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PRODUCTION AND QUALITY OF RYEGRASS PASTURE WITH DIFFERENT NITROGEN FERTILIZATION LEVELS AND ITS INFLUENCE IN THE PERFORMANCE OF LAMBS

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ABSTRACT

The effect of nitrogen levels (0, 75, 150, and 225 kg ha^{-1}) in broadcast application on the production and quality of ryegrass pasture as well as lamb production was evaluated. Thirty-six matrices and 60 lambs were maintained in continuous grazing for 112 days. A randomized complete block design with a factorial arrangement 4 (nitrogen levels) x 6 (evaluation dates) for forage evaluation, and 4 (nitrogen levels) x 7 (evaluation dates) for animal performance with 3 replications was used. Forage mass production was positively influenced by nitrogen fertilization, which had increase of 32% compared to the absence of fertilization. Animal stocking and weight gain of lambs had increase of 136% with application of 225 kg ha^{-1} of nitrogen compared to no application of nitrogen. Crude protein and mineral material increased whereas acid detergent fiber, neutral detergent fiber and dry mass decreased, which maintained the quality of forage longer. The highest ruminal disappearance rate of neutral detergent fiber and dry mass occurred with application of 150 kg ha^{-1}of nitrogen. The results of this study, nitrogen fertilization enables the use of ryegrass as exclusive source for animal feed, because it provided increase in production and quality of forage as well as animal gain.

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INTRODUCTION

Crop-livestock integration systems enable the use of agricultural areas for forage production during the winter season in Southern Brazil, which is a period characterized by shortage of food for animal production. The most common forage species used in these systems are black oat (*Avena strigose* Schreb) and ryegrass (*Lolium multiflorum* Lam.) (Lupatini *et al*., 2013; Marques *et al*., 2014). Black oat is considered the ideal forage crop due to its resistance to animal traffic and rapid growth, which allows for anticipation of the use of the pasture (Marques *et al*., 2014). The search for forage crops that have complementary distribution of forage production during the winter season has led to the adoption of consortium of black oat and ryegrass (Tonato *et al*., 2014). It is known that ryegrass provides excellent soil cover and elevated nutritional quality, which enables the intensification of its use as a forage crop associated with its high versatility and capacity of consortium with other grass crops (Cassol *et al*., 2011). However, the consortium of this species with oat may lead to intraspecific competition due to the distinct dynamics of growth other time, which may have negative impact on final yield if not managed well (Tonato *et al*., 2014). Ryegrass can be considered as one of the most important forage crops in the agricultural production systems in Southern Brazil due to its complementarity in vegetative cycle with natural forage, high nutritional value, facility of establishment and excellent capacity of natural reseeding. The single use of ryegrass becomes interesting because it resists to low temperatures and has late cycle, which enables the use of the pasture for longer period allowing its use previously to common bean and soybean. Nitrogen fertilization can improve the bromatological composition of forage crops during the whole phonological cycle of the plants. It is also known that the increase of neutral detergent fiber (NDF) has negative impact in animal consumption as well as on digestibility of dry mass (Vahl *et al*., 2014). . However, little is known about the effect of nitrogen fertilization on the ruminal disappearance rate. Thus, considering the necessity of study of management practices that allow the use of ryegrass as exclusive source of animal feed, the objective of this study was to evaluate the effect of nitrogen fertilization on forage mass production, accumulation rate, capacity of animal support (animal stocking), as well as the effect on bromatological composition and ruminal disappearance rateduring the long period of use of ryegrass as pasture.

