NUTRITIONAL PARAMETERS OF LEAF BLADE FROM DIFFERENT TROPICAL FORAGES

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ABSTRACT - The aim of this study was to evaluate the leaf blade nutritional value of different genotypes of Cynodon cultivars in four regrowth ages (28, 48, 63 and 79 days), by chemical composition and in vitro dry matter digestibility. The treatments were distributed in a randomized block design, with treatments arranged in a split plot scheme, with the studied genotypes (cultivars) (Tifton 68, Tifton 85, Russell, Jiggs and Vaquero) parcels and the four ages cutting the subplots (28, 48, 63 and 79 days). There was an increase in dry matter digestibility with the advance of plant age ranged from 30% to 70% at 28 and 79 days of regrowth, respectively. A reduction of 25 g kg⁻¹ in crude protein content of the genotypes when it was increased the regrowth age of 28 for 79 days, with a daily reduction of 1.79%. There was daily increases of 1.72% and 1.17% in the fiber neutral detergent and acid detergent fiber, respectively. The management of all genotypes at the age of 28 days of regrowth provided greater in vitro digestibility of dry matter leaf blade (79.65%). For a best nutritional value of the Cynodon cultivars, it is recommended to have a period of 28 days between the cuts.

Key words: chemical composition, Cynodon, digestibility.

PARÂMETROS NUTRICIONAIS DA LÂMINA FOLIAR DE DIFERENTES FORRAGEIRAS TROPICAIS

RESUMO - Objetivou-se por meio deste trabalho avaliar o valor nutritivo da lâmina foliar de diferentes genótipos do gênero Cynodon em quatro idades de rebrota (28, 48, 63 e 79 dias), por meio da composição química e da digestibilidade in vitro da matéria seca. O delineamento experimental foi em blocos ao acaso, com tratamentos arranjados em esquema de parcelas subdivididas, sendo os genótipos estudados (cultivares) (Tifton 68, Tifton 85, Russell, Jiggs e Vaquero) as parcelas, e as quatro idades de corte as subparcelas (28, 48, 63 e 79 dias). Verificou-se aumento no teor de matéria seca com o avanço da idade da planta que variou de 30% a 70%, aos 28 e 79 dias de rebrota, respectivamente. Houve redução no teor de proteína bruta dos genótipos de 25 g kg⁻¹ quando aumentou a idade de rebrota de 28 para 79 dias, com redução diária de 1.79%. Houve incrementos diários de 1.72% e 1.17% no teor de fibra em detergente neutro e fibra em detergente ácido, respectivamente. O manejo de todos os genótipos na idade de 28 dias de rebrota proporcionou maior digestibilidade in vitro da matéria seca da lâmina foliar (79,65%). Recomenda-se o intervalo de corte para todos os genótipos a cada 28 dias, em função do melhor valor nutricional.

Palavras-chave: composição química, Cynodon, digestibilidade.

INTRODUCTION

The forage plants have a fundamental role in the ruminants feeding. It is the source of energy and protein of low cost in contrast to other concentrate feeds, and it is a supply of the necessary fiber to the ruminal function maintenance which is the determinant of the dry biomass intake and the animal production (SILVA et al., 2014).

Determining the forage plants' nutritional value is a basic premise to know what the forage is offering in nutrients to the animal. This concept has greater importance in the case of tropical grasses such as the genus Cynodon, widely used in several areas of Brazil. However, some genotypes of the genus Cynodon are less studied for example the Vaquero, Russell and Jiggs. Therefore, it is essential the development of researches focused on its nutritional value and management.

According to Mertens (1994), the forage nutritional value may vary from species, genotypes, the plant’s parts and it is directly related to the consumption. Studies characterizing the pastures in terms of chemical composition and digestibility are relevant in the forage evaluation, as they help in the supplementation necessity of dietary indication at certain times for some animals’ categories. In addition, the elucidation of forage nutritional
value contributes to the potential identification impasses that limits the nutrients consumption and the animal production consequently (BRÂNCIO et al., 2002).

