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ANIMAL PRODUCTION

Nitrogen nutrition index and morphogenesis in Alexandergrass of the Central Depression of Rio Grande do Sul

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ABSTRACT: This study assessed the nitrogen nutrition index, morphogenic characteristics and tiller structure of Alexandergrass (*Urochloa plantaginea* (LINK) Hitch) pasture submitted to different nitrogen (N) levels (zero, 150 or 300 kg ha^{-1} of N). The experimental design was entirely randomized with repeated measures arrangement. The experimental animals were Angus heifers under rotational stocking grazing method. The number of animals was variable to keep 30±5 cm post-grazing sward height. Nitrogen nutrition index increased linearly according N levels ($\hat{Y} = 59.8 + 0.1216N$; $P < 0.0001$; $r^2=0.53$). The leaf appearance rate adjusted to a positive linear regression model according the thermal sum (TS) with zero of N ($\hat{Y}0N = 0.0077 + 0.0000087TS$; $P = 0.0308$; $r^2 = 0.72$) and 150 kg ha^{-1} of N ($\hat{Y}150N = 0.0020 + 0.000021\ TS$; $P = 0.0022$; $r^2 = 0.92$). The use of 300 kg ha^{-1} of N did not alter the leaf appearance rate (0.0124 leaf degree-days^{-1}). The use of up to 300 kg ha^{-1} of N increases the Alexandergrass nitrogen content. The leaf appearance rate in Alexandergrass is modified using N while the morphogenic characteristics leaf expansion, stem expansion, phyllochron, leaf lifespan, leaf elongation duration and tiller structure are not altered by N utilization.

Key words: Leaf appearance, leaf expansion, phyllochron, nitrogen fertilization, Urochloa plantaginea.

Índice de nutrição nitrogenada e morfogênese em papuã na Depressão Central do Rio Grande do Sul

RESUMO: Este trabalho teve como objetivo avaliar o índice de nutrição nitrogenada, as características morfogênicas e a estrutura dos perfilhos de papuã (*Urochloa plantaginea* (LINK) Hitch) submetidos a diferentes níveis de nitrogênio (N) (zero, 150 ou 300 kg ha^{-1} de N). O delineamento experimental foi inteiramente casualizado com medidas repetidas no tempo. Os animais experimentais foram novilhas Angus em regime de pastejo rotacionado. O número de animais foi variável para manter 30 ± 5 cm de altura do pasto pós-pastejo. O índice de nutrição com nitrogênio aumentou linearmente de acordo com os níveis de N ($\hat{Y} = 59{,}8 + 0{,}1216N$; $P < 0{,}0001$; $r^2 = 0{,}53$). A taxa de aparecimento de folhas ajustou-se ao modelo de regressão linear positiva de acordo com a soma térmica (TS) com zero de N ($\hat{Y}0N = 0{,}0077 + 0{,}0000087TS$; $P = 0{,}0308$; $r^2 = 0{,}72$) e 150 kg ha^{-1} de N ($\hat{Y}150N = 0{,}0020 + 0{,}000021\ TS$; $P = 0{,}0022$; $r^2 = 0{,}92$). O uso de 300 kg ha^{-1} de N não alterou a taxa de aparecimento de folhas (0,0124 folha grau-dia^{-1}). O uso de até 300 kg ha^{-1} de N aumenta o teor de nitrogênio do papuã. A taxa de aparecimento de folhas em papuã é modificada usando N, enquanto que as características morfogênicas expansão foliar, expansão de colmo, filocrono, duração de vida das folhas, duração da elongação foliar e a estrutura dos perfilhos não são alteradas pela utilização de N.

Palavras-chave: Aparecimento foliar, expansão foliar, filocrono, fertilização nitrogenada, Urochloa plantaginea.

INTRODUCTION

The livestock activity in Brazil is based on the use of pastures as the main food resource, with the predominance of tropical forage species, especially the genus *Urochloa* (JANK et al., 2011). In the southern region, in the summer period, some areas used for livestock farming have a good coverage with Alexandergrass (*Urochloa plantaginea* (Link) Hitch). The species presents itself spontaneously and, due to its tillering potential, forage accumulation (RESTLE et al., 2002; SARTOR et al., 2011; COSTA et al., 2011) and good adaptation to the soil and climate conditions of the southern region (BERGOLI et al., 2019) has been used as forage in pastoral systems (SOARES et al., 2020). Alexandergrass allows the adequate conversion into animal product, enabling the mating of beef heifers from 15 months of age (COSTA et al., 2011; ELOY et al., 2014, SALVADOR et al., 2014), average daily gain above 0.6 kg animal^{-1} day^{-1} in beef heifers (COSTA et al., 2011; SALVADOR et al., 2014; NEGRINI et al., 2018) and stocking rate of 1.600 to 2.400 kg ha^{-1} of body weight (RESTLE et al., 2002; NEGRINI et al., 2018).

