, method 978.04) and ashes (method 942.05) according to Association of Official Analytical Chemist (AOAC, 2005). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and hemicellulose were determined according to the procedures described by Robertson and Van Soest (1981). The cellulose was solubilized in 72% sulfuric acid and the lignin content was obtained by difference (Goering & Van Soest, 1970).

To evaluate the degradability of hay potential (DMPD), hay samples were placed in nylon bags of TNT type (Non-woven fabric; Casali et al. (2008), with weight of 56 microns, and dimensions of 15x8cm, in a ratio of 20 mg of MS cm⁻² (Nocek, 1988). The bags were tethered and fixed on a nylon cord and placed in the rumen of an adult bovine (6 years old) weighing 450 kg and carrying a ruminal cannula. The incubation period corresponded to 264 hours, with the bags placed in duplicate (Casali et al., 2008). After the incubation period, all the bags were removed from the rumen, washed in running water until it was clean, and then dried. Data for

DMPD were obtained by weight difference between weighing done before and after ruminal incubation and it was expressed as a percentage. The yield of hay digestible DM (DMD) was estimated by multiplying the DMPD of the hay of each replicate by its respective yield of DM at 105°C.

The obtained data were submitted to the analysis of variance using PROC MIXED of Statistical Analysis System (SAS, 2000). When the "F" test was significant for the treatments, the regrowth ages were analyzed by means of orthogonal contrasts. As ages are not equidistant, the SAS PROCIML was used to estimate the Linear, Quadratic and Cubic order matrix. Nitrogen doses were analyzed by the T test. For all the tests the probability of 5% was used.

Results and discussion

There was no significant interaction between regrowth ages and N doses for any of the analyzed variables ($p > 0.05$), thus, the factors were discussed separately.

A linear reduction was observed for DMP ($p < 0.01$) and DMPD ($p < 0.01$) of hay with the advancement of regrowth ages (Table 1). This was due to the lower number of cut (4 cuts) at the ages of 42 and 49 days in relation to the others, and also due to the DM content that did not differ within the ages ($p = 0.40$) nor within the doses of N ($p = 0.56$). However, there was an increase in PMS ($p < 0.01$) of hay between N doses, with mean values of 44.3 and 53.9 t ha⁻¹ at 100 and 300 kg ha⁻¹, respectively, with an increase of 21.7%. For the DMPD of the hay, the averages were 31.8 and 39.2 t ha⁻¹, corresponding to the doses of 100 and 300 kg ha⁻¹ of N, respectively.

According to Vitor et al. (2009), the highest DMP with nitrogen fertilization can be attributed mainly to the effects of nitrogen, which promotes a significant increase in enzymatic reactions and stimulation in cell divisions, especially in phytomers.

Table 1. Productive and nutritional characteristics of Tifton 85 hay harvested at four regrowth ages and fertilized with two doses of nitrogen.

Item (% DM)	Dose N 100 (kg ha ⁻¹)				Dose N 300 (kg ha ⁻¹)				SEM	P-value				
	Age of regrowth (days)				Age of regrowth (days)					Age L	Age Q	Age C	Dose N	I x D
	28	35	42	49	28	35	42	49						
DMP, t ha ⁻¹	52.5	45.6	43.9	35.1	57.4	56.6	55.5	45.9	3.5	<0.01	0.18	0.25	<0.01	0.58
DMPD, t ha ⁻¹	37.3	33.5	31.6	24.8	41.6	41.4	40.4	33.2	2.5	<0.01	0.09	0.35	<0.01	0.67
Dry matter	89.6	88.0	90.1	89.6	90.0	88.7	89.9	89.5	0.4	0.40	0.07	0.01	0.56	0.56
Ashes	7.97	6.68	7.69	7.96	7.75	7.78	7.71	8.41	0.40	0.26	0.06	0.40	0.24	0.38
Crude protein	12.3	11.0	7.5	7.5	13.3	12.8	10.8	10.3	1.3	<0.01	0.70	0.23	0.01	0.75
PIDN	0.81	0.81	0.75	0.71	0.93	0.64	0.81	0.71	0.10	0.14	0.54	0.24	0.95	0.39
Neutral detergente fiber*	71.6	75.4	73.9	73.3	73.5	74.6	74.1	72.5	1.0	0.96	0.01	0.27	0.87	0.39
Acid detergente fiber	30.8	33.2	35.4	33.5	32.1	34.6	33.9	33.0	1.1	0.08	0.01	0.89	0.77	0.45
Hemicellulose	40.8	42.2	38.6	39.9	41.4	39.9	40.2	39.5	1.3	0.14	0.83	0.36	0.90	0.47
Cellulose	26.7	27.6	30.9	26.8	28.5	29.2	29.9	28.1	1.1	0.66	0.02	0.09	0.25	0.55
Lignin	4.05	5.63	4.46	6.65	3.63	5.39	4.07	4.88	0.40	<0.01	0.73	<0.01	0.01	0.11
MS potential degradability	71.2	73.3	72.0	70.6	72.6	73.3	72.9	72.0	0.6	0.18	0.01	0.34	0.04	0.56

