Critical Nitrogen and Nutritive Index in Cultivars of *Cenchrus purpureus* (Schumach) Morrone

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ABSTRACT

Objective: To determine and compare the parameters of the nitrogen curve and the nutritive nitrogen index of cultivars of *Cenchrus purpureus* with and without nitrogenous fertilization.

Design/Methodology/Approach: Five cultivars of *C. purpureus* with or without fertilization were evaluated, and the dose was 141N-43P-20K. The grass was harvested every 21 d with production of dry matter (DM) and nitrogen (N). Growth rates and Critical Nitrogen (Nc) were obtained. The negative power equation with its parameters Nc and the Nitrogen Decrease Coefficient (Cd) were used. The Nutritive Nitrogen Index (NNI) was obtained with the Nc parameter of each curve, divided by the N content of the cultivar in each sampling. The data were analyzed with a completely randomized design in a factorial arrangement of five (cultivars) and two levels of fertilization.

Results: Nc of 2.6 and a Cd of 0.65 fertilized, and 1.14 of Nc and 0.68 of Cd without fertilization, and in turn all of them supplemented the amount of N for their growth from soil. The NNI was 0.8 for the fertilized cultivars, with classification of excess and sufficient for the first 56 d.

Study Limitations/Implications: Variables to determine the availability of water and the balance of soil-plant N could further expand the knowledge of these tools.

Findings/Conclusions: Parameters obtained such as Nc and NNI help to determine nutritional status of the fodders.

Keywords: Nitrogenous Fertilization, Tropical Fodders, Model Curves.

INTRODUCTION

The genus *Pennisetum* (Poaceae) has about 140 species, most of them native to Africa and Central and South America. However, species of *Pennisetum* may be found in any country in the tropics and subtropics. It was introduced to Mexico in 1950 (Ramos-Sánchez, 1985), to the state of Veracruz, where it was introduced together with a wide collection of Poaceae for evaluation and selection; one of them was the *Pennisetum merkeri*, a product of

that evaluation. It was recommended for use as cut fodder and released as Zacate Merkeron, which spread throughout the country's southeast. Mexico has a broad diversity of environments, in which the production of perennial Poaceae is highly feasible. These offer high potential for biomass production per surface unit, since it has been documented that the intensely fertilized species *P. purpureum* (Schumach), syn. *Cenchrus purpureus* (Schumach) Morrone, Chemisquy *et al*. (2010), produces large amounts of biomass that can exceed 50 t of dry matter ha^{-1} and year (Calzada *et al*., 2014; Rueda *et al*., 2016; Reyes-Castro *et al*., 2018). Given that the species *C. purpureus* has a wide potential for biomass production, its preferential use is for cattle feed, whether in grazing, cut, or else as fodder ensilage. In recent years, various cultivars of *C. purpureus* have been introduced to Mexico, from different origin and with scarce technical information, such as: Taiwan, King Grass, CT-115, CT-169, Maralfalfa, Mott, Vruckwona, OM-22, Roxo or Purple, and Clone 69, among others. However, these new cultivars are unknown regarding their management, particularly fertilization, and therefore, they require high amounts of nutrients, specifically nitrogen. Nitrogen (N) is essential for crop growth (Tillman *et al*., 2002) and is considered a limiting factor after water for forage production in meat and dairy production systems in the tropics. The practice of fertilization provides enough N to reach the potential allowed in appropriate climatic conditions, so that the producers apply amounts that are high or less than that required by the plant to obtain the maximum forage production to ensure that this potential is reached every year, although it is known that excess N reduces the efficiency and can cause environmental problems (Jeuffroy and Meynard, 1997). The management of N in soil and in fertilizer is an important point for the profitability and sustainability of extensive meat and dairy production systems in the tropics, and for this purpose, methods are required that estimate the critical concentration of N in the fodders, in order to adjust the dose of N and the frequency of applications according to their growth potential. Authors such as Justes *et al*. (1994) developed the methodology to know the nitrogen status during the growth and production of biomass considering the term critical nitrogen (Nc), defined as the minimum concentration of nitrogen required to obtain the maximum growth rate, expressed as g N per 100 g in relation to the accumulated dry matter (DM) (t DM ha^{-1}). This curve is a negative power function that is described as follows:

$$\%N = a\,DM^{-b}$$

Both are specific physiological coefficients where: *a*, is the concentration of *%N* in the plant when the fodder produces a t DM ha^{-1} and *b*, is the concentration of Nitrogen in the plant when it decreases.