In this context, the adoption of management strategies maximized the production of pastoral systems is extremely important. The response of forage grasses to nitrogen (N) fertilization is difficult to

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predict due to soil capacity to supply nitrogen and the variable demand of plants for this nutrient. The greater availability of N can increase its concentration in plant tissues, modify morphogenesis and; consequently, the tillers structure. This structural modification can result in a greater amount of sward leaf blades with an increased photosynthetic rate, in addition to facilitating the process of bite formation by the herbivore. The use of N in pastures, without elevating production costs, must be accompanied by practices that reduce the environmental impact and preserve the quality of the food produced (LEMAIRE et al., 2008).

The effect of nitrogen fertilization on morphogenic characteristics is variable depending on the grass species. The low level of nitrogen nutrition of plants can reduce forage production due to lesser leaf expansion and; consequently, determine a negative impact on animal productivity by area. Accompanying the increase in growth rate and forage production, a greater pasture stocking rate result and a greater efficiency of forage use happens due to the shorter time interval between two consecutive defoliations in the same leaf blade (GASTAL & LEMAIRE, 2015).

Studies with nitrogen fertilization in Alexandergrass (*Urochloa plantaginea* (Link) Hitch) have been carried out in the southern region of Brazil (SARTOR et al., 2014; SALVADOR et al., 2016; HUNDERTMARCK et al., 2017). However, the literature shows no data about the hypothesis that the morphogenic response of this grass may be altered by the use of N. The understanding of these relationships, in the context of individual tillers, can provide more detailed information and support decisions on the management of N fertilization. These decisions should avoid recommendations for utilization of N levels higher than the response capacity of the plants. The study of the N effect on the plant based on individual tillers measurements belongs to the so-called short-term knowledge whereas in the long term the response must be measured at the canopy level through the biomass flows (LEMAIRE & CHAPMAN, 1996).

Within this context, the objective was to evaluate the effect of nitrogen fertilization on the morphogenic characteristics, tiller structure and nitrogen nutrition index of Alexandergrass under rotational stocking grazing method.

MATERIALS AND METHODS

Experimental sites

The experiment was approved by the Ethics Committee for Animal Experimentation of Universidade Federal de Santa Maria (UFSM), Protocol 138/2014. The experiment was performed from January to March 2014 out at UFSM-RS at 29º43’S and 53º42’W, Santa Maria – RS, Brazil. The climate of the region is subtropical humid, according to the Köppen classification and the soil is classified as a Paleudalf (SANTOS et al., 2018). Average soil chemical characteristics for the 0–20 cm layer were: pH-H_2O: 5.8; % clay: 21 m V^{-1}; P: 14.4 mgL^{-1}; K: 128.3 mg L^{-1}; 2.5 % of organic matter; Al^{3+}: 0.7 cmol L^{-1}; Ca^{+2}: 5.3 cmol L^{-1}; Mg^{+2}: 2.5 cmol L^{-1}; CTC pH7: 9.2. Meteorological data of the trial period were obtained from the UFSM Meteorological Station (Figure 1).

Field experimentation

The experimental area, divided into six experimental units (paddocks), comprised 4.8 ha. Each paddock was subdivided into four plots of 0.2 ha. Alexandergrass (*Urochloa plantaginea* (Link) Hitch) pasture comes from by an existing seed bank in the area. There is no commercial cultivar available for commercialization. Fertilization consisted of 90 and 60 kg ha^{-1} of P_2O_5 and K_2O, respectively. Fertilization was carried out to produce 10.000 kg of dry matter. A mowing canopy height homogenization was performed on January 8th, 2014.