DM- Dry matter content; DMP – Dry matter production; DMPD – Dry matter production digestible; P – Probability; N – Nitrogen; I x D – Interaction between regrowth ages and nitrogen doses. * Corrected for ash and protein.

It is worth mentioning that to increase the PMS with the application of N it is necessary that the macros and micro minerals are available in the soil for the plant.

The DM and ash contents were not altered ($p > 0.05$) between regrowth ages and nor with nitrogen fertilization, with a mean of 89.4% and 7.7%, respectively. This was probably due to the fact that the age of regrowth studied was not sufficient for the plant to reach physiological maturity, which usually raises the content, mainly of DM, as verified in the researches of Oliveira et al. (2014b), Oliveira et al. (2016) and Monção et al. (2016). However, it can be highlighted that the DM content verified in this research is in accordance with the recommended for proper storage of hay during storage (above 85% DM; Van Soest (1994), Evangelista & Lima (2013) and Oliveira et al. (2014b). The storage of hay with inadequate DM content may provide an increase in respiration rate, favoring the development of fungi and losses of nutritive value caused mainly by fermentation of soluble carbohydrates (Castagnara et al., 2011). The DM contents observed in this research are similar to those verified by Oliveira et al. (2014b), who verified mean DM values of 92.7% for Tifton 85 hay harvested with 52 days of regrowth and dehydrated for 48 hours after cutting. The average mineral content observed in this research was 7.74%, close to that verified by Taffarel et al. (2014), which was 7.6% during the baling of the Tifton 85 hay harvested at 35 days.

The mean DMP of hay decreased by 0.63 t ha^{-1} per day of advancement at regrowth ages. For the DMPD the mean values were 39.5, 37.5, 36 and 29 t ha^{-1} , at the ages of 28, 35, 42 and 49 days of regrowth, respectively, and the daily reduction was 0.47 t ha^{-1} . According to Oliveira et al. (2014a), at age 28 and 35 days, Tifton 85 grass presents a high content of components of the cellular content with high digestibility (Van Soest, 1994), consequently, higher DMPD at these ages. In contrast, at ages greater than 42 days, there are the initial effects of plant physiological maturity, such as an increase in the deposition of cell wall components (cellulose, hemicellulose and lignin), which reduce the CP content and ruminal degradation of the plant (Monção et al., 2014).

In this research, the CP content decreased 43.8% (linear decreasing behavior) with the increase of the regrowth ages ($p < 0.01$). Among N doses, the CP content increased by 11.8% ($p = 0.01$) as the N dose increased from 100 to 300 kg ha^{-1} . For neutral detergent insoluble protein (NDIP, % DM), there was no difference between N doses, with a mean of 0.77% of DM.