MATERIAL AND METHODS

Site description and forage establishment: This study was conducted under field conditions during 2015 in the crop-livestock integration experimental area at Universidade Estadual do Centro-Oeste located in Guarapuava, State of Paraná, Brazil. The experimental area is situated at the geographic coordinates 25° 33' S and 51° 29' W with altitude of 1,000 meters. The soil is classified as Typical Brown Latosol and it presented the following chemical characteristics at 0-20 cm depth: pH $CaCl_2$ 0.01M = 4.5; P = 4.4 mg dm^{-3}; K^+ = 0.5 $cmol_c$ dm^{-3}; organic matter content = 4.15%; Al^{3+} = 0.15 $cmol_c$ dm^{-3}; H^++Al^{3+} = 6.99 $cmol_c$ dm^{-3}; Ca^{2+} = 2.8 $cmol_c$ dm^{-3}; and Mg^{2+} = 2,2 $cmol_c$ dm^{-3}. According to Köppen's classification, the climate of the region is classified as Cfb (humid subtropical) with mild summer and moderate winter, without a defined dry season and with a risk for occurrence of severe frost. Sowing of ryegrass (*Lolium multiflorum* cv. Ponteio) was mechanically performed at 04/04/2015 with 17 cm row spacing, 10 cm depth and seed population of 40 kg ha^{-1}. The experimental area has been under no-tillage system for over 10 years. Fertilization at sowing was performed with 260 kg ha^{-1} with the formulated fertilized 04-20-20 (N-P_2O_5-K_2O) in all the experimental area. The experiment was performed in a randomized block design, with 3 replications, and nitrogen levels and evaluation rates as factors. Nitrogen fertilization treatments were 0, 75, 150 and 225 kg ha^{-1} applied in broadcasting. Forage production was evaluated at 0, 23, 46, 69, 92 and 115 days after beginning of animal grazing. Animal performance was evaluated at 0, 14, 28, 42, 56, 70, 84, 98 e 112 days after beginning of animal grazing. The nitrogen source was urea applied at tillering stage. The experimental unit was 2000 m^2.

Animal performance and grazing management: Animal grazing initiated 58 days after sowing. Thirty-sixmixed race (Texas x Île de France) sheep matrices at end stage of gestation (average of 15-day prior parturition) with average weight of 70 kg were used for the experiment. Continuous grazing with variable animal stocking was used. The animals were separated into two groups which were named as testers and regulators. The tester group consisted of three matrices and five lambs (two twins and a single parturition) in each experimental unit, and they remained in the area during the entire period of the experiment for evaluation of animal performance. The regulator group consisted of animals used to regulate the animal stocking during the experiment. The regulation was performed according to the pasture height. Nine stakes were placed at random in each experimental unit. A 15 cm section was marked in each stake, where the first 10 cm were painted in red and the remaining 5 cm were painted in yellow. Forage height was visually evaluated every week. If the red color was visible in 5 stakes, the regulator animals were removed from the experimental unit. On the other hand, if the red color was not visible in 5 stakes, regulator animals were added to the experimental unit. Animal performance was evaluated during a 112 days period. Animals were weighted every 14 days with a 100 g precision scale. In total, 9 grazing periods for the matrices and 7 grazing periods for the lambs were evaluated: day zero (06/04 to 06/17), day 14 (06/17 to 07/02), day 28 (07/02 to 07/18), day 42 (07/18 to 07/30), day 56 (07/30 to 08/13), day 70 (08/13to 08/20), day 84 (29/08 a 09/10), day 98 (09/10 to 09/23), and day 112 (10/09to 10). Animal stocking was calculated by summing the average weight of tester animals with regulator animals by category. The number was then multiplied by the number of days that the animals remained in the pasture, and the result was divided by the number of days of grazing period.

