DRY MATTER AND PROTEIN ACCUMULATION AS A FUNCTION OF NITROGEN FERTILIZATION IN *Brachiaria brizantha* CV. MARANDU (*Urochloa brizantha)*

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ABSTRACT – In Brazil, forage is the main source of nutrients for ruminants, but most production systems are extensive and low amounts of inputs are used to improve soil fertility. This study aimed to evaluate the effect of nitrogen doses on dry matter yield and protein content in leaves, stems and shoots of Brachiaria brizantha cv. Marandu. The soil was fertilized with dolomitic limestone and gypsum to raise base saturation to 60% in the 0-20 cm layer and decrease exchangeable aluminum in the subsurface layer. The experimental design was the randomized blocks with four replications, and the plots were five meters long by five meters wide. Nitrogen fertilization doses were 0 (control), 50, 100, 150, 200 and 250 kg of N ha^{-1}, and applied on the soil surface at the beginning of the regrowth period. Phosphate and potassium fertilization of 50 kg P ha^{-1} and 150 kg K ha^{-1} were also carried out. Plants were cut thirty-seven days after fertilization. The evaluations were carried out in the central 9.0 m^2 of the plot. A positive effect of nitrogen fertilization was found on all the variables assessed in the study. The effect was linear for fresh matter and dry matter accumulation in leaves + stems of Brachiaria brizantha cv. Marandu. The accumulation of dry matter in leaves + stems was 4.46 t ha^{-1} in the control treatment, increasing to 6.03 t ha^{-1} at the highest nitrogen dose. Nitrogen fertilization positively influenced the percentage accumulation of dry matter in leaves of Brachiaria brizantha cv. Marandu, achieving the equation Y= 25.529 + 0.0372x. Consequently, there was a percentage reduction in the dry matter allocated in stems. At the highest nitrogen dose, the percentage increase of dry matter in leaves was 17%. The effect of nitrogen fertilization on protein content in leaves, stems and leaves + stems was also found. At the highest nitrogen dose (250 kg of N ha^{-1}), there was an increase in protein per kg of dry matter of 86.78 g, as content in the control treatment was 67.5 g kg^{-1}, increasing to 154.3 g kg^{-1}. In addition to increasing the yield due to dry matter accumulation in the plant and improving the bromatological quality, nitrogen fertilization also increased protein production. We should also note that there was an increase in yield and protein production per hectare with the highest dose of nitrogen fertilization.

***Keywords**: dairy cattle, forage, mineral nutrition, silage.*

ACÚMULO DE MATÉRIA SECA E DE PROTEÍNA EM FUNÇÃO DA ADUBAÇÃO NITROGENADA EM *Brachiaria brizantha* CV. MARANDU (*Urochloa brizantha)*

RESUMO – No Brasil, as pastagens são as principais fontes de nutrientes para os ruminantes, mas a maior parte dos sistemas de produção são extensivos, onde há baixa utilização de insumos para melhoria da fertilidade do solo. No presente trabalho, objetivou-se avaliar o efeito de doses de nitrogênio sobre produção de matéria seca e teores de proteína bruta total, nas folhas e colmos da planta de *Brachiaria brizantha* cv. Marandu. O solo recebeu calcário dolomítico e gesso visando elevar a saturação por bases para 60% na camada de 0 a 20 cm e diminuir o alumínio trocável na camada subsuperficial. O delineamento experimental foi o de blocos ao acaso, com quatro repetições, sendo as parcelas constituídas de cinco metros de comprimento por cinco metros de largura. As doses de nitrogênio utilizadas foram: zero (testemunha), 50, 100, 150, 200 e 250 kg de N ha^{-1}, aplicados na superfície do solo, no início da rebrota. A braquiária recebeu também