Derived from the previous function, the nutritional status of the fodders is defined and the term Nutritional Nitrogen Index is obtained by its acronym NNI (Colnenne *et al*., 1998), which can be used as a basis to make decisions about the need to fertilize with N and its frequency in applications (Lemaire *et al*., 2008). It is described next:

$$NNI = N/Nc$$

where *N* is the current nitrogen concentration of the fodder in percentage of DM and *Nc* is the critical nitrogen calculated with the negative power function equation.

For N management in fodders, the NNI threshold has been defined as follows: Values greater than 1 indicate that N is in excess, values between 1 and 0.8 indicate that the nutrition is satisfactory, and values below 0.8 indicate N deficiency, so growth and production are limited (Duru *et al*., 1997). It has been observed that lower concentrations of N than critical nitrogen (Nc) indicate that growth is restricted by a deficit of N, while higher values of N than Nc indicate that the contribution of N is in excess (Greenwood *et al*., 1990). Forage production is related to NNI (Lemaire and Gastal, 1997; Ziadi *et al*., 2008; Agnusdei *et al*., 2010). The concept of Nc and NNI has been confirmed to be an effective tool for agronomic analysis and interpretation (Lemaire *et al*., 1997; Lemaire and Meynard, 1997; Gonzalez-Dugo *et al*., 2005). The objective was to determine and compare the parameters of the nitrogen curve and the nutritive nitrogen index of cultivars of *C. purpureus* with and without nitrogenous fertilization, to know the nitrogen status in the fodder.

MATERIALS AND METHODS

The study was carried out in the experimental field "La Posta" in Paso del Toro, Veracruz, located at coordinates 19° 00' 49'' N and 96° 08' 19'' W at 12 m altitude (INEGI, 2009). The area's climate corresponds to Aw$_2$ sub-humid tropical (Vidal Zepeda, 2005), with rainfall of 1461 mm, and relative humidity of 77.4%. The average, maximum and minimum temperatures are 25, 35

and 15 °C. The soils are Vertisol, acidic pH (5.4), clay-sandy crumb texture and with organic matter content of 2.6%.

Treatment Design
The evaluated cultivars were: CT-115, Elefante, King-grass, Roxo and Taiwan, all with and without nitrogenous fertilization; fodder samples were carried out every 21 d for six months in the rainy season. The land was prepared with subsoiling, fallow, double raking and plowing at 80 cm. Two cuttings or stakes of three nodes were used, which were planted every 80 cm on the embankment of the furrow, ensuring that two of the three nodes were buried at a 45° angle in relation to the surface. The establishment period was July-August. The sampling area at each cutting date was 5.76 m², equivalent to three rows, 2.4 m wide by 2.4 m long, and eight cuts were made.

At the beginning of the experiment, a uniformity cut was made at 25 cm. The fertilization dose was 141N-43P-20K (N200 kg of Urea (46%), 50 kg of the mixture 18N-46P-00K, and K200 kg of the mixture 20N-10P-10K) which was dosed in two applications; in the first, eight days after the uniformity cut, the composition was 100 kg of Urea, 25 kg of the 18-46-00 mixture and 100 kg of the 20-10-10 mixture; in the second, 60 d after the first application, the rest of the nitrogen, phosphorus and potassium were added. The fodder (green matter) was harvested at a height of 25 cm from the ground, and every 21 d of growth.

During sampling, dry matter (DM) production was recorded of the complete plant in each plot, from which a subsample (300 g) was taken to dry at 55 °C until reaching constant weight using a forced air oven, and later it was ground. With the registered weights the crop's growth curves and rates were obtained. The amount of nitrogen in the fodder was determined by the Kjeldhal method, in samples of 0.5 g of complete plants, in the Animal Nutrition Laboratory of CIRGOC-INIFAP, in Campo La Posta, Veracruz. The first cut was made at 14 days of growth and then every 21 d, until 161 d, in the unfertilized plots every 21 d until 168 d of age.