The grazing method was rotational stocking. The criterion to determine the rest period was 210 degree-days (DD) (ELOY et al., 2014). To calculate the rest period, in days, the historical mean temperatures were utilized. The grazing period plus the interval until the beginning of the next occupation constituted one grazing cycle. The grazing cycles lasted 16 days (4 days graze, 12 days rest). Thermal sum (TS) was calculated by the equation: $TS = \Sigma(Tmd) - 10$, where: Tmd is the daily average of the cycle; the value of 10 grade is the minimum temperature required for growth of warm season forage species. The grazing cycles and the accumulated thermal sum at the end of each cycle were: 1- Jan./21 to Feb./ 05 (297 DD); 2- Feb./06 to Feb./21 (563 DD); 3- Feb./22 to March/09 (772 DD); 4- March/10 to March/25 (1104 DD).

The treatments were Zero, 150 or 300 kg ha^{-1} of nitrogen (N) in urea form (46% N). The total amount of N was split into three levels of similar amount, the first applied on January 8th, and the others on February 10th and February 26th.

Evaluation of parameters

Sixteen Angus heifers with initial age and body weight (BW) of 15 months and 276.0 ± 17 kg, respectively, were the test animals utilized. Canopy height was measured through readings at 30

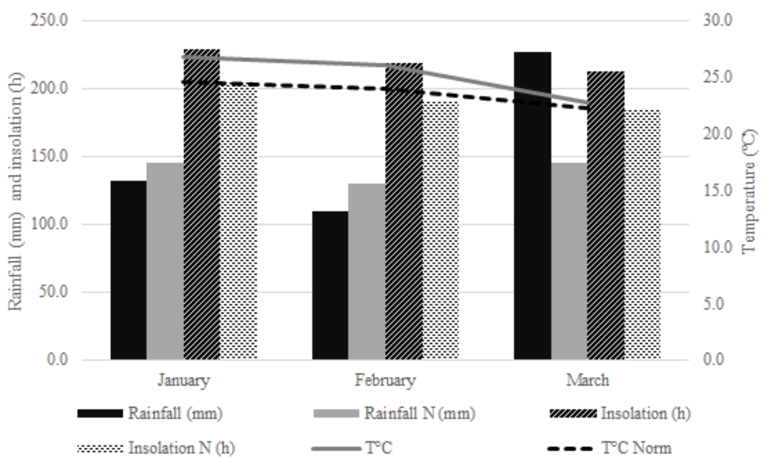

Figure 1 - Mean temperature, rainfall and insolation during the experimental period and historical means, Santa Maria/RS, Brazil.

Source: UFSM Meteorological Station.

points at pre- and post-grazing. For the maintenance of post-grazing canopy height at 30 ± 5 cm, 22 regulator animals were used. The stocking rate (kg BW ha^{-1}) was calculated by the sum of test-heifer average weight with the average weight of each regulator animal, multiplied by the number of days which they remained in each replication, divided by the number of days of the experimental cycle. The number of animals ha^{-1} in each paddock was obtained by dividing the stocking rate and the average weight of the test heifers.

Every grazing cycle, before the entrance of heifers in the paddock, two forage samples were taken (0.25 m²) at the ground level. Forage from these samples was manually separated in leaf blades and stems to determine their mass (kg ha^{-1} DM).

N concentration (%) in leaf blades and stems was determined by laboratory analysis. The nitrogen nutrition index (INN) was calculated according to the equation (LEMAIRE et al. 1989): $INN = (100 * N\%) / (3.6 * DM^{-0.36})$. Where: DM (t ha^{-1}) = sum of leaf and stem biomass; N% = N concentration in leaf and stems biomass calculated using a weighted average.

Morphogenetic and structural characteristics were evaluated in 20 selected tillers per experimental unit according the ‘marked tiller’ technic (CARRÈRE et al., 1997). These tillers were measured daily during grazing period and twice a week during the interval between grazing. Every new grazing cycle, a new group of tillers was tagged for evaluation. On these tillers, leaves were measured (cm) and classified as: expanded leaves (visible ligule), expanding leaves (no visible ligule), senescent leaves (only green part, from the ligule to the point where the senescent process had advanced was measured). Leaves with more than 50% of the leaf blade senescent were considered dead leaves. The stem length was measured from the ground level to the ligule of the last expanded leaf.

Foliar leaf elongation, stem elongation and senescence rates, in cm degree day^{-1}, were calculated by the ratio between the leaf elongation, stem elongation or mean tiller senescence between two consecutive evaluations and the accumulated thermal sum in the same period.

Phyllochron (degree-days) was calculated in the period between grazing as the thermal sum between the appearance of two consecutive leaves. Leaf appearance rate (leaf degree-days^{-1}) was calculated from the slope of the linear regression between the number of leaves per tiller and the thermal accumulation of the period. The leaf lifespan was calculated by the product of phyllocron and the number of green leaves per tiller. The duration of leaf elongation was obtained through the products of phyllochron and the average number of leaves in expansion per tiller.