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adubação fosfatada e potássica em quantidades de 50 kg de P ha^{-1} e 150 kg de K ha^{-1}. O corte da braquiária foi aos trinta e sete dias após as adubações. As avaliações foram realizadas nos 9,0 m^2 centrais da parcela. Constatou-se efeito positivo da adubação nitrogenada sobre todas as variáveis analisadas. Para o acúmulo de matéria natural e de matéria seca na parte aérea da *Brachiaria brizantha* cv. Marandu o efeito foi linear. O acúmulo de matéria seca na parte aérea foi de 4,46 t ha^{-1} no tratamento testemunha, elevando-se para 6,03 t ha^{-1} na dose máxima de nitrogênio. As doses de nitrogênio influenciaram positivamente no acúmulo percentual da matéria seca nas folhas da *Brachiaria brizantha* cv. Marandu, obtendo a equação Y= 25,529 + 0,0372 x, consequentemente houve redução percentual na matéria seca alocada no restante da parte aérea da planta. Na dose máxima de nitrogênio o incremento percentual de matéria seca nas folhas foi de 17%. Constatou-se também efeito da adubação nitrogenada sobre os teores de proteína bruta total, nas folhas e colmos em toda a parte aérea da braquiária. Na dose máxima de nitrogênio houve aumento de 86,78 g de proteína bruta por kg de matéria seca, uma vez que no tratamento testemunha o teor era de 67,5 g kg^{-1}, elevando-se para 154,3 g kg^{-1} na dose de 250 kg de N ha^{-1}. Além de aumentar a produtividade devido ao acúmulo de matéria seca na planta e de melhorar a qualidade bromatológica da forragem, a adubação nitrogenada também aumenta a produção de proteína, tendo em vista que na dose mais alta da adubação nitrogenada houve incremento em produtividade e produção de proteína por hectare.

Palavras-chave: forragicultura, nutrição mineral, pecuária de leite, silagem.

INTRODUCTION

Large areas all over the territory of Brazil and the state of Minas Gerais are used as pastures, both for beef and dairy cattle. In sown pastures, there is a predominance of the genus *Urochloa*. Farmers have preferred plant genus due to its robustness, wide adaptability to different edaphoclimatic environments, in addition to being associated with high yield potential and good nutritional quality of plants (Braz, 2003; Barcelos et al., 2011; Pacheco et al., 2013; Oliveira et al., 2017).

Brachiaria brizantha cv. Marandu is currently one of the most planted *brachiaria*. It is originally from tropical Africa and adapts well to soils of medium fertility, obtaining high yield in fertile soils (Portes et al., 2000). With a short and perennial cycle, it can be planted in rows 50 cm apart or at a maximum depth of 2 cm when broadcast. It grows in the shape of a clump, its stems have dense hairiness, and the plant has good digestibility and palatability. When grown in medium to high fertility soils, plants exceed 1.5 m in height (Crispim & Branco, 2002; Fagundes et al., 2005; Porto, 2017).

Nitrogen and potassium are absorbed in greater quantity by *brachiaria*. Nitrogen is important in plant nutrition and physiology because it is a constituent of proteins and nucleic acids, among other functions (Malavolta et al., 1997; Oliveira et al., 2007). The nitrogen absorbed by the plant results in increased tillering, leaf area index (LAI) and leaf longevity (Oliveira et al., 2007; Taiz et al., 2017).

The increase in LAI increases solar radiation use efficiency, measured as carbon dioxide fixation rate (μmol of $CO_2 m^{-2} s^{-1}$), thus increasing dry matter accumulation (Malavolta et al., 1997; Taiz et al., 2017). Plant N accumulation varies with the age of the crop and the availability of N and other nutrients in the soil solution, as well as edaphoclimatic factors (Oliveira et al., 2007). Cecato et al. (2000) reported positive results to the application of up to 500 kg of N ha^{-1} for tropical grasses.

This study aimed to evaluate the accumulation of dry matter and protein in leaves, stems and leaves + stems of *Brachiaria brizantha* cv. Marandu as a function of nitrogen fertilization in the edaphoclimatic environment of the state of Minas Gerais, Brazil.

MATERIALS AND METHODS

The study was conducted in a rural property located in the city of Porto Firme, state of Minas Gerais (Latitude 20°75'19" S, Longitude 43°01'08" W, average altitude of 717.94 m).