As the physiological age of the plant progresses, the percentages of the cell wall components are enhanced (cellulose, hemicellulose and lignin) thus reducing the proportion of nutrients that are potentially digestible (soluble carbohydrates, proteins, minerals and vitamins) representing a drop-off in the digestibility (REIS et al., 2009; OLIVEIRA et al., 2014). Besides of the increased concentration of the fiber in the culm and most of the leaves, the concentration of fiber is also greater in the total forage because of the decrease of the leaf/culm ratio that is due to the maturity of the plants (BUXTON; REDFJEAR, 1997).

The advantages of using the in vitro technique to assess the nutritional value of the ruminant feed are in its speed, the uniformity of the physical-chemical spot of fermentation, the convenience of maintaining a few cannulated animals and it is less expensive. These techniques can be effective just if they are easily reproducible and high correlated with in vivo results (GETACHEW et al., 1998).

The aim of this study was to evaluate the nutritional value of the leaf blade of different genotypes of Cynodon cultivars in four regrowth ages (28, 48, 63 and 79 days) by chemical composition and in vitro digestibility of dry matter.

MATERIALS AND METHODS

This study was conducted according to the ethical standards and approved by the ethics committee and biosecurity of the Federal University of the Grande Dourados institution under protocol number 223/07.

The experiment was conducted in the Faculty of Agriculture Sciences of the Federal University of the Grande Dourados, where the latitude is 22° 14’ S, longitude is 54° 49’ W and an altitude of 450 m, being executed in two stages, one at the field level and another at the animal nutrition laboratory.

The treatments were distributed in a randomized block design, with treatments arranged in a split plot scheme, with the studied genotypes (Tifton 68, Tifton 85, Russell, Jiggs and Vaquero) were the parcels and the four cutting ages the subplots (28, 48, 63 and 79 days). The occupied area of the experiment was 540 m², and each portion was 9 x 3 m, totaling 27 m² per plot. Each subplot was 2.25 x 3 m totaling 6.75 m² with floor area of 1 m², located in the center of the subplot.

The soil preparation (Dystroferric RED LATOSOL/OXISOL) was realized in September 2009 while implementing the experiment, through the soil sampling followed by plowing, harrowing and planting. The soil of the area had the following chemical characteristics: pH (CaCl²) = 5.7; P (resin) = 2.0 mg dm⁻³; K = 0.3 cmolc dm⁻³; Ca = 3.7 cmolc dm⁻³; Mg = 0.2 cmolc dm⁻³; H + Al = 5.0 cmolc dm⁻³; T = 9.2 cmolc dm⁻³ and V = 45.5%.

Before the planting it was applied the equivalent of 1.600 kg ha⁻¹ of calcitic limestone, being incorporated with plowing and harrowing in all plots to provide an equal condition of growth to the grasses.

The spacing of the holes within the plots was 0.5 m. The planting was done through grass seedlings of the genus Cynodon. The grasses used were Tifton 68, Tifton 85, Russell, Jiggs and Vaquero.

After the complete establishment of the used materials, there was a cut close to the ground for uniformity proceeded by a nitrogen fertilization using the equivalent of 50 kg ha⁻¹ of nitrogen, using urea as a source and then the cutting activities were initiated in different regrowth ages (28 days (13/05/09), 48 days (02/06/09), 63 days (17/06/09) and 79 days (03/07/09) (Table 1).

### TABLE 1. Monthly mean values of temperature and precipitation in Dourados, MS region, during the experimental study period.