Upon defoliation, the leaf blades were marked with a marker pen to identify new grazing.

The intensity of defoliation (ID; % removed from the leaf blade) was estimated as: ID = [(initial length-final length)/initial length]. The defoliation interval (DI; days to return to the same leaf) was obtained by the equation: DI=1/ (nº of grazed leaves /nº of tiller´s leaves x number of days). To calculate the percentage of the area grazed daily by heifers, one hectare was considered as 100% of the grazed area. The area grazed daily by each animal was obtained considering the paddock area as 100% and dividing this value by the defoliation interval (days).

Statistical analyses

A completely randomized design following a repeated measure arrangement (thermal accumulation) was used, three N levels and two area replications for treatments. After testing normality of data distribution, the variables were analyzed using the Mixed procedure of the statistical package SAS®. We performed a structure selection test, following the Bayesian Information Criterion (BIC) to determine the model that best fit the data. The interaction treatment x thermal sum was evaluated at 5% of probability. In regression analyses, the model was selected based on the significant level of linear and quadratic coefficients, using the Student´s t-test at the 5% significance level. The variable responses were modeled according N levels and thermal sum observed in the period.

The general mathematical model refering to the analysis of variance was represented by: Yijk= μ + Ti + ek:I + Pj + (T^*Pij) + kijk, where: μ = mean of all observations; Ti= is the fixed effects of treatments; ek:i= is the random effect of the paddock nested in the treatments (error a); Pj= is the fixed effect of termal sum; T^*Pij= interaction treatments*termal sum; kijk= residual experimental error (error b).

RESULTS

The post-grazing canopy height was 31.1 ± 2 cm, similar ($P = 0.4696$) in all paddocks used to evaluate the N levels. The meteorological data from the experimental period (January to March 2014) showed average monthly values of temperature (25.2 °C), insolation (220.2 hours) and rainfall (156.0 mm) (Figure 1).

There was no interaction between nitrogen levels (N) × thermal sum (TS) for the number of animals ha^{-1} and this variable increased linearly as a function of N levels ($\hat{Y} = 7.3 + 0.0084N$; $P = 0.0145$; CV = 20%; $r^2 = 0.27$). The number of animals ha^{-1} was adjusted to the quadratic regression model as a function of the TS ($\hat{Y} = 2.5 + 0.0188ST - 0.000012TS^2$; $P = 0.0379$; CV = 21%; $r^2 = 0.25$).

There was interaction between N levels × thermal sum for pre-grazing canopy height ($P = 0.0009$). Pre-grazing canopy height was adjusted to the quadratic regression model as a function of thermal sum when zero ($\hat{Y} = 27.1 + 0.0357TS - 0.000022TS^2$; $P = 0.0494$; $r^2 = 0.67$) and 150 kg ha^{-1} of N ($\hat{Y} = 46.7 + 0.0211TS - 0.000017TS^2$; $P = 0.0173$; $r^2 = 0.78$). In the paddocks where 300 kg ha^{-1} of N were used, the pre-grazing canopy height was on average 41.7 ± 3.7 cm ($P > 0.05$).

There was no interaction between N levels × thermal sum for leaf expansion rate (0.0456 ± 0.1 cm degree-days^{-1}) and stems (0.0408 ± 0.1 cm degree-days^{-1}) and these variables did not were influenced by N levels (Table 1). The stem expansion rate was not altered as a function of the thermal sum (Table 1). The leaf expansion rate adjusted to the negative linear regression model as a function of the thermal sum ($\hat{Y} = 0.0590 - 0.000028TS$; $P = 0.0008$; CV = 34%; $r^2 = 0.22$). The rate of leaf expansion was positively correlated with the nitrogen nutrition index of Alexandergrass ($r = 0.43$; $P = 0.0344$) (Figure 2).

There was interaction of N levels × thermal sum for leaf senescence and leaf appearance rate (Table 1). The leaf senescence rate was not changed as a function of the thermal sum when Alexandergrass was fertilized with 0 (0.0474 ± 0.01 cm degree-days^{-1}) or 150 kg ha^{-1} of N (0.0492 ± 0.01 cm degree-days^{-1}). The use of 300 kg ha^{-1} of N linearly reduced the leaf senescence rate as a function of the thermal sum ($\hat{Y}300N = 0.0468 - 0.000021TS$; $P = 0.0139$; CV = 23%; $r^2 = 0.38$).