Response Variables
The Nc parameters of the different cultivars of *C. purpureus* were obtained with a non-linear regression analysis using the equation (Justes *et al.*, 1994):

$$\%N = a\ DMP^{-b}$$

where $\%N =$ is the percentage of nitrogen in the total plant in DM; $a =$ is the critical nitrogen parameter, expressed in g N 100 g of DM; $b =$ is the parameter that indicates the dilution of nitrogen as the fodders grow and $PDM =$ is the dry matter production of the fodders at each point or sampling time, and these in turn were adjusted with the Gompertz model to obtain the estimated data in t MS ha^{-1}.

The observed N percentages of each time in the fodder's growth were adjusted with the Levenberg-Marquart algorithm, and the Kaleidagraph® v3.0 software was used. The NNI of the fodders at each time of growth was determined for each repetition, dividing the amount of nitrogen observed at each time of growth or sampling and the parameter of Nc determined with the equation defined above.

The growth rate was calculated in kg DM ha^{-1} d^{-1}, with the DM production data estimated with the Gompertz model in the fodder's ages, with the following equation:

$$TC = \frac{MSP_{t2} - MSP_{t1}}{T2 - T1}$$

where $TC =$ Growth rate in kg DM ha^{-1} d^{-1}, $MSP_{t2} =$ Dry matter present in time 2, kg DM ha^{-1}, $MSP_{t1} =$ Dry matter present in time 1, kg DM ha^{-1}, $T2 =$ end time, $T1 =$ initial time.

Experimental Design and Statistical Analysis
The parameters obtained from the nitrogen curves of each fodder by repetition a = Nc and b = Cd, as well as the coefficient of adjustment, were analyzed in an ANOVA in a completely randomized design in a factorial arrangement of 5×2:

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + \varepsilon_{ijk}$$

where factor A was the five fodders, factor B the application or not of nitrogen fertilizer, and AB the interaction. The data were analyzed with the MINITAB software version 17 in the GLM procedure. The means comparison was carried out with Tukey's test at an alpha of 0.05%.

RESULTS AND DISCUSSION

Table 1 shows the results of the data adjustment of the nitrogen percentages in the plant and DM production, during growth every 21 d and up to 168 d of age of the *C. purpureus* cultivars, with the negative power function equation of the parameters Nc, Cd and R^2. The Nc values did not show a difference (P≥0.05) between the studied cultivars; these are found in an interval between 1.85 and 2.22 g of N per 100 g DM, necessary to produce 1.0 t DM ha^{-1}. In relation to the coefficients of N, no differences (P≥0.05) were found between the cultivars; however, CT-115 and King Grass showed high negative rates of nitrogen dilution through growth with 73.5%, while the Roxo cultivar presented negative rates of less than 57.6%, that is, its decrease is very fast, which indicates that the percentage of N decreases drastically in these fodders. Nc values of 2.5 and 2.78 g of N per 100 g DM have been reported for *Brachiaria humidicola* and *Brachiaria humidicola* × *Brachiaria ruziziensis* (Juárez, 2005). In this regard, Duru *et al.* (1997) determined that the Nc for tropical fodders under non-limiting conditions of water and nitrogen is 3.6 g of N per 100 g DM and a nitrogen coefficient of 34%; in turn, Lemaire and Salette (1984 a, b) and Lemaire *et al.* (1985) demonstrated that tropical fodders decrease Nc as DM production increases.

Nitrogenous Fertilization Effect

Table 2 shows the effect of fertilization on the parameters of the nitrogen curve in *C. purpureus* cultivars, which presented differences (P≤0.05), for Nc, but not for Cd. Nc values of 2.63 g N per 100 g DM represent the minimum amount necessary to produce one t DM ha^{-1} for fodders that were fertilized with doses of 141 kg of N ha^{-1}, whereas for unfertilized fodders, it was 1.42. This decrease is possibly due to the fact that the organic matter content in the soil was not sufficient and would be limited by the lack of water. Juárez *et al.* (2004) reported a concentration of Nc of 2.9 with a fertilization dose of 200 kg of N ha^{-1} and 2.6 for the unfertilized, for species of *Brachiaria* sp., in rainfed conditions and soil rich in organic matter.

Growth Rate and Nitrogen Curves of Cultivars

Figures 1, 2, 3, 4, and 5 show the growth rate behavior and the nitrogen curve of cultivars *C. purpureus* with and without fertilization.