Forage production: Forage was evaluated approximately every 23 days: day zero (06/03), day 23 (06/24), day 46 (07/20), day 69 (08/10), day 92 (09/04), and day 115 (09/28). Forage production was evaluated by measuring the accumulation rate, which was determined by the total amount of dry mass in an area previously protected with isolation cages following the methodology of triple pairing (Moraes *et al*., 1990). Partial forage mass production was obtained by multiplying the average accumulation rate by the number of days of grazing. Total forage mass production was obtained by summing the accumulation rate of the grazing period plus the forage mass at the time of animal entry. Forage samples were collected by cutting the plants at the soil line in nine representative areas of 0.25 m^2 in each experimental unit. The total weight was considered as forage mass. Subsamples were obtained and dried in forced air oven at 60°C for 72 hours. The samples were grounded at 1 mm size in mill type "Willey", and then they were submitted for laboratory analyses. The pre-dried samples were used to determine the following chemical-bromatological parameters: total dry mass (DM) in oven at 105°C for 12 hours; crude protein (CP) by the Kjeldahl's method; mineral material (MM) by incineration at 550°C for 4 hours; neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to Van Soest *et al*. (1991) using the alpha amilase thermostable (Termamyl® 120L, Novozymes® Latin América Ltda.). The ruminal disappearance rate of DM and NDF were determined by the digestibility technique *in situ*. Two fistulated bovines with 350 kg average weight were used for the analysis. The bovines were maintained in confined and individual areas, and they were fed with corn silage. DM and NDF were determined prior and after incubation according to Van Soest *et al*. (1991), respectively. Ruminal disappearance was calculated by the difference between the material prior and post incubation as follows: (prior inculation – post incubation)/dry mass x 100.

Statistical analyses: Homogeneity of variances was verified by Bartlett's test with the software Assistat®. Once homogeneity was confirmed, data was submitted to analysis of variance with F test in a randomized block design with a factorial arrangement of 4 (nitrogen levels) x 6 (evaluation rates) for analysis of forage production and 4 (nitrogen levels) x 7 (evaluation rates) for analysis of animal performance. Significant results equal or lower than 5% probability level, were submitted to regression analysis with linear and quadratic models with the statistical software Sisvar®.

RESULTS

There was significant effect ($p<0.01$) of evaluation dates and nitrogen for forage mass production and accumulation rate.

However, the interaction of evaluation dates and nitrogen was only significant for accumulation rate. Although the forage mass did not present significant effect of the interaction of evaluation dates and nitrogen levels, there was increase in availability of forage during the distinct periods of the experiment (Fig 1A). Initial production of forage was similar for all nitrogen levels studied, however, it was verified that during the experimental period those areas where nitrogen fertilization was not applied lower forage production was observed. By the end of the experiment, it was verified increase of 69%, 99.4% and 100.8% in forage production with application of 75, 150 and 225 kg ha^{-1} of nitrogen, respectively, compared to absence of nitrogen fertilization. Accumulation rate presented significant differences with application of nitrogen (Fig 1B), which demonstrates that ryegrass has high response to this element. From the 23rd day of evaluation, it was verified that all experimental units with nitrogen fertilization had increased accumulation rate.

A quadratic model with significance of 1% was fitted to the response variable. In the present study, 223 kg ha^{-1} was the estimated level of nitrogen for maximum efficiency of forage production of 2,622 kg ha^{-1}. Total animal stocking had significant effect of evaluation dates and nitrogen levels at 1% probability level. Although there was not significant effect for the interaction, it was observed that the levels of 75, 150, and 225 kg ha^{-1} of nitrogen presented quadratic response, which differed from the absence of nitrogen that presented negative linear response over time (Fig 2A). Increase in animal stocking of 75%, 122% and 114% was observed for 75, 150 and 225 kg ha^{-1} of nitrogen, respectively, which was expected because animal stocking is directly related to forage production. Considering that category of lambs is the final product in sheep production, the gain of live weight of labs per hectare was considered separately, and it presented significant effect for evaluation dates ($p<0.01$).