<table>
<thead>
<tr>
<th>Date</th>
<th>Tº average</th>
<th>Tº max</th>
<th>Tº min</th>
<th>Precipitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>09/05/2009</td>
<td>20,00</td>
<td>27,40</td>
<td>16,10</td>
<td>51,30</td>
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<td>17,00</td>
<td>17,90</td>
<td>15,90</td>
<td>53,90</td>
</tr>
<tr>
<td>15/07/2009</td>
<td>18,90</td>
<td>25,30</td>
<td>13,50</td>
<td>152,90</td>
</tr>
<tr>
<td>16/08/2009</td>
<td>16,30</td>
<td>19,70</td>
<td>9,90</td>
<td>25,90</td>
</tr>
</tbody>
</table>


In the prearranged dates one square meter was collected of the material in the field with the help of pruning shears being taken within each subplot for each genotype. The collected material was taken to the animal nutrition laboratory, where it was weighed to obtain a productivity estimation per hectare of each genotype and done subsamples to estimate the dry matter (DM) and the remainder was separated into leaf blade and culm + sheath to estimate the blade: culm ratio.

The dry matter (DM) was estimated by the pre-drying of subsamples in a greenhouse with forced air circulation, temperature of 55 °C for 72 h and subsequently grounded in a Wiley mill using sieve with 1 mm riddles for the value of the final dry matter. During 8 h all the sub-samples were dried at 105 °C, according to the
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methodology described by the Association of Official Analytical Chemists - AOAC (1990). The method described by AOAC (1990) was used to estimate the crude protein (CP).

The fiber content of the neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose (HEM) and lignin (Lig) were estimated based on the method described by Van Soest et al. (1991).

The total carbohydrate content (TC) was estimated by the equation: CT (%) = 100 - [PB + % humidity (%)] and the non-fiber carbohydrates (NFC) according to Sniffen et al. (1992). The total digestible nutrients (TDN) were estimated using the formula NDT = 40.2625 + 0.4028 + 0.1969PB CNF + EE 1.903 - 0.1379 FDA (WEISS, 1998).

The in vitro digestibility of leaf blade dry matter (DM) was determined according to the methodology described by Tilley and Terry (1963) modified second Detmann et al. (2012) by means of the in vitro incubator use, Tecnal® (cT=150) with a change in the material of the bag used (7.5 x 7.5 cm) made using a non-woven fabric (TNT -100 g m⁻²) as Casali et al. (2008).

Data were submitted to variance analysis, “F” test, and the days of regrowth were submitted to the study of regression (p<0.05) (procedure REG) through the SAS program (SAS, 2000).

The selection of the best adjustment model (linear and quadratic) was based on the orthogonal contrasts, the trend of the data, the significance of the “F” test in the variance analysis for regression of the data and determination coefficient.

RESULTS AND DISCUSSION

The chemical composition of forage plants that form a pasture can be changed according to the edaphoclimatic conditions and management imposed. Analyzing the experimental conditions of this research: temperature and precipitation (Table 1) there was a significant interaction for the dry content matter (DM) of the genotypes x regrowth age (Figure 1).

For all genotypes, it was verified an increase tendency in DM content with increasing plant age which ranged from 30% to 70%, in the ages 28 to 79 days of regrowth, respectively. At 63 days of regrowth, there was decline in DM content, probably due to the effects of precipitation (Table 1), favoring greater emergence of new tillers, with a higher concentration of water in the tissues. Among the genotypes, it is observed that the Vaquero and Russell grass had higher DM content for all the analyzed ages. The increase in DM content derives from a relationship change of cell wall components (cellulose, hemicellulose and lignin) and middle lamella (pectin and β-glucans) with the cell content constituents (soluble carbohydrates, proteins, vitamins and minerals) according to the plant reaches physiological maturity (WILSON, 1993; 1997). As the Vaquero and Russell genotypes presented a different morphological characteristics of other genotypes, probably because of the smaller canopy height, thinner stem and leaf blade may have favored the maturation of these at an earlier stage, indicating that management should be anticipated (28 days) when compared with other genotypes.