The soil of the experimental area is classified as a dystrophic Red Yellow Latosol of medium to clay texture. For decades the area has been used for grazing, in an extractivist manner, which has left it completely degraded. In July 2019, soil samples were collected in the 0-20 cm layer from an area soon to be recovered. The area was plowed using a tractor disc plow. Based on chemical analysis results, a dose of four tons per hectare of dolomitic limestone was applied and the land was harrowed. Lime samples were collected and analyzed. The lime used had total relative neutralizing power ranging from 92 to 94%.

In October 2019, 10 kg ha^{-1} of seed of *brachiaria brizantha* cv. Marandu (*Urochloa brizantha* cv. Marandu) was sown, with 60% purity and 60% germination. For every 10-kg bag of *brachiaria brizantha* cv. Marandu seeds, four bags (200 kg) of simple superphosphate were mixed, and sowing was carried out immediately mixing. A bean planter was used for sowing, with a spacing of 0.50 m between the furrows.

In early March 2020, the entire pasture area was mowed, at which time the average height of *brachiaria brizantha* cv. Marandu was 1.5 meters. Plant material from the mowing remained on the soil surface. In mid-September 2020, the experimental area was chosen: a slope where *Brachiaria brizantha* cv. Marandu had regular growth in the first regrowth period.

The area was mowed once again close to the soil surface and all the plant material was immediately removed from the area. New soil samples were collected in the 0-20 cm and 20-40 cm layers for chemical analysis. The results are shown in Table 1.

Table 1 - Soil chemical analysis of the study area in the 0-20 cm and from 20-40 cm layers.

Identif.	pH	P	K	Na	Al^{3+}	$H^{+} + Al^{3+}$	Ca^{2+}	Mg^{2+}	BS	CEC (t)	CEC (T)	V	m
Layer	H_2O	.---- mg dm^{-3} ---.			.------------------------ cmol$_c$ dm^{-3} ---------------------------.							.--- (%) ---.	
0 a 20 cm	5.1	1.0	23	0.0	0.8	5.94	0.68	0.28	1.02	1.82	6.96	14.64	43.98
20 a 40 cm	5.0	0.6	17	0.0	0.6	4.62	0.25	0.11	0.40	1.00	5.02	8.03	59.79

pH in H_2O (1:2.5 ratio); P, K, Fe, Zn, Mn and Cu: Mehlich Extractant; Ca, Mg and Al: KCl extractant; H+Al: Calcium acetate extractant at pH 7.0.

After removing the plant material from the study area, six and four tons per hectare of lime and agricultural gypsum were applied, respectively, following recommendations of Oliveira et al. (2007), Raij (2008) and Oliveira et al. (2018).

The study of nitrogen fertilization doses began when the regrowth of *Brachiaria brizantha* cv. Marandu was approximately 10 cm tall to avoid any transport of chemical fertilizers by rainwater. The study was conducted in a randomized block design with four replications, and the plots were five meters long by five meters wide.

The nitrogen fertilization doses were zero (control), 50, 100, 150, 200 and 250 kg of N ha^{-1}, applied on the soil surface at the beginning of the regrowth period. Due to extremely low phosphorus contents (Table 1), a single dose of phosphate fertilizer (115 kg of P_2O_5) was applied to *brachiaria brizantha* cv. Marandu at the beginning of the study.

Potassium fertilization of 180 kg K_2O was also carried out at each cut due to low potassium availability in soil (Table 1). Phosphate, potassium and nitrogen fertilization were carried out at the same time. The fertilizer sources used were ammonium sulfate, triple superphosphate and potassium chloride. Although we applied gypsum to the soil, we chose ammonium sulfate as a source to avoid possible nitrogen losses by volatilization (Oliveira et al., 2007). Fertilization was carried out on October 12, 2020. There was no need for pest control.