The leaf appearance rate was adjusted to the positive linear regression model as a function of the thermal sum with zero of N ($\hat{Y}0N = 0.0077 + 0.0000087TS$; $P = 0.0308$; CV = 10%; $r^2 = 0.72$) and 150 kg ha^{-1} of N ($\hat{Y}150N = 0.0020 + 0.000021TS$; $P = 0.0022$; CV = 10%; $r^2 = 0.92$). With the use of 300 kg ha^{-1} of N, the leaf appearance rate (0.0124 leaves degree-days^{-1}) did not vary according to the thermal sum.

There was no interaction of N levels × thermal sum for phyllochron (90.6 ± 18 degree-days;) and leaf lifespan (362.2 ± 84 degree-days) (Table 1) and for leaf elongation duration (153.9 ± 27 degree-days) and these variables were not altered by the N levels. The phyllochron adjusted to the negative linear regression model as a function of the thermal sum ($\hat{Y} = 132.2 - 0.0764TS$; $P < 0.0001$; CV = 11%; $r^2 = 0.69$). The leaf lifespan ($\hat{Y} = 517.0 - 0.028TS$; $P = 0.0025$; CV = 18%; $r^2 = 0.44$) and the duration of

Table 1 - Morphogenetic characteristics of Alexandergrass according N levels and thermal sum (TS).

N Level^{1}	Leaf expansion^{2}	Stem expansion^{2}	Leaf Senescence^{2}	Leaf Appereance^{3}	Phyllochron^{4}	Leaf Lifespan^{4}
0	0.0396	0.0353	0.0474	0.0474	94.5	385.8
150	0.0489	0.0432	0.0492	0.0492	87.2	321.1
300	0.0468	0.0438	0.0404	0.0404	90.0	379.7
-----Thermal Sum^{3}-----						
297	0.0598	0.0354	0.0442	0.0097	110.7	439.9
563	0.0401	0.0362	0.0377	0.0133	86.4	339.8
772	0.0461	0.0464	0.0585	0.0155	74.7	306.8
1104	0.0277	0.0470	0.0442	-	-	-
SD	0.01	0.01	0.01	0.002	18.0	84.0
-----(P=)-----						
N*TS	ns	ns	<0.0001	0.0240	ns	ns
N Levels	ns	ns			ns	ns
Thermal Sum	0.0008	ns			ns	ns

^{1}kg ha^{-1} of N; ^{2}cm degree-day^{-1}; ^{3}leaves degree-day^{-1}; degree-day^{4}; SD= standard deviation; (P=)- significance of effect; ns= non-significant ($P > 0.05$).

leaf elongation ($\hat{Y} = 208.9 – 0.100ST$; $P = 0.0005$; CV = 12%; $r^2 = 0.54$) decreased linearly as a function of the thermal sum.

There was no interaction between N levels × thermal sum for the intensity and defoliation interval ($P > 0.05$). Defoliation intensity (66.5 ± 10%) adjusted to the quadratic regression model as a function of the thermal sum ($\hat{Y} = 0.00718 + 0.2383ST - 0.00019ST^2$; $P = 0.0088$; CV = 10%; $r^2 = 0.63$). The defoliation interval (1.69 ± 0.2 days) was not altered by the levels of N and the thermal sum ($P > 0.05$).

There was no interaction between N levels × thermal sum for the length of the expanded leaf blades (17.5 ± 3 cm; Table 2) and stem length (25.2 ± 5 cm; Table 2) and these variables were not altered by the N levels ($P > 0.05$). The length of the expanded leaf blades adjusted to the quadratic regression model as a function of the thermal sum ($\hat{Y} = 11.5 + 0.034TS - 0.000031TS^2$; $P = 0.0014$; CV = 17%; $r^2 = 0.64$). There was a positive correlation between leaf blade length and nitrogen nutrition index ($r = 0.49$; $P = 0.0156$) (Figure 2). The stem length was adjusted to the quadratic regression model as a function of the thermal sum ($\hat{Y} = 8.7 + 0.0526TS - 0.000038ST2$; $P = 0.0098$; CV = 20%; $r^2 = 0.28$).

DISCUSSION

Meteorological data showed that, during the experimental period, the average temperature was 1.6 degrees higher to the historical average (23.6 °C). The rainfall exceeded the expected precipitation by 9.6% (13.7 mm). The mean insulation was 13.7% (26.6 hours) higher than the historical average insulation (Figure 1). According to these meteorological data, climatic conditions were adequate for the development of plants.