With the increase of regrowth age, the average of all the genotypes were adjusted to the crescent linear model of regression, and observed daily increases of 3.92; 4.55; 5.05; 5.23 and 2.97% in DM content, respectively, for the genotypes Tifton 85, Jiggs, Tifton 68, Vaquero e Russell.

For the ratio leaf blade-culm (L/C), has not been verified significant interaction between genotype x cutting ages. However, differences were observed (p<0.05) of age on the L/C ratio of genotypes studied (Figure 2). With increasing of regrowth age from 28 to 79 days, the values of the relationship L/C were adjusted to quadratic regression model.
The age of the plants is an important factor, relating with the forages quality and quantity due to the changes that occur in their morphology and their chemical constituents. Productivity studies should, whenever possible, take into account morphological characteristics of the plant as the ratio L/C (SALES et al., 2014). The reduction in relation L/C with the increase in age can be explained by the structural arrangement of the canopy forage which with advancing age, leaf elongation rate is not sufficient to capture sunlight for photosynthesis (SALES et al., 2014). When the pasture intercepts 95% of incident light is obtained a value of leaf area index (LAI) critical (BORGES et al., 2011). This critical value, the rate of growth of any forage would be close to its maximum value due to photosynthesis (DA SILVA; PEDREIRA, 1997). Therefore, the plant elongates the culm for exposing the blade to solar radiation to increase the leaf area index and light interception seeking maximum photosynthesis. However, for the genotypes studied, the ratio L/C changed from 1.75 to 1.6, which would indicate favorable conditions for leaf blade selection for animals and differentiated with respect to other species of forage such as of the genus Brachiaria e Panicum (VELÁSQUEZ et al., 2010). Thus, the deferral of genotypes studied is an alternative to offer better quality forage, with higher leaf blade, probably with a high concentration of nutrients. The ratio L/C is a very important tool for the management of forage plants, considered the critical limit when the values are lower than 1.0, which implies a reduction in the quantity and quality of forage produced (BRÂNCIO et al., 2003). Based on the upper stratum is potentially consumed all the genotypes under study are within the recommended range in all ages, even reaching the plateau at the regrowth age of 50 days.

Considering that the highest concentration of crude protein (CP) are contained in the leaf blade, the use of Cynodon genotypes under study at an advanced age (79 days) after regrowth, in ruminant feed, can result in animal performance, depending on the category and animal requirement, even with an increase in the fraction culm of forage produced (Figure 2), which has largest contents of grass fibrous fractions.

There was no significant interaction between genotype x regrowth ages. However, with increasing of regrowth age, there was a reduction in the CP content of the genotypes (Figure 3).

It was detected a mean reduction in CP content of the genotypes of 25% when increased the age of 28 for 79 days, with a daily reduction of 1.79%. This reduction occurred probably due to higher plant nitrogen content is concentrated on mobile content that is diluted as the plant reaches physiological maturity (VELÁSQUEZ et al., 2010). So, between 28 and 79 days of age, there is assistance with the limit range 6 to 8% of CP in DM, below which the rumen microorganisms have nitrogen limitations to fiber degradation, as recommended by Van Soest (1994) for beef cattle, which would require energy-protein supplementation, including for maintenance. Although crude protein values found in forages species (15-21%) was above the critical range of 6-8% of dry matter, recommended so that there is not a decrease in the efficiency of microbial growth and capacity degradation of fiber (VAN SOEST, 1994). Velásquez et al. (2010) verified that half of the present protein in forages evaluated (Tifton 85, Tanzânia e Marandu) appeared as fractions B3 e C. Even so, the genotypes evaluated in this study are within the recommended range. B3 fraction has a very slow degradation rate since it is associated to the plant cell wall. The fraction C corresponding to the nitrogen unavailable, and is composed of proteins and nitrogenous compounds related to lignin, tannic protein complexes and Maillard products which are highly resistant to attack from enzymes of microbial origin and host (SNIFFEN et al., 1992; VAN SOEST, 1994).