On November 20, 37 days after NPK fertilization, sampling was performed to quantify the accumulation of fresh matter in plant shoots. The evaluations were performed in the central 9.0 m^2 of the plot. *Brachiaria brizantha* cv. Marandu was cut 10 cm from the soil, weighed and subsampled, and the leaves were separated from the rest of the plants.

The subsamples of the leaves and stems were weighed, chopped, homogenized and subsampled. Then, they were dried in a forced ventilation oven at 65 °C until constant weight was reached to determine dry matter. These subsamples were passed through a Wiley mill, and nitrogen contents were subsequently quantified according to the method described by Malavolta et al. (1997) and Silva & Queiroz (2006). Nitrogen content was then converted into protein content using factor 6.25 (Silva & Queiroz, 2006).

The results of fresh matter accumulation, dry matter accumulation, dry matter allocation and protein contents in forage shoot dry matter were submitted to analysis of variance (Ferreira, 2011), and regression equations were obtained to relate nitrogen doses to the variables.

RESULTS AND DISCUSSION

Firstly, we will discuss about the accumulation and allocation of fresh matter and dry matter in leaves + stems of *Brachiaria brizantha* cv. Marandu, after which we will focus on protein contents in leaves and stems, as a function of nitrogen fertilization.

Based on the analysis of Table 2, we found a significant effect of nitrogen fertilization on all the variables. The history of land use associated with the control of agricultural practices during the installation and conduction of the study resulted in low experimental variability, as the highest coefficient of variation was 8.12% for dry matter accumulation in leaves of *Brachiaria brizantha* cv. Marandu.

Table 2 **-** Mean squares of analysis of variance and average fresh matter accumulation in leaves + stems (FM Acc. L+S), dry matter accumulation in leaves (DM Acc. Leaves), stems (DM Acc. Stems), leaves + stems (DM Acc. L+S) and percentage allocation of dry matter in leaves (% Alloc. Leaves) and stems (% Alloc. Stems) in *Brachiaria brizantha* cv. Marandu, as a function of nitrogen doses on the 37th day after the start of the study.

Source of variation	FM Acc. Shoots	DM Acc. Leaves	DM Acc. Stems	DM Acc. L+S	% Alloc. Leaves	% Alloc. Stems
	Mean Squares					
Nitrogen Doses	22.78***	0.548***	0.258**	1.50***	25.20**	25.20**
	Overall Average					
	t ha^{-1}				%	
	30.04	2.09	3.17	5.26	39.53	60.47
CV (%)	5.97	8.12	6.09	5.39	5.32	3.48

***, ** significant at 0.1 and 1.0 % by the F-test, respectively.

Linear responses were found for fresh and dry matter accumulation in leaves + stems of *Brachiaria brizantha* cv. Marandu, with high R^2 (Figure 1). Using the equation obtained for dry matter accumulation as a reference, an average increase of 6.3 kg of dry matter was found for each kg of nitrogen applied. Viana et al. (2011) evaluated the effect of nitrogen fertilization on dry matter yield and bromatological composition of *Brachiaria decumbens* cv. Basilisk. Viana et al. (2011) observed an increase in dry matter accumulation of 363 kg ha^{-1} for the dose of 100 kg ha^{-1} N, which was almost half of what was found in this study. The differences in the results of Viana et al. (2011) and those found in our study are most likely due to the improvement of soil physical properties, as we plowed the soil, corrected soil acidity, increased base saturation by applying lime and gypsum, and carried out phosphate and potassium fertilizations.

Tropical grasses have a high response potential to the improvement of physical properties, to the neutralization of aluminum and to the adequate supply of nutrients owing to the metabolism of C4, among other factors. Barcelos et al. (2011) reported studies in which there were increases from 1.0 to 6.5 t of dry matter per hectare, when *Brachiaria decumbens* received adequate nutrient supply and Al^{+3} was neutralized by liming. Francisco et al. (2017) stated that *Brachiaria brizantha* is a plant of moderate tolerance to aluminum and recommended that base saturation in soils be 45 to 50%. Fagundes et al. (2005) and Oliveira et al. (2018) noted that low nutrient availability associated with toxic levels of aluminum in tropical regions are most often the main factors that negatively interfere in forage yield and quality.