According to increased availability of external nitrogen (N), ranging from 0 to 300 kg ha^{-1}, a linear increase in the nitrogen nutrition index (INN) of Alexandergrass was observed. In the system with zero N, the N made available to plants from the soil mineral N added to N from organic matter mineralization was not enough to reach the same INN as the use of 150 or 300 kg ha^{-1} of N. According to the INN regression model as a function of N levels, it would be necessary to use 166.1 kg ha^{-1} of N so that the INN value would be raised to the minimum value of 80. When Nitrogen nutrition index values are between 80–100, they are considered satisfactory and there is no limitation of this nutrient for plant growth (LEMAIRE et al., 1989). These same authors consider INN values below 60 to be very low, between 60 and 80 inadequate, and above 100 indicate an excess of N. The average INN observed when using zero and 150 kg ha^{-1} of N was 64 and 69, respectively, characterizing as values very low, and only the use of 300 kg ha^{-1} of N made INN be considered satisfactory.

The N levels resulted in different pre-grazing canopy heights. In the paddocks without nitrogen fertilization, a highest pre-grazing canopy height (41.5 cm) was observed when the thermal sum

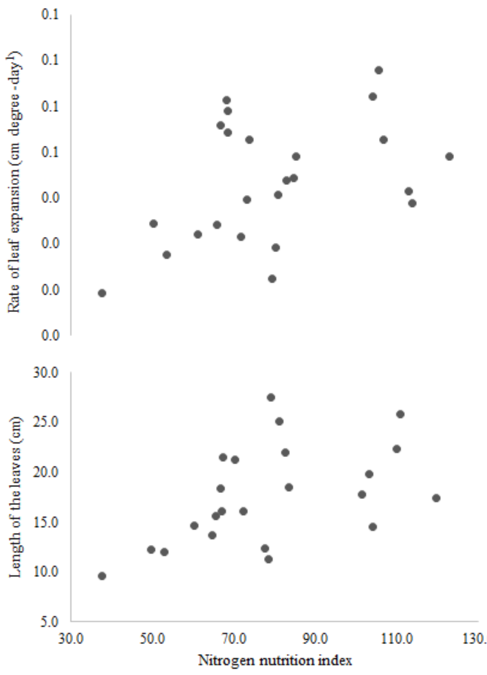

Figure 2- Values of leaf expansion, leaf length and nitrogen nutrition index of Alexandergrass.

was 811.3 DD (degree day). When 150 kg ha^{-1} of N was used, the pre-grazing canopy height showed the lowest value (40.1 cm) at 620.5 DD. In the paddocks fertilized with 300 kg ha^{-1} of N, the pre-grazing canopy height (41.4 cm) was not influenced by the thermal sum.

The highest INN value (90.7) was observed when the thermal sum (TS) was 625 degree-days. Considering the average temperature observed, this thermal accumulation occurred at 41 days of pasture use, nine days after the last nitrogen fertilization (1/3 of the total amount). The INN value reduction after the thermal accumulation of 625 degrees-day, can be explained by the N remobilization from leaf blades located in the more shaded strata of the canopy and a great presence of structural plant tissues (LEMAIRE et al., 2008). This hypothesis can be confirmed by regressions that show a reduction in the leaf expansion rate and maintenance of stem expansion rate as a function of TS.

The absence of N levels effect on leaf and stem expansion rates is not the usually reported answer when studies involve these two factors. These responses showed that, generally, as the nitrogen fertilization increase, a higher expansion rate of these components is observed (HUNDERTMARCK et al., 2017; TIECHER et al., 2017). Observations showed; however, a similar rate of leaves and stems expansion in the absence or presence of N fertilization. One possible cause of the absence of an N effect on leaves and stems expansion rates is the amount of light radiation intercepted being a limitation for the greater photosynthetic rate of the plants. In conditions where N quantity is not limiting, competition for light becomes more important to determine the rate of expansion of the plant’s organs (IRVING, 2015).

The N effect on the grasses morphogenetic traits is determined according to their growth habit, which can result in a stolon higher rate of expansion (stoloniferous species) or leaf blades expansion (tufted species) (CRUZ & BOVAL, 2000). Alexandergrass has a decumbent growth habit during the beginning of its development, similar to stoloniferous species. After the establishment period, with the increase of the soil cover, its growth habit becomes tufted and this induces different N responses in these two phases.