The thickening of the secondary cell wall with the maturation of plant tissues increases the concentration of neutral detergent fiber (NDF) and acid detergent fiber (ADF) to the detriment of cell content (WILSON, 1993; 1997). This behavior was evident in all the genotypes studied with the increase of regrowth age there being no
interaction (p>0.05) between genotypes x regrowth age (Figure 4).

The NDF content of the forage studied ranged from 71.73% to 77.21% between the ages 28-79 days, respectively. For the ADF content, the marginal variation was 12.47%. There were daily increments of 1.72% and 1.17% in the NDF and ADF content, respectively. The growth of forage plants implies increase of cell wall, as evidenced in the genotypes studied. This fact occurs to the detriment of organic molecules that may or may be not nutrient that actively works on metabolic processes, with deposition of non-nitrogenous organic molecules (cellulose, hemicellulose, lignin, etc.), resulting in a reduction in levels of nitrogenous compounds (Van Soest, 1994). Pena et al. (2009), evaluating the morphogenetic and structural characteristics and forage accumulation of capim-tanzânia, under different cutting heights and cutting intervals, concluded that both the interval as the cutting height can influence the accumulation and morphological composition of forage produced, and their relative importance varies with the time of year and the phenological stage of the plants.

For the main structural carbohydrates (cellulose (CELL) and hemicellulose (HEML)) of the leaf blade, was not observed interaction (p>0.05) between genotype x plant age (Figure 5) within each structure. The CELL and HEML content increased with age of regrowth, and the averages had adjusted to the linear regression model. Daily increments of 0.86% and 1.66% were checked, respectively for the CELL and HEML content. For HEML is justified due to the increase observed in NDF, since the HEML is the difference between NDF and ADF.

An important factor observed in this study was the high NDF content (above 70%) in the leaf blade of genotypes in relation to other forage grasses from tropical climate, in which the content ranges from 55% to 65%. However, plants ruminal degradation of NDF from the genus *Cynodon* seems to be bigger than the plants of the
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According to Van Soest (1994), this occurs because to the higher content of hemicellulose present in the cell wall of these plants and the majority of this carbohydrate can be found in free form and not forming ester bonds with the lignin, which reduces microbial action in the rumen.

Behavior similar to the NDF, ADF, HEML and CELL was also observed in lignin (Lig) content of forages studied. There was no significant interaction between genotype x cut times for this variable (Figure 6).

There was a marginal change of 31.16% in the Lig content when increased the regrowth age of 28 to 79 days. Thus, the management of the genotypes in age 28 days favors the supply of forages higher quality, since the Lig is a anti qualitative factor, present in the cell wall, causing poisoning of rumen microorganisms and a reduction in digestibility (VAN SOEST, 1994). Under the conditions of this research and evaluated genotypes, it was observed that for each day increased at the regrowth age, there was deposit of 0.65% of Lig in DM. According to Martins-Costa et al. (2008), lignin contributes significantly to the effects of rumen repletion, due to it being a toxic phenolic compound with rumen microorganisms, in addition to being available as an energy source for microbial growth. Thus, probably, animals fed gender genotypes of Cynodon at the ages of 79 days or longer, suffer from effect of ruminal repletion by that fraction, and have thereby reducing energy consumption by the physical effect of digestion (VIEIRA et al., 1997; OLIVEIRA et al., 2014).

For total carbohydrates content (TC) was a significant interaction between genotype x regrowth age (Figure 7). It was found that with advancing age, the mean adjusted to the linear regression model, and observed daily increments of TC of 2.27, 2.16, 2.27, 2.51 and 0.95% for the respective genotypes, Tifton 85, Jiggs, Tifton 68, Vaquero e Russel. The increase in TC content is justifiable due to the thickening of the cell wall and observed for DM and NDF content, the major components being cellulose.
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which is a water-insoluble carbohydrate, consisting of a set of glucose, linked together by type connections β-1,6 (VAN SOEST, 1994) (Figure 5). In this study, the genotype TC content ranging 64-78% in the plant, and at the age of 28 days, the TC is more digestible due larger proportion of soluble TC, while in older age (79 days) are carbohydrates, mostly, are insoluble and less degradation in the rumen.