Gypsum application was another agricultural practice used in our study which also contributed to high dry matter yields. Even in the control treatment, the average dry matter accumulation was high: 4.5 t of dry matter per hectare in only 37 days (Figure 1). Francisco et al. (2017) reported studies which used small doses of 1.5 t of gypsum per hectare. These authors reported that, in the average of two years, the application of 1.5 t of gypsum per hectare resulted in an increase of 2.5 t of dry matter per hectare, corresponding to a percentage increase of 55% in relation to the control treatment. In addition to neutralizing exchangeable aluminum and increasing base saturation in the non-arable layer of the soil, gypsum provides sulfur, which most pasture soils have low contents of. The increased availability of sulfur influences nitrogen metabolism, as these nutrients have joint action on several metabolic routes.

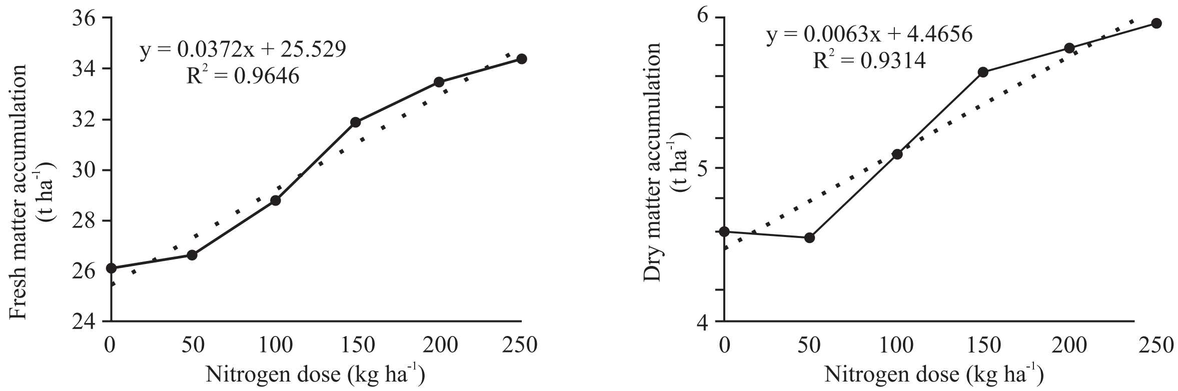

Figure 1 - Accumulation of fresh matter and dry matter in leaves + stems of *Brachiaria brizantha* cv. Marandu on the 37th day after the start of the study.

In addition to the positive interaction with sulfur, nitrogen uptake and metabolism are greatly influenced by phosphorus availability (Malavolta et al., 1997; Oliveira et al., 2007). Oliveira et al. (2007) and Oliveira et al. (2018) cited several studies in which it was observed that plants with inadequate P supply showed a reduction in nitrate absorption from the soil solution; a reduction in nitrate translocation from roots to shoots and an increase in amino acid accumulation in leaves and roots. In soils without exchangeable aluminum, the use efficiency of fertilizer phosphorus is higher because there is no reaction of phosphate anions with aluminum and iron. The compounds formed in this reaction, aluminum phosphate and iron phosphate, are of low solubility, thus their formation results in a marked decrease in the diffusion of phosphorus in the soil. Therefore, in studies involving nitrogen, one should be aware of adequate phosphorus supply so that nitrogen uptake and metabolism are not negatively influenced by endogenous phosphorus availability.