Cultivar CT-115

It is observed that the effect of nitrogenous fertilization on the maximum growth rate was 112 kg DM ha^{-1} day^{-1} and was reached at 35 d of age, with the production of 3,920 kg DM (Figure 1). In addition, with the adjustment of the nitrogen curve, an Nc content of 2.62 g of N per 100 g DM necessary to produce 1.0 t of DM ha^{-1} was obtained, for a DM production interval of 1 to 8 t of DM ha^{-1} (Figure 1). On the contrary, without fertilization, this cultivar showed a growth rate three times lower, of 44 kg DM d^{-1}, presenting its peak at 42 d of age and requiring 1.54 g of N per 100 g DM ha^{-1} day^{-1} to produce only 1,800 kg DM ha^{-1} at 42 d. In other words, this cultivar showed rapid growth by only providing 65% of the nitrogenous fertilization at the beginning, plus the soil content, requiring 102 kg of N ha^{-1} to produce 4 t of DM, with an efficiency of 72 % of the fertilizer; this suggests that the second portion of the nitrogenous fertilization (35% remaining) had no effect on the maximum growth rate, possibly due to the efficiency resulting from the effect of the cultivar. In this case, a fertilization frequency appropriate to its growth is suggested.

Table 1. Effect of the cultivar on the parameters and goodness of fit of the nitrogen curve during its growth, up to 168 days of five fodder genotypes.

Factor A	N	Nc	SEM	Cd	SEM	R^2	SEM
CT-115	6	2.080[a]	0.308	−0.735[a]	0.057	0.965[a]	0.011
Elephant	4	2.220[a]	0.377	−0.656[a]	0.070	0.979[a]	0.013
King grass	5	1.851[a]	0.344	−0.736[a]	0.064	0.977[a]	0.012
Roxo	6	2.001[a]	0.308	−0.576[a]	0.057	0.986[a]	0.011
Taiwan	5	1.961[a]	0.344	−0.623[a]	0.064	0.979[a]	0.012

N = number of observations; Nc = Critical Nitrogen; Cd = Nitrogen Coefficient; R^2 = Goodness of fit; SEM = Standard error of the mean; Different literals are statistically significant (Tukey; P≤0.05).

Table 2. Effect of fertilization on the parameters of the nitrogen curve during growth of five fodder genotypes.

Factor B	N	Nc	SEM	Cd	SEM	R^2	SEM
Fertilized	14	2.627[a]	0.204	-0.645[a]	0.038	0.977[a]	0.007
No Fertilized	12	1.419[b]	0.222	-0.686[a]	0.041	0.977[a]	0.008

N = number of observations; Nc = Critical Nitrogen; Cd = Nitrogen Coefficient; Goodness of fit R^2; SEM = Standard error of the mean; Different letters are statistically significant (Tukey; P≤0.05).
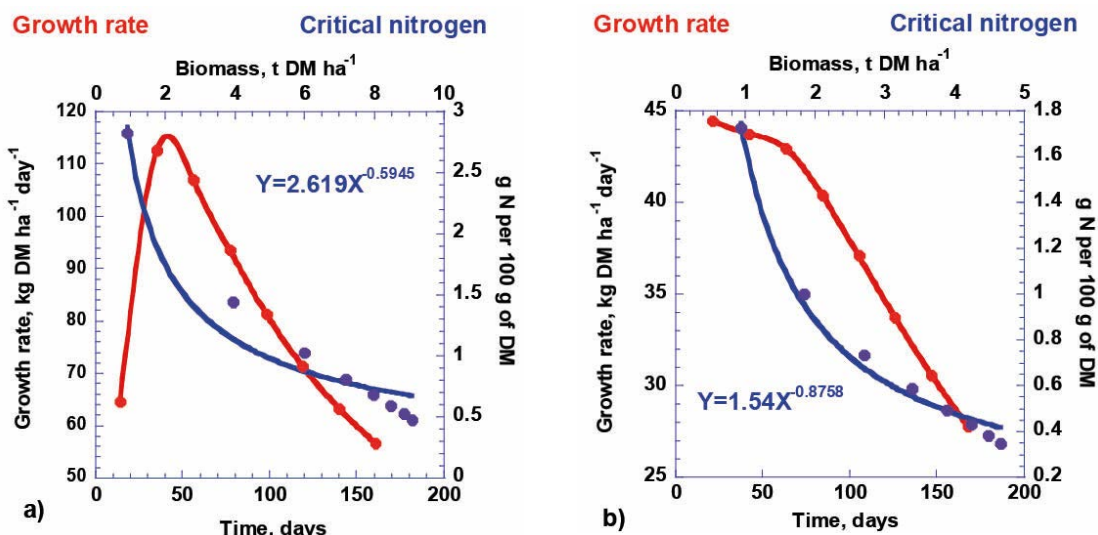
Figure 1. Growth rate and Nc of cultivar CT-115: a) Fertilized b) Not Fertilized.