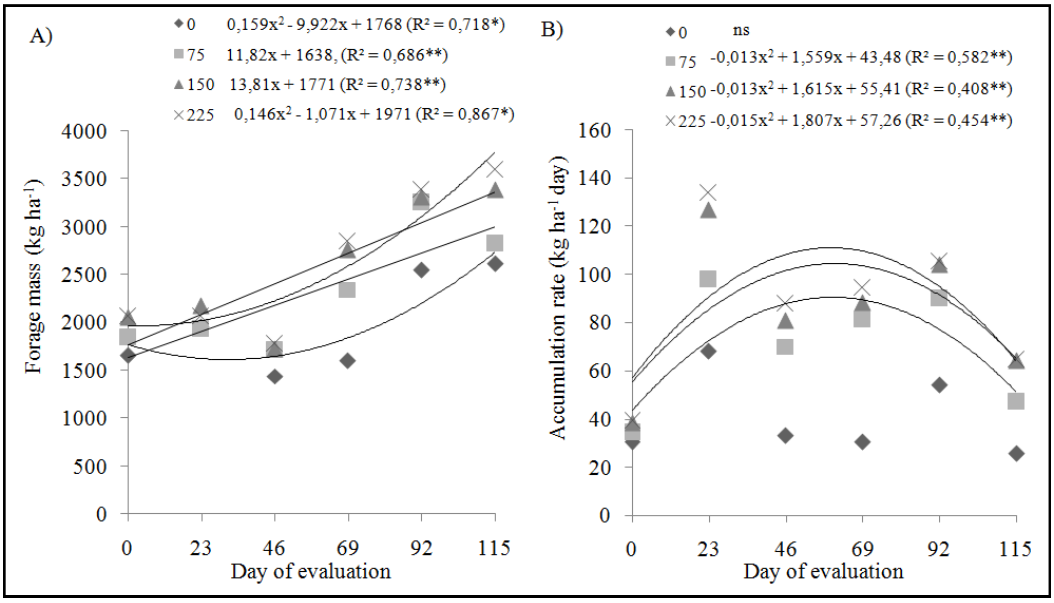

Figure 1. (A) Forage mass production (kg ha^{-1}) and (B) accumulation rate (kg ha^{-1} day^{-1}) of ryegrass pasture with different doses of nitrogen (0, 75, 150, 225 kg ha^{-1}) during the period of evaluated

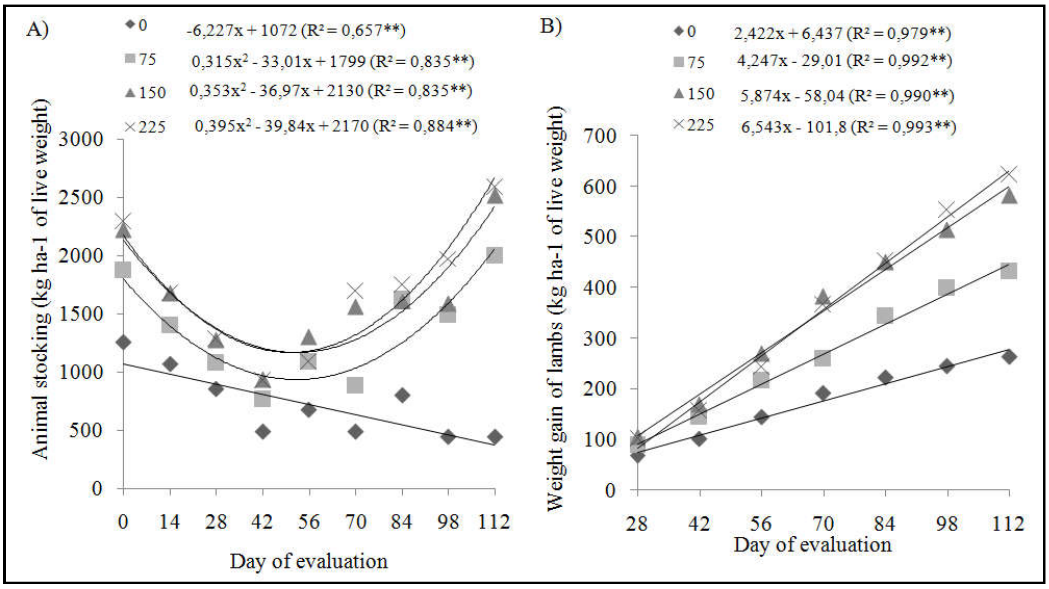

Figure 2. (A) Animal stocking (kg ha^{-1} of live weight) and (B)weight gain of lambs (kg ha^{-1} of live weight) with different doses of nitrogen (0, 75, 150, 225 kg ha^{-1}) during the period of evaluated

Based on regression analysis (Fig 2B), there was linear response for all nitrogen levels studied. Regarding to the absence of nitrogen, there was increase of 64%, 121% and 136% in lamb production with application of 75, 150, and 225 kg ha^{-1} of nitrogen, respectively, which was also expected because the higher the forage mass production was, the higher animal stocking was allowed in the pasture.