Nitrogen is important in plant nutrition and physiology, because, among other functions, it is a constituent of nucleic acids, amino acids, proteins and enzymes, including PEP carboxylase and RuBisCO. The absorbed N increases the meristematic activity of the shoots, resulting in higher tillering and leaf area index (LAI) of the plants. In addition, N increases leaf longevity. This increase in LAI increases solar radiation use efficiency, measured as carbon dioxide fixation rate (μmol s^{-1} CO_2 m^{-2} s^{-1}). The carbon dioxide fixation rate, performed by PEP carboxylase and RuBisCO, is greatly influenced by the adequate plant nutrition, especially nitrogen, phosphorus and sulfur. Approximately 45% of the entire plant mass is carbon. Thus, the increase in dry matter accumulation by the plant is a direct result of increasing atmospheric CO_2 fixation (Malavolta et al., 1997; Oliveira et al., 2007; Taiz et al., 2017).

Most tropical grasses have highly significant responses to nitrogen fertilization. These responses are influenced by other factors such as base saturation, balanced availability of other nutrients, the management of pastures and climatic conditions, especially when the increase in water availability is associated with increased temperature and luminosity (Malavolta et al., 1997; Oliveira et al., 2007; Oliveira et al., 2018).

Francisco et al. (2017) cited studies with Guinea grass (*Megathyrsus maximus*) fertilized with nitrogen at doses of zero, 150, 300 and 450 kg ha^{-1}. Without nitrogen fertilization, forage yield was approximately 0.5 t ha^{-1}. The increase by nitrogen fertilization was almost linear and dry matter accumulation of 7.0 t ha^{-1} was found at a dose of 450 kg ha^{-1} N.

One of the variables used to measure the response to nitrogen fertilization is dry matter yield per kg of applied nitrogen (kg of DM per kg of N). Viana et al. (2011) reported values of 26 kg of DM per kg of N to 83 kg of DM per kg of N. For *Brachiaria brizantha*, values of 12.7; 18.2 and 18.0 kg of DM per kg of N for doses of 100, 200 and 300 kg ha^{-1} N were reported by Magalhães et al. (2007).

In this study, the average value was 28.1 kg of DM per kg of N applied. However, Viana et al. (2011) reported higher values (65 kg of DM per kg of N) in *Brachiaria decumbens* fertilized with nitrogen and phosphorus.

Nitrogen fertilization positively influenced the percentage allocation of dry matter in leaves of *Brachiaria brizantha* cv. Marandu. Consequently, there was a percentage reduction in the dry matter allocated in the stems (Figure 2). At the highest nitrogen dose, dry matter participation of the leaves in the total accumulated in the shoots increased from 36.36% to 42.68%, according to values predicted by the regression equation. In percentage terms, this increase in leaf dry matter represents 17.38%, considering 36.36% as a reference.

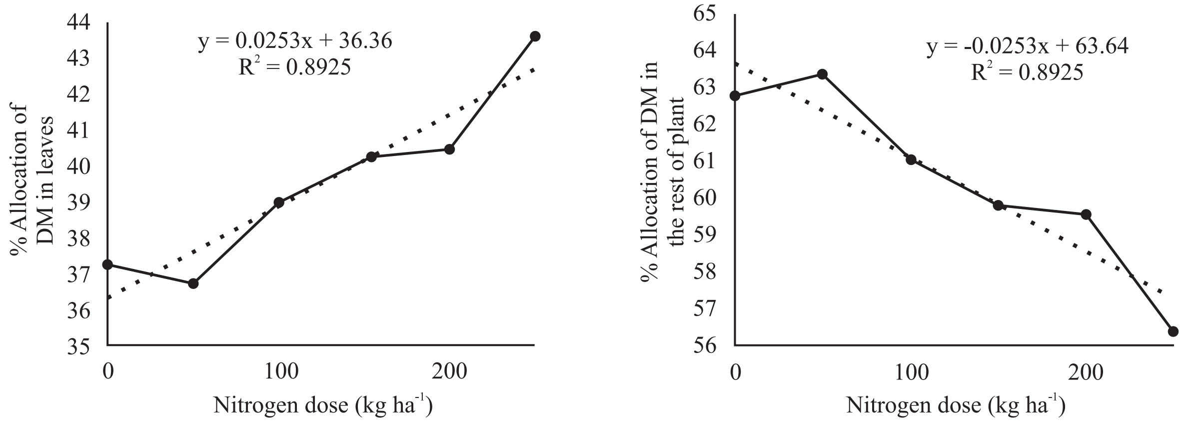

Figure 2 - Percentage allocation of dry matter in leaves and stems of *Brachiaria brizantha* cv. Marandu, on 37th day after the start of the study.