For non-fiber carbohydrates (NFC), represented mainly by the simple sugars, disaccharides, starch and pectin, significant interactions were observed between genotypes x regrowth age (Figure 8).

FIGURE 7 - Total carbohydrates content (TC) of the leaf blade from five Cynodon genotypes into four regrowth ages. \( \hat{Y}_{\text{Tifton 85}} = 2.27x + 67.65 \) (\( R^2 = 0.84 \)); \( \hat{Y}_{\text{Jiggs}} = 2.16x + 64.63 \) (\( R^2 = 0.90 \)); \( \hat{Y}_{\text{Tifton 68}} = 2.27x + 67.65 \) (\( R^2 = 0.84 \)); \( \hat{Y}_{\text{Vaquero}} = 2.51x + 63.95 \) (\( R^2 = 0.84 \)); \( \hat{Y}_{\text{Russel}} = 0.95x + 67.88 \) (\( R^2 = 0.29 \)).

There was a linear reduction of the NFC content with an increase of the regrowth age, which is justified by the increases in the cell wall components, that are, fibrous carbohydrates (cellulose and hemicellulose). Daily reductions were 1.58, 1.52, 2.36, 1.0 and 2.13% for the genotypes of Tifton 85, Jiggs, Tifton 68, Vaquero and Russell, respectively. Among the genotypes Tifton 68 and Russell presented the highest daily reductions when the regrowth age was increased from 28 to 79 days. Despite of Tifton 68 has showed the higher daily reduction (2.36%) in the 79 days, it also displayed a higher NFC content (26.51%) in contrast to the other genotypes at the same age. The Jiggs and Vaquero genotypes showed lower marginal variation and NFC content comparing to the other forages in test. However, all the genotypes had a higher content of NFC in the regrowth age of 28 days. The knowledge of the NFC content of the forages has an important fundament in the adequacy of energy availability to the ruminal microorganisms for the microbial synthesis of protein.

The results referring to the in vitro digestibility of the dry matter (IVDDM) and the total digestible nutrients (TDN) in the leaf blade of the evaluated forage genotypes (Figure 9) are correlated to the cell wall concentrations...
Nutritional parameters of leaf blade... MONÇÃO, F. P. et al. (2016) significant interaction between the genotypes vs. regrowth age for both IVDDM and the TDN.

(Figure 4, 5 and 6), the non-fiber carbohydrates (Figure 8) and the protein content (Figure 3). There was no

The IVDDM of the evaluated genotypes reduced according to the most advanced physiological stages. The management of all genotypes in the regrowth age of 28 days provided a major IVDDM of the leaf blade (79.65%), wherein there was a 14.31% reduction when they were managed at the age of 79 days. For the TDN content the marginal variation was 6.90%. The daily reductions observed in IVDDM and TDN were 3.73% and 1.20% respectively. This behavior of the IVDDM and TDN is justifiable and coherent, since all the genotypes increased the cell wall components (Figure 4, 5 and 6) and decreased the CP content (Figure 3) and physiological maturity.

Some authors have established a relationship between the anatomy, the chemical composition and the digestibility of the forage grasses. Highly significant correlations among the proportion of individual tissues or in combination, and the nutritional entities have been observed (WILSON et al., 1989). In general, the fibrous constituents (NDF, ADF and Lignin) are negatively correlated to the digestibility (WILSON et al., 1983; WEISS, 1994; ALVES DE BRITO et al., 2003).

CONCLUSIONS

The Tifton 68 and Tifton 85 genotypes showed better results of total carbohydrates and non-fibrous leaf blade than the other genotypes. Therefore, it is recommended cutting range for all genotypes every 28 day according to the best nutritional value.

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