Bromatological composition of the forage was significantly affected by evaluation dates and nitrogen levels for CP, MM and ADF, whereas DM and NDF were significantly affected (P<0.05) by the interaction. During the growing cycle of the pasture, there was reduction in quality with increase in NFD and ADF, and reduction in CP and MM (Fig 3).

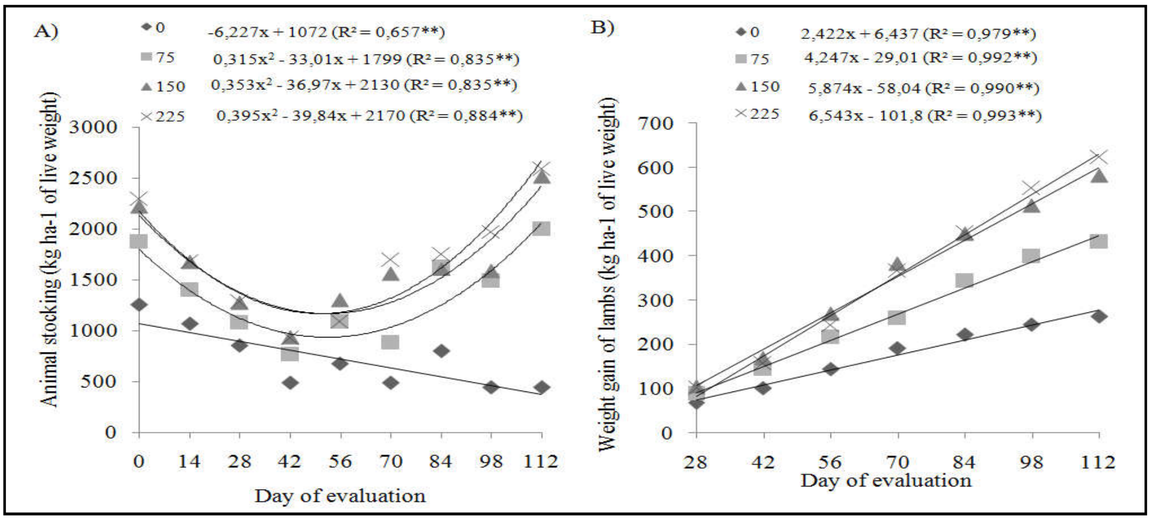

Figure 2. (A) Animal stocking (kg ha^{-1} of live weight) and (B)weight gain of lambs (kg ha^{-1} of live weight) with different doses of nitrogen (0, 75, 150, 225 kg ha^{-1}) during the period of evaluated