Strozzi (2014) conducted a study with *Brachiaria brizantha* cv. Marandu similar to ours in terms of soil correction, as well as in phosphate and potassium fertilization. For nitrogen fertilization, Strozzi (2014) used doses ranging from 90 to 360 kg ha^{-1}. Dry matter yield was approximately 6.0 t ha^{-1} at the dose of 250 kg ha^{-1} N, which is comparable to that found in this study (Figure 1). The author reported a significant effect of nitrogen fertilization on the morphological composition of forage, with an increase in the percentage allocation of dry matter in leaves of *Brachiaria brizantha* cv. Marandu. For a dose of 250 kg ha^{-1} N, the percentage allocation of dry matter in leaves was 52%, higher than that found in our study (43%). The values of the percentage allocation of dry matter in leaves in this study are closer to those of Sales et al. (2013) who reported a percentage allocation of dry matter in leaves of *Brachiaria brizantha* of 46.24% at the dose of 300 kg ha^{-1} N.

In addition to increasing forage yield, nitrogen fertilization improved bromatological quality, both by increasing the leaf share in plant dry matter and increasing the protein content in stems, sheaths and leaves. Table 3 shows the mean squares of analysis of variance and average protein content in leaves (P in leaves), in stems (P in stems), in leaves + stems (P in L+S) and protein accumulation in leaves + stems (P Acc. in L+S) of *Brachiaria brizantha* cv. Marandu, as a function of nitrogen fertilization.

Based on the analysis of Table 3, there was a significant effect of nitrogen fertilization on all variables. As previously mentioned, the history of land use of the area associated with the control of agricultural practices during the installation, conduction of the study and chemical analysis, resulted in low experimental variability, as the highest coefficient of variation was 7.11% for protein content in stems of *Brachiaria brizantha* cv. Marandu.

Results of increased protein content in leaves and stems of *Brachiaria brizantha* cv. Marandu due to nitrogen fertilization were also reported by Magalhães et al. (2007), with positive linear effect. Nitrogen fertilization also resulted in increased protein in leaves + stems of *brachiarias* in several studies, such as Viana et al. (2011) and Francisco et al. (2017). Figure 3 shows the increases in protein contents in leaves, stems, leaves + stems, and protein accumulation in leaves + stems of *Brachiaria brizantha* cv. Marandu, as a function of nitrogen fertilization. Fertilization had a quadratic effect for all these variables.

Table 3 - Mean squares of analysis of variance and average protein content in leaves (P in leaves), in stems (P in stems), in leaves + stems (P in L+S) and protein accumulation in leaves + stems (P Acc. in L+S) of *Brachiaria brizantha* cv. Marandu, as a function of nitrogen fertilization, on the 37th day after the start of the study.

Source of variation	P in leaves	P in stems	P in L+S	P Acc. in L+S
	------------- Mean Squares -------------			
Nitrogen Doses	7,508.59***	2,919.40***	4,878.26***	252,226.55***
	------------- Overall average -------------			
	------------- g kg^{-1} -------------			---------kg ha^{-1}--------
	166.64	91.14	121.24	654
CV (%)	4.96	7.11	5.22	6.77