The reduction in CP content as a function of age may be related to the increase of the cellular appearance constituents, proportionally diluting the components present in the cellular content, a fact also observed by Bosa, Guimarães, Polizel, Bonfim-Silva and Canuto (2016) and Monção et al. (2016). The CP content of 8.8% at the 49 days of regrowth verified in this research is satisfactory according to Van Soest (1994), who showed that the dry matter intake of tropical forages is positively influenced by the protein content of the plants, when this parameter is above 7%. This information is related to cellulolytic and fibrolytic bacteria that use ruminal ammonia as a source of nitrogen to degrade the fibrous fraction of the diet and synthesis of microbial protein, requiring at least 70 grams of CP per kilogram of dietary DM. This protein, when degradable in the rumen, is converted into ammonia by proteolytic bacteria (Van Soest, 1994).

Regarding the doses of N, Taffarel et al. (2014) also observed a positive effect of N on CP levels in cultivars of the genus *Cynodon*. This increase, according to Malavolta and Moraes (2007), is probably due to the reduction of N to the ammoniacal form, where it is assimilated to the carbonic skeletons by the GS-GOGAT cycle (glutamic acid and glutamine), which is the precursor of several amino acids, of which about 20 are used in the in the so-called 'all or none' process.

The contents of NDFap ($p = 0.01$), the ADF ($p = 0.01$), cellulose ($p = 0.02$) and the degradability of DM ($p = 0.01$) showed quadratic regression behavior with the advancement of age of regrowth. The same variables were not influenced by nitrogen fertilization ($p > 0.05$), presenting mean values of 72.3, 32.9 and 72.2%, respectively. The lignin content increased 50.1% with regrowth age ($p < 0.01$) and decreased 15.5% when the dose of 100 kg ha^{-1} of (5.2%) was increased to 300 kg ha^{-1} ($p = 0.01$).

The behavior observed for NDFap content, ADF and lignin with the increase in regrowth age is due to increased cell wall accumulation of the effect of physiological plant maturity influenced by edafoclimatic conditions of each region and can be enhanced in conditions of high temperatures (Van Soest, 1994) and in semi-arid and arid climates. The quadratic behavior of NDFap and ADF may be related to the dilution effect provided by N fertilization altering the ratio of cell content to cell wall (Van Soest, 1994). This change is explicit when the mean values of CP content at different doses of N.

It was found that the quadratic behavior for degradability followed the same pattern of NDFap

and ADF, since these variables, especially the ADF, affect the action of ruminal microorganisms on the substrate (Monção et al., 2014; Van Soest, 1994).

The NDFap values observed in this study were lower than those described by Castagnara et al. (2011), who observed NDF values of 80.6%, for hay harvested at 42 days of regrowth and by Taffarel et al. (2014), which verified a mean content of 76.8% for Tifton 85 hay harvested at 35 days, both determined in the hay storage stage. These differences allow us to infer that the management of forages based on the age of regrowth is not always the same in different regions, with regrowth heights being a more consistent slope for comparison. In addition, several variations occur in the different regions, which justifies variations in the nutritional values of the same forage.

From the nutritional point of view, Van Soest (1994) reported that forage with NDF content above 55% of DM is related to lower forage intake by the animal, due to the effect of ruminal filling caused by the slow ruminal degradation of the fibrous fraction. This is because ester-link bonds between hemicelluloses and lignin make it difficult for ruminal microorganisms to degrade the cellulose fraction and hemicellulose of tropical forages. However, in some genotypes of the *Cynodon dactylon* species, such as Tifton 85 grass, even with high NDF content (above 70%), ester-like bonds do not appear to occur with high intensity, since Oliveira et al. (2014a) verified mean values for potential degradability of the NDF of the Tifton 85 grass leaf of 71, 69, 67 and 66% in the regrowth ages of 28, 42, 63 and 79 days. In this research, the means for potential degradability presented quadratic behavior of regression, being the highest average verified in the regrowth age of 37 days. Sanches et al. (2016) verified an average value of 78.1% degradability of Tifton 85 grass in a rain fed system (without irrigation).

Conclusion

The hay Tifton 85 grass fertilized with 300 kg nitrogen ha⁻¹ and harvested after 37 days of growth accounted for most hay production and nutritional value.

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