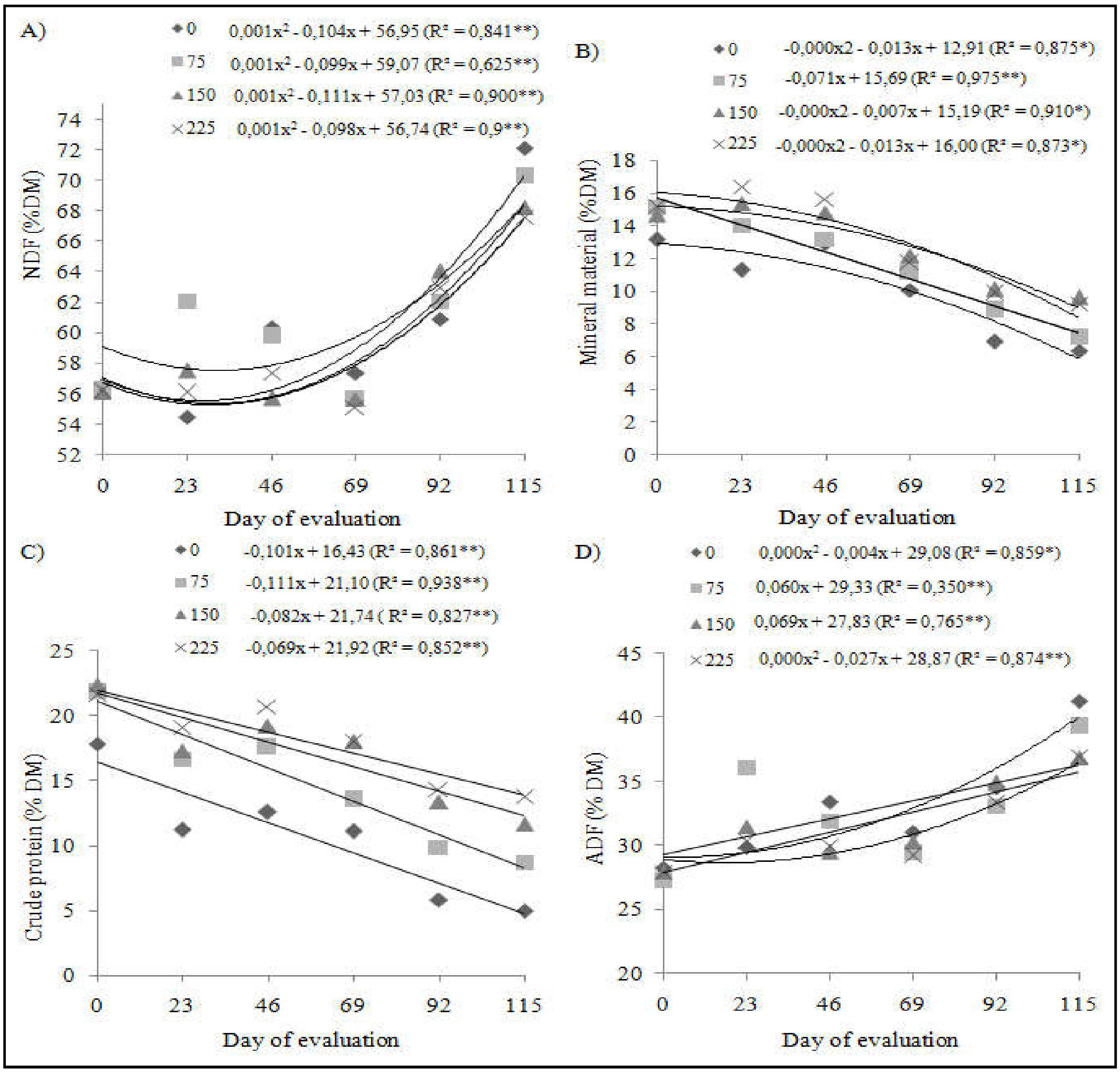

Figure 3. (A) Neutral detergent fiber (NDF), (B) mineral material (MM), (C) crude protein, and (D) acid detergent fiber (ADF) content of ryegrass forage with different doses of nitrogen (0, 75, 150, 225 kg ha^{-1}) during the period of evaluated