Cultivar Elefante

The fertilized cultivar Elefante used 2.6 g of N per 100 g of DM to produce 1.0 t of DM ha^{-1} similar to cultivar CT-115, but required 149 kg of N ha^{-1} to produce 5,700 kg of DM at its maximum peak at 56 d of age, with a growth rate of 102 kg of DM ha^{-1} day^{-1} (Figure 2a). A similar situation occurred with the unfertilized control in CT-115, with only 1,828 g of N per 100 g DM per t of DM ha^{-1}, resulting in this cultivar extracting nitrogen from the soil, making the latter poorer at the expense of increasing production of DM ha^{-1} and with an efficiency of nitrogen use of 87% (Figure 2b).

Cultivar King Grass

The growth rate of fertilized King Grass was 56 kg DM ha^{-1} day^{-1} against 27.7 kg DM ha^{-1} day^{-1} without nitrogenous fertilization (Figure 3b). A maximum peak of production of 4,300 kg DM ha^{-1} was observed at 77 d of age and 1,749 kg DM ha^{-1} at 63 d of age, respectively. With Nc values of 2.62 and 1.08 g of N per 100g of DM, for fertilized and unfertilized, respectively. For fertilized King Grass, 113 kg of N ha^{-1} were required, and for this same cultivar without fertilization only 18.9 kg of N ha^{-1} to obtain its maximum DM production. In both cases, the percentage of nitrogen decreases at a rate of 69% in the fodder. On the other hand, with the contribution of 100% of nitrogen in two applications as fertilizer, it seems that there was an effect on the production peak, since the second application of nitrogen (35% of the 141 kg) was carried out after 56 d of regrowth. The efficiency of nitrogen use against the soil content was 83.4%.
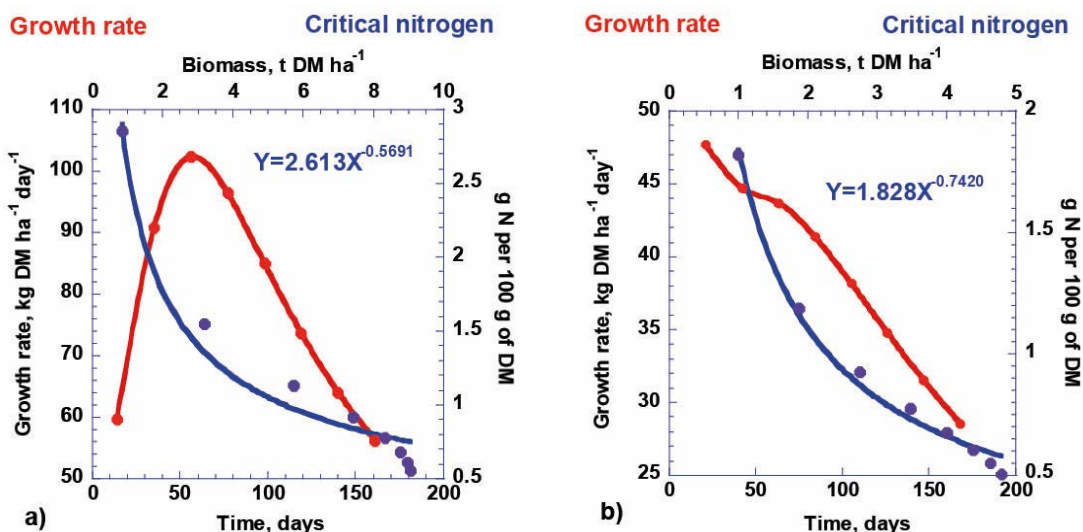