The leaf expansion rate reduction of 0.000028 cm degree-days^{-1} per accumulated degree-day can be explained by Alexandergrass being a tropical climate species, which response to the temperature lowering and reduced daily light is the reduced leaf expansion (HUNDERTMARCK et al., 2017). Also, the approximation of the reproductive stage of Alexandergrass may have provided a greater nutrients allocation to the reproductive organs, in detriment of the leaf blades production.

The similarity observed in the stem expansion rate, probably can be better explained by the pre-defined interval between grazing (210 DD) than by the use of N. This grazing interval did not promote an increase in the expansion rate of this component, not allowing different canopy structures due to the use of N.

This interval induced a stems expansion of 6.1 cm, and may not have triggered a photomorphogenic response, that would increase the elongation of the stems (CHORY, 1997). Probably, a reduction in the interval between grazing could accentuate the N effect on the pre-grazing canopy height, altering the Alexandergrass canopy structure.

There is an advantage due to stem length similarity in all N levels, as it prevents the greater stems accumulation in the forage mass. In addition, stems act as a physical barrier to bite formation (BENVENUTT et al., 2006) and can increase the participation of dead and senescent material in the forage mass (SALVADOR et al., 2016). According

Table 2 - Nitrogen (N) nutrition index and structural characteristics of Alexandergrass according N levels and thermal sum (TS).

N Level[1]	N nutrition Index	Leaf Lenght[2]	Stem Lenght[2]
0	64.0	17.0	24.5
150	69.7	18.0	25.7
300	100.5	17.6	25.6
----------Thermal Sum[3]----------			
297	79.3	18.8	20.2
563	88.9	23.9	30.4
772	84.2	16.2	25.2
1104	60.0	11.8	23.3
SD	17.6	3.9	5.5
----------(P=)----------			
N*TS	ns	ns	ns
N Levels	<0.0001	ns	ns
Thermal Sum	0.0438	0.0014	0.0098

[1]kg ha^{-1} of N; cm[2]; degree-day[3]; SD= standard deviation; (P=)- significance of effect; ns= non-significant (P > 0.05).

to the quadratic regression model, the stems showed greater length (26.9 cm) when the thermal sum was 692 degrees-day, when tillers were reaching the reproductive stage through the externalization of its reproductive organs.

The negative effect of N utilization on the leaf senescence rate, regardless of the level used, indicates alteration of the N remobilization strategy when the availability of this nutrient is modified. The greater availability of N may have altered the balance between N remobilization and N absorption by the roots, reducing leaf senescence. With 300 kg ha^{-1} of N, the remobilization of N from the older leaves to the expanding leaves may have been reduced (LEMAIRE & CHAPMAN, 1996), and making these tillers became more independent of N from the older tissues.

The similarity observed in the leaf senescence rate in tillers fertilized with zero or 150 kg ha^{-1} of N may have occurred due to the fact that even at the lowest levels of nitrogen fertilization there was enough N available in the soil so that it was not necessary to increase the remobilization of N to maintain the same leaf expansion rates. ADAMI et al. (2010) observed that N remobilization is greater in grasses that receive low levels of nitrogen fertilization.

The interaction between the N levels × thermal sum for the rate of leaf appearance is not commonly reported in the literature. The leaf appearance rate is temperature dependent and little affected by the variation in N availability (CRUZ & BOVAL, 2000). In response to use of 150 kg ha^{-1} of N, the leaf appearance rate increased in greater magnitude, with 0.00002 leaf degree-days^{-1} per accumulated degree-day. The linear increase observed in the leaf appearance rate as a function of the thermal sum at the levels of zero (0.0000087 leaf degree-days^{-1} degree-day^{-1}) and 150 kg ha^{-1} of N (0.000021 leaf degree-days^{-1} degree -days^{-1}) may have occurred due to the acceleration in the emission of new leaves with the elevation of the apical meristem.

The increase in the speed of leaf blade emission with the use of 150 kg ha^{-1} of N may signal that, at this level of fertilization, the tillers will anticipate its entry into the reproductive stage. From the management point of view, this is not advantageous, as stopping the emission of new leaf blades reduces the period of pasture utilization. However, when 300 kg ha^{-1} of N was used, the leaf appearance rate remained constant throughout the evaluation period, in accordance with the available bibliography (LEMAIRE & CHAPMAN, 1996).