***, ** significant at 0.1 and 1.0 % by the F-test, respectively.

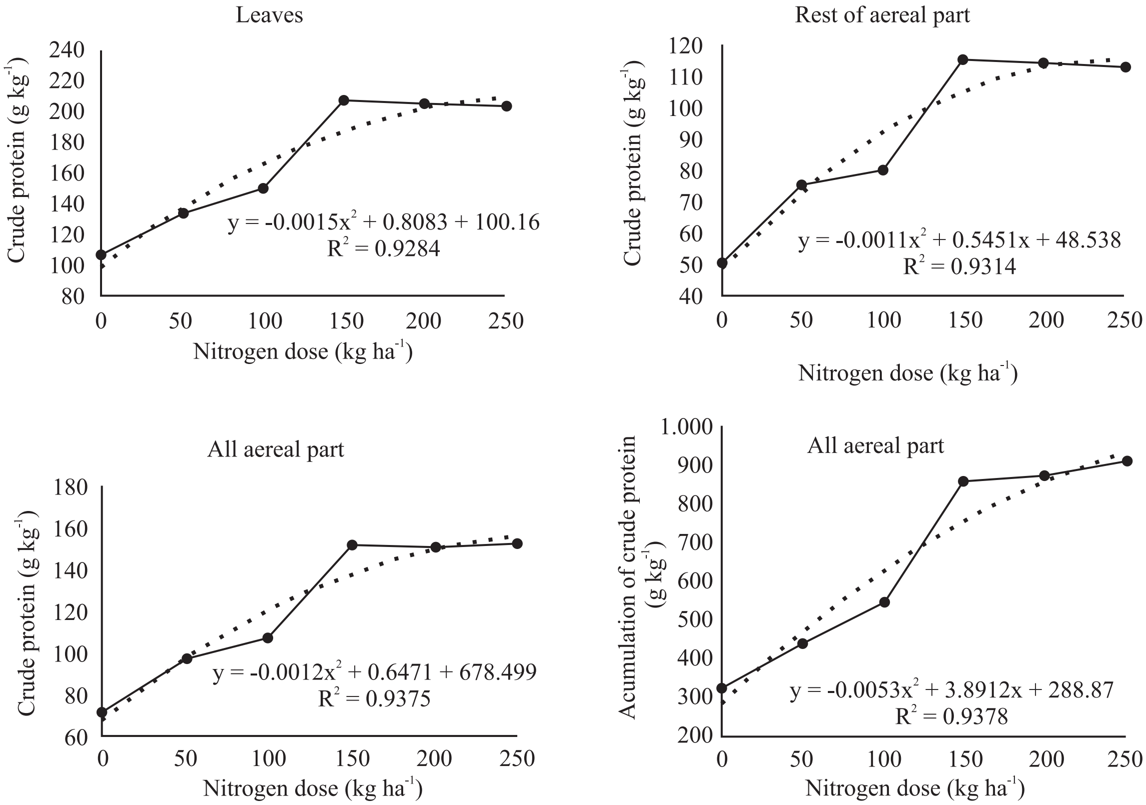

Figure 3 - Protein content in leaves, stems, leaves + stems and protein accumulation in leaves + stems of *Brachiaria brizantha* cv. Marandu, as a function of nitrogen fertilization, on the 37th day after the start of the study.

Viana et al. (2011) reported quadratic effects of nitrogen fertilization on protein content in shoots of *Brachiaria brizantha*. In the control treatment, protein contents were 87 g kg^{-1}, increasing to 108 g kg^{-1} at the dose of 200 kg ha^{-1} N. Compared to the results of Viana (2011), protein contents of the control treatment in this study were lower (67.50 g kg^{-1}). However, at the dose of 200 kg ha^{-1} N, protein content in leaves + stems of *Brachiaria brizantha* cv. Marandu in this study was 149 g kg^{-1}.

Francisco et al. (2017) cited studies in which nitrogen fertilization had a linear effect on protein content in shoots of *Brachiaria brizantha*, but the highest nitrogen dose was small. In analyzing Figure 3, we could estimate that the effect was also linear for doses of 50 and 100 kg ha^{-1} N.

CONCLUSIONS

Nitrogen fertilization increased yield due to dry matter accumulation in the plant and improved the bromatological quality of forage, in addition to increasing protein production. Moreover, there was an increase in yield and protein production per hectare at the highest fertilization dose.

These results are extremely important to promote increased yield and quality of *Brachiaria brizantha* cv. Marandu in the zona da Mata region of the state of Minas Gerais.

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