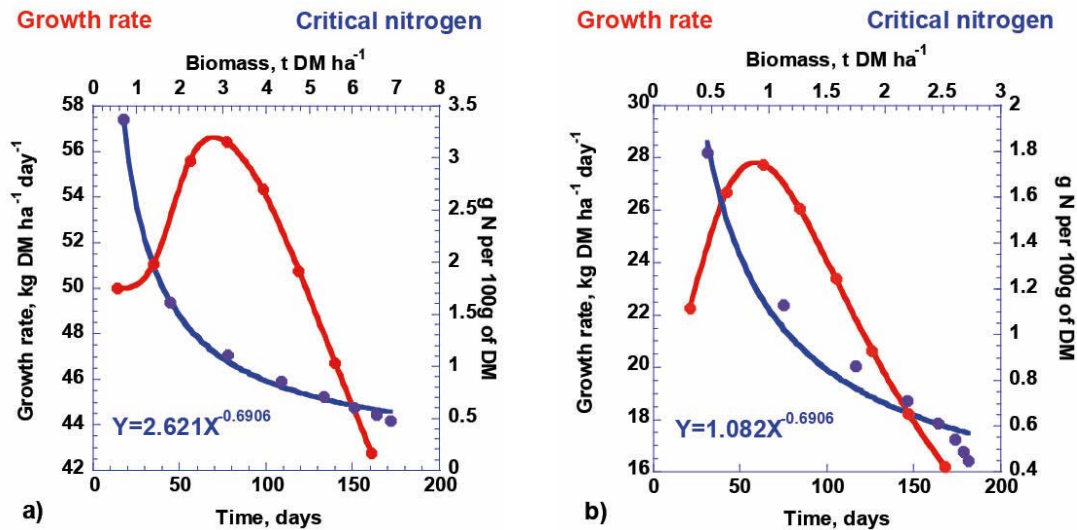**Figure 2**. Growth rate and Nc of cultivar Elefante: a) Fertilized b) Not Fertilized.

Figure 3. Growth rate and Nc of the cultivar King Grass: a) Fertilized b) Not Fertilized

Cultivar Roxo

The cultivar Roxo presented a higher growth rate compared to the cultivar King Grass of 76 kg DM ha^{-1} day^{-1} when fertilized and 38.5 kg DM ha^{-1} day^{-1} without fertilizing, at 77 and 63 d of age respectively. The Nc values were 2.65 and 1.35 g of N per 100g of DM, fertilized and unfertilized, respectively; therefore, it required a greater amount of nitrogen (155 kg N ha^{-1}) to produce 5,844 and 2,425 kg of DM respectively. The efficient use of nitrogen was close to 78%, so the fertilization dose was not enough, since nitrogen was extracted from the soil.

Cultivar Taiwan

The fertilized cultivar Taiwan presented the highest production of DM ha^{-1} compared to the previously mentioned cultivars. With production of 6,314 kg of DM

ha^{-1} at 77 d, in which it reached its maximum growth rate with 82.72 kg of DM ha^{-1} day^{-1}. The Nc was similar to the cultivars with 2.6 g of N per 100 g of DM in a production of 1.0 t DM ha^{-1}, but when this cultivar did not receive nitrogenous fertilization, its production decreased by 73% (1,700 kg of DM ha^{-1}). Its growth rate was reduced to half (40.5 kg DM ha^{-1} day^{-1}) and its Nc content was 1.29 g of N per 100 g of DM. The efficiency of the use of nitrogen was 86% and showed balance in the nitrogen consumption of the soil, that is, the dose of nitrogenous fertilization was sufficient to grow and obtain these fodder productions.