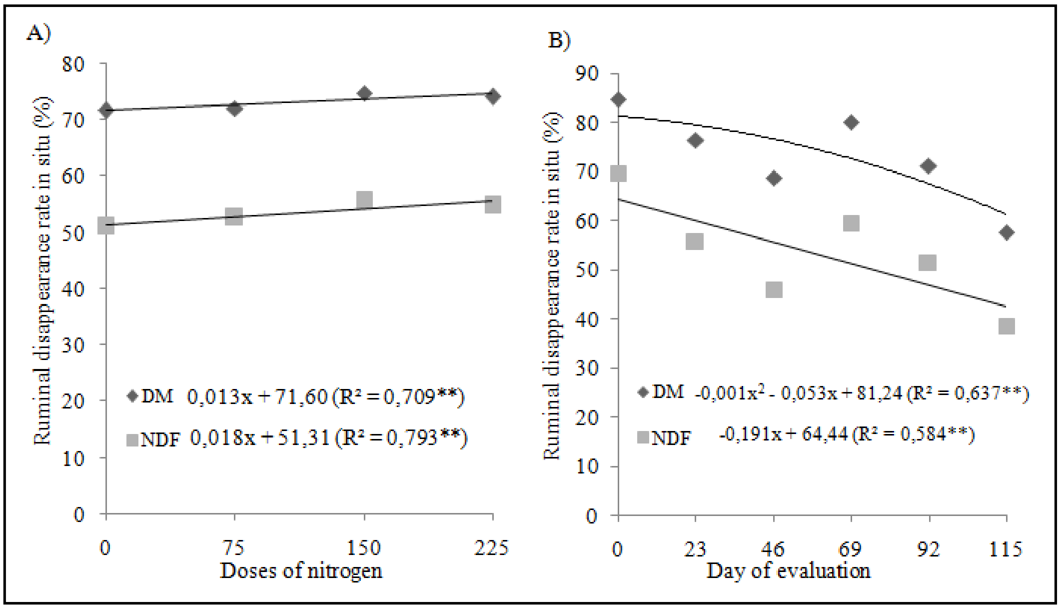

Figure 4. Ruminal disappearance rate *in situ* of neutral detergent fiber (NDF) and dry mass (DM) of ryegrass forage with different doses of nitrogen (0, 75, 150, 225 kg ha^{-1}) during the period of evaluated

Increase in NFD and ADF is a result of physiological modifications with the advancement of the cycle of the plants. ADF content decreased linearly with increase of nitrogen levels. When nitrogen fertilization was not performed, average ADF content was 33.04%, whereas with application of 75, 150, and 225 kg ha^{-1} of nitrogen ADF content was 32.8%, 31.7%, and 3.2%, respectively (Fig 3E). These results demonstrate that with the advancement of the cycle of the plants, nitrogen fertilization can reduce ADF content. NDF content was very similar among the treatments until the third period evaluated (Fig 3A). MM (Fig 3C) and CP (Fig 3D) were reduced overtime and linear models were fitted to the data. CP content presented significant effect of nitrogen levels (P<0.01) as well as MM (P<0.05). DM had significant effect of evaluation dates and nitrogen, with a quadratic model fitted to the data (Fig 3B). The modification in bromatological composition by the days of use of the pasture as well as by the nitrogen levels was responsible for changes in ruminal disappearance rate *in situ* of NDF (P<0.01) and DM (P<0.05). The response of NDF and DM was fitted with a negative linear and quadratic model, respectively (Fig 4A). Nitrogen fertilization provided increase in ruminal disappearance rate of NDF and DM with a linear model fitted to the data (Fig 4B).
Evaluation of forage mass production as well as animal grazing initiated when average forage supply was 1,903 kg ha^{-1}. which is higher than the practical indication by Rosso *et al.* (2016) who suggested 1,500 kg ha^{-1}.According to Cassol *et al.* (2011), knowledge about the exact moment of animal entry is important because anticipated grazing could reduce tiller density as well as foliar area, which could compromise forage and animal production.