The magnitude of the interaction between levels of N × thermal sum for leaf appearance rate was not sufficient to alter the phyllochron value of Alexandergrass. This similarity may have occurred due to the stocking rate management, which kept similar, in all paddocks, the post-grazing canopy height and stem length. The phyllochron value depends on the leaf sheath length of the previous leaf, which is directly influenced by the grazing intensity (CRUZ & BOVAL, 2000). The similarity observed in the length of the leaf elongation can be explained by the existent link between this characteristic and the phyllochron value (LEMAIRE & CHAPMAN, 1996).

The absence of N effect on the leaf lifespan may have occurred because this variable is genetically determined and is mainly influenced by temperature (POMPEU et al., 2009). The leaf lifespan is related to the efficiency of leaf blades utilization, which is defined by GASTAL & LEMAIRE (2015) as being the proportion of the leaf tissue produced by the plant that is harvested before starting the process of leaf senescence.

In order to maintain the same post-grazing canopy height in all paddocks, it was necessary to increase 0.0084 animals ha^{-1} for each additional kg of N. The highest number of animals ha^{-1} (9.9) was observed when the thermal sum was 783.3 degree-days. Considering that the average daily thermal accumulation was 15.2 degrees-day, the leaf blades remained alive for 23.8 days, they were grazed 2.4 times in each grazing cycle and 3.5 times while they remained alive. Dividing the duration of the grazing cycle (days) by the number of leaf blade defoliations during the cycle, it is observed that the interval between defoliation was 6.7 days, this value being similar to the values presented in the literature for Alexandergrass managed in continuous stocking method (SALVADOR et al., 2014; ELOY et al., 2014; HUNDERTMARCK et al., 2017). According to the defoliation interval, the animals used 60% of the available area for daily grazing, equivalent to 152.8 m² per heifer.

The observed value of defoliation intensity was higher than the 40-60% reported by GASTAL & LEMAIRE (2015), who consider these values constant and independent of the grazing method. However, this constancy was not observed during the forage cycle because, according to the quadratic regression model, the highest defoliation intensity (74.7%) occurred when the thermal sum was 627.1 degree-days. If leaf blades were defoliated 3.5 times and in each defoliation was removed 66.5% of the length, the harvest efficiency of leaf blades was 99.3%. This efficiency value is 26.3% higher than the value reported by MAZZANTI & LEMAIRE (1994) evaluating *Festuca arundinacea.* This lower harvest efficiency value can be explained by the lower defoliation interval (20.5 days) observed by the authors.

The lack of effect of N levels on the rate of expansion and duration of leaf expansion explains the similarity in the length of Alexandergrass leaves. For the same duration of leaf expansion, provided by the same stem length, greater leaf length would be expected if the leaf expansion rate were increased by the N levels. According to the quadratic regression model, the greatest leaf length (20.7 cm) was observed at 36 days of pasture use when the thermal sum was 548.3 degrees-days, close to the thermal sum value (692 degrees-day) when the stems had greater length. As a result, there was an increase in the leaf sheath length, which increases the leaf blade length (LEMAIRE & CHAPMAN, 1996).

The similarity observed in the expansion rate and length of the leaf blades is associated with the similar leaf blades mass of Alexandergrass in the different levels of N used. This evidence can confirm the hypothesis that the amount of light radiation intercepted by leaf blades may have limited the effect of N on expansion, length, and leaf blade mass.

The correlations (Figure 2) observed between the rate of leaf expansion and the length of the leaves with the nitrogen nutrition index are related to the proportionality between the uptake of N by the plant and its expansion in the leaf area index, both processes, for C4 species, occurring coordinated in a linear function (LEMAIRE et al., 2007).

The information obtained in the present study confirmed the potential response of Alexandergrass to nitrogen fertilization when the nutrient is applied during the vegetative stage. Under the conditions in which the study was conducted, only the fertilization level of 300 kg of N ha^{-1} allowed the Alexandergrass to have an adequate supply of N. Nitrogen fertilization levels did not promote changes in tiller structures. Understanding the relationships measured at the tiller level supports decisions about nitrogen fertilization management and can contribute to the understanding of plant-animal interactions in tropical grazing systems.

CONCLUSION

The use of up to 300 kg ha^{-1} of N increases the nitrogen nutrition index and reduces the rate of leaf senescence of Alexandergrass. The leaf appearance rate in Alexandergrass is modified using N while the morphogenic characteristics leaf expansion, stem expansion, phyllochron, leaf lifespan, leaf elongation duration and tiller structure are not altered by N utilization.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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