N, Nc, NNI Parameters in Cultivars

In general, when the evaluated cultivars are fertilized with doses of 141 kg of N ha^{-1} in two applications and
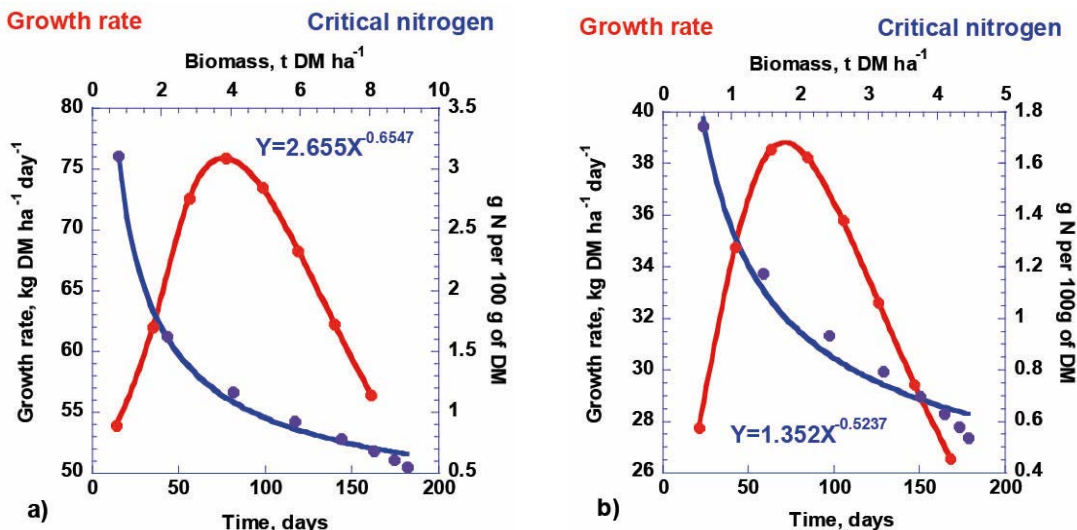

Figure 4. Growth rate and Nc of the Roxo cultivar: a) Fertilized b) Not Fertilized.
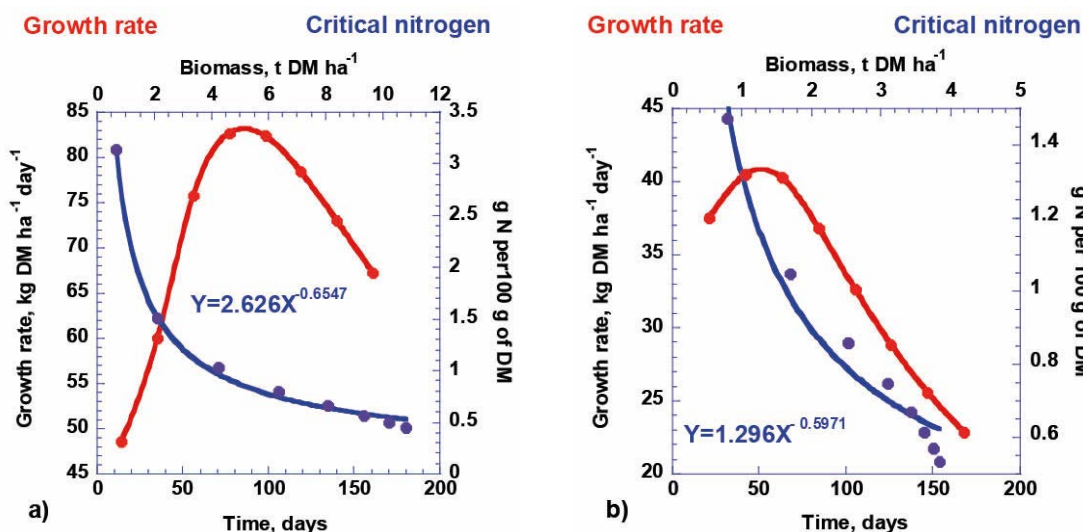
Figure 5. Growth rate and Nc of the cultivar Taiwan: a) Fertilized b) Not Fertilized.

in the rainy season in the Mexican tropics, they have Nc of 2.6 g of N per 100 g of DM in t MS ha^{-1}. The Nc values of this study were lower than those reported by Lemaire and Gastal (1997) who mentioned that the Nc values are 3.6 g of N per 100 g of DM in tropical fodders. These differences may be due to factors, such as the high DM production of these cultivars of *C.pupureus*, the deficiency or lack of nitrogen, water stress, among other factors. They also presented a decrease in the N content, with an average of 59% in the fodder, which caused that in all cultivars, except Taiwan, more nitrogen was extracted from the soil due to the deficit in the dosage or contribution of mineral nitrogen.