In a study by Quatrim *et al.* *et al.* (2015) who evaluated 50, 100 and 150 kg ha^{-1}, observed that fertilization with 100 kg ha^{-1}of nitrogen produced average of 1,928 kg ha^{-1}, and from this level a minimum increase in forage production was observed. However, when those nitrogen levels are compared to our study, similar response was observed between 150 and 225 kg ha^{-1}. Although these responses may be associated with other factors such as pasture management, fertilization, year, and dynamics of nitrogen in the soil, which could interfere with the capacity of uptake and efficiency of nitrogen use by the plants (Lupatini *et al.*, 2013). In this study, 197 kg ha^{-1} of nitrogen was estimated as the maximum efficiency dose for animal stocking, which allowed for animal production of 1,708 kg ha^{-1}. On the other hand, Quatrim *et al.* (2015) obtained 3.32 animal units (AU) ha^{-1}, which corresponds to 1,494 kg ha^{-1} with application of 150 kg ha^{-1} of nitrogen. In a study by Lupattini *et al.* (2013) with consortium of ryegrass and black oat, there was also better efficiency in weight gain with application of 150 kg ha^{-1} compared to 300 kg ha^{-1} of nitrogen. If we analyze the data obtained from our study with the results from the studies previously cited, we can infer that the levels of nitrogen between 150 and 190 kg ha^{-1} would be the most indicated to maximize animal stocking in ryegrass pasture. If we consider that the average weight for slaughtering of lambs is at 40 kg of live weight, these results allowed to infer that an increase of 4, 8 and 9 lambs per hectare could be produced when nitrogen was applied at 75, 150 and 225 kg ha^{-1}, respectively. These results are similar to other studies (Amaral *et al.*, 2016) where mean values of animal production were positively influenced by nitrogen fertilization in broadcasting.
At the reproductive stage, lower amount of foliar area and increase in stalk is observed (Gerdes*et al.*, 2005). These results are similar to those found by Marchesan *et al.* (2015) who evaluated the nutritional values of ryegrass cv. BAR Jumbo and common ryegrass in consortium or not with black oat cv. IAPAR 61, and observed that there was increase in ADF content as well as NDF over time, which interfered with the forage quality.

However, by the end of the experiment, in the absence of nitrogen fertilization, NDF content was 4.5% higher than 225 kg ha^{-1} of nitrogen. Although in this study the difference in NDF was low, even small reductions in the variable are important because it had negative relationship with volunteer animal consumption (Vahl *et al.*, 2014). Reductions in these two variables were expected because over time there is accumulation of structural carbohydrates in the cell wall of the plants, and as a result there is reduction in concentration of nitrogen with reduction in CP content (Loaiza *et al.*, 2016). Increase in CP content from 10.58% in the absence of nitrogen to 17.93% with 225 kg ha^{-1} of nitrogen was observed. Marchesan *et al*. (2015) also observed positive effect of nitrogen fertilization in the increase of CP content. The capacity of restructuring the pasture due to the presence of nitrogen allows for the development of new tillers and, consequently, increases in CP content. In this study, it was verified that in the first four evaluation dates, even in the absence of nitrogen, CP content was still 12.8%, which is in accordance to Cassol *et al*. (2011) who suggested 12% CP for animals in termination stage. The highest DM content was observed in the absence of nitrogen fertilization in all the periods evaluated, which is likely due to the lower capacity of restructuring and regrowth of the pasture with less development of new tillers. These results corroborate with Quatrim *et al*. (2015) where high levels nitrogen allowed for lower values of senescent material to occur. Marchesan *et al*. (2015) observed the same behavior with digestibility in vitro of dry mass, where all the treatments presented quadratic effect with little elevation in the beginning and posterior reduction until the end of the experiment.

Grabber *et al*. (2009) stated that the greater the lignin content in the forage is, the lower the degradability will be because lignin is associated with carbohydrates in the cell wall, which causes lower fiber degradability. The lower the food digestibility, the longer the time will be required inside the gastrointestinal tract and consequent reduction in food consumption and weight gain. Although linear response was found, it was also observed that the dose of 150 kg ha^{-1} of nitrogen provided the highest rates of 55% and 74.3% for NDF and DM, respectively, when compared to the other doses.
In conclusion, the application of 150 kg ha^{-1} of nitrogen in ryegrass pasture provided the highest forage accumulation rate, forage mass production, animal stocking, MM, CP, ADF, NDF, and ruminal disappearance rate of DM and NDF, which resulted in higher weight gain by lambs.

Statement of animal rights: This study was conducted in accordance with the Ethics Committee on Animal Use of the Universidade Estadual do Centro-Oeste under protocol number 018/2015.

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