Regarding the growth rates, cultivars CT-115 and Elefante registered their maximum growth before applying 35% of the rest of the nitrogen, that is, it was before 56 d of age, in contrast to King Grass, Roxo and Taiwan whose maximum growth was recorded at 77 d of age. These results suggest applying the fertilization dose before its maximum growth occurs. In antecedents to the thresholds established by Duru *et al*. (1997) for the management of the NNI of fodders, Figure 6a shows where the NNI was higher than 1.0 in the first 14 d, which indicates that the studied cultivars had excess N for their growth. However, from 35 d of age the NNI of cultivars King Grass, CT-115, Elefante, Roxo and Taiwan, these values were located in the classification of nitrogen deficient (NNI<0.8). With the results obtained, the application of nitrogen can be planned to obtain the highest efficiency; since, as it was observed, at the beginning of the growth nitrogen is present in excess and after day 35 of growth the deficiencies appear.

Figure 6b shows the NNI behavior of cultivars CT-115, Elefante, King Grass, Roxo and Taiwan, during growth without application of the fertilizer. The NNI data are less than 0.8, therefore in accordance with the threshold described by Duru *et al*. (1997), it is in deficiency. This is demonstrated by the low Nc value of 1.14 g of N per 100 g of DM and low growth rates and production of dry matter. Although the NNI data in the cultivars studied during the first 35 d of age are sufficient, the dose used did not reach the fodder requirements, since they extracted N from the soil to complement growth and DM production.

CONCLUSIONS

The critical nitrogen content of the cultivars of *Cenchrus purpureus* fertilized was 2.6 g of N per 100 g of DM to produce 1.0 t DM ha^{-1} and growth rates up to 112 kg of DM per day in 77 d. The NNI values were higher than 0.8 during the first 56 d, which is considered good nutritional status, but considering the data of the unfertilized group it is considered that these cultivars require a greater amount of N in order not to extract the complement from the soil. This demonstrates the usefulness of these agronomic tools such as Nc and NNI to help diagnose the N requirements of said fodders, and thus be able to make adjustments to fertilization and its frequency of application.
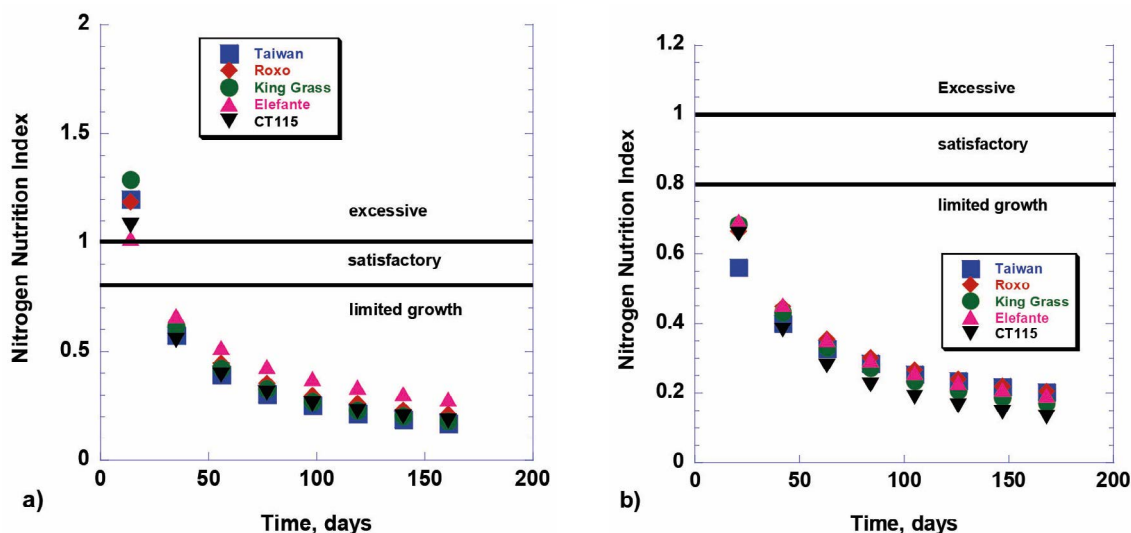
ACKNOWLEDGMENTS

Figure 6. NNI of cultivars of *Cenchrus purpureus* during growth: a) Fertilized b) Not Fertilized.

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