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Protodioscin Content, Degradation Kinetics, and *In Vitro* Digestibility of Marandu Palisadegrass Hay as were Affected by Cutting Interval of the Canopy

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ABSTRACT

Even though marandu palisadegrass is widely used in Brazil, the consumption of this forage may cause photosensitization in ruminants due to the protodioscin. This study aimed to recommend a harvest cutting interval for haymaking that provides a better nutritional value and lower levels of protodioscin. The experimental treatments were four cutting intervals (21, 35, 49, and 63 days of regrowth period up to forage harvest). The experimental design was a completely randomized block design with 4 treatments and 4 replications. We assessed the forage production, chemical composition, *in vitro* digestibility, *in vitro* degradation kinetics, and protodioscin content. The forage accumulation rate did not differ with the increased cutting interval. The crude protein contents and *in vitro* digestibility of dry matter decreased linearly with the increased cutting interval in fresh and hay material. The greatest gas production in hay material was occurred in 21 d cutting interval, which was 26%, 14%, and 5% greater than cutting intervals of 63, 49, and 35 d, respectively. A linear increase was observed in protodioscin concentration in hay according to the cutting interval, which in the hay with 63 d cutting interval was 7%, 29%, and 43% greater than hays with cutting interval of 49, 35, and 21 d, respectively. In addition, protodioscin contents were lower in hay compared to fresh material. The haymaking process reduced protodioscin content in relation to forage before haymaking. Our results showed that the better condition to harvest marandu palisadegrass for hay was at 21 d. The better condition to develop a productive canopy is associated with better nutritive value and lower protodioscin content.

Keywords: Brachiaria brizantha; haymaking; tropical grass; gas production; saponins

INTRODUCTION

The majority of cultivated grassland in Brazil are Brachiaria genus pastures (Jank *et al.,* 2014). Despite the importance of Brazilian livestock, some cultivars of *Brachiaria* genus may cause a problem for ruminants due to hepatogenic photosensitization, being sheep is the most sensitive species (Riet-Correa *et al.,* 2011). The occurrence of photosensitization has been reported in animals that consumed *Brachiaria decumbens*, *Brachiaria brizantha*, *Brachiaria humidicola,* and *Brachiaria ruziziensis* (Riet-Correa *et al.,* 2011, Faccin *et al.,* 2014; Diamantino *et al.,* 2020). Clinical signs observed in hepatogenic photosensitization include dermatitis with thick skin on the flank and perineum, jaundice, ocular discharge, apathy, anorexia, photophobia, and facial and auricular subcutaneous edema (Faccin *et al.,* 2014, Diamantino *et al.,* 2020).

Initially, photosensitization was associated with the presence of mycotoxin produced by *Pithomyces chartarum* in *B. decumbens*. Subsequently, it was demonstrated that poisoning in ruminants was caused by steroidal saponins (Brum *et al.,* 2007). Protodioscin, a saponin present in plants of the Brachiaria genus, may cause liver lesion and, subsequently, photosensitization (Riet-Correa *et al.,* 2011, Lima *et al.,* 2015).

Leal *et al*. (2020) observed the protodioscin contents of five Brachiaria brizantha cultivars: arapoty, paiaguas, xaraes, and marandu palisadegrass, and piata throughout the year in all seasons. All grasses showed the highest protodioscin contents during autumn, ranging from 5.6 g kg^{-1} in the spring to 19.2 g kg^{-1} in the autumn. The protodioscin content also varied according to the cultivar, in which arapoty, paiaguas, xaraes, marandu palisadegrass, and piata showed values of 13.2, 11.3, 8.0, 9.5, and 9.4 g kg^{-1}, respectively (Leal *et al.,* 2020).

The saponin contents in plants vary with the climatic factors (rainfall, temperature, and relative humidity) and plant age (Lozano *et al.,* 2017). Higher concentrations of protodioscin occur in greater cutting intervals (Riet-Correa *et al.,* 2011). This condition can be a problem in grazing systems that make hay from surplus forage since farmers usually harvest old fodder for

haymaking (Lima *et al.,* 2015). Therefore, this condition may cause economic losses due to the decrease in animal performance due to the photosensitization injuries, which may cause animal loss (Mustafa *et al.,* 2012).

It is known that haymaking is a viable strategy to supplement forage in the drought period. The production of tropical grasses in rainy periods results in a forage surplus, which can be used when the existing forage is insufficient for animals' demand. After long rest periods between cuts, it may cause a greater deposition of low digestibility material in plants and decrease grasses' nutritional value, ultimately affecting animals' intake (Costa *et al.,* 2007). Therefore, grass should be cut at a time with the optimal balance between nutritional value and forage production.

Nevertheless, the haymaking process slightly reduces the protodioscin content in Brachiaria decumbens harvested at 150 days after regrowth, compared with fresh forage (Lima *et al.,* 2015). The protodioscin content of *B. brizantha* cultivars can negatively affect their digestibilities and some parameters of cumulative *in vitro* gas production (Leal *et al.,* 2020). In this context, to turn haymaking into an efficient alternative, it is necessary to know the dynamics of forage accumulation, nutritional value, and, finally yet importantly, the protodioscin contents in forage. Only then it is possible to determine the appropriate time for harvesting and haymaking of marandu palisadegrass.

This study tested the hypothesis that shorter harvest cutting intervals provide better nutritional value and lower levels of protodioscin. This was assessed by quantifying the forage structural characteristics, chemical composition, *in vitro* digestibility, *in vitro* degradation kinetics, and protodioscin content of *B. brizantha* cv. marandu hay harvested in different cutting intervals. Therefore, this study aimed to recommend a harvest cutting interval for haymaking that provides a better nutritional value and lower levels of protodioscin.

MATERIALS AND METHODS

The experimental procedures of this study were approved by the Ethics and Animal Welfare Committee of National Council of animal experimentation control of Federal University of Mato Grosso do Sul (protocol number 611/2014).

The rumen material was collected at the Experimental Farm of the Federal University of Mato Grosso do Sul, Brazil (20º26′48.2′′S 54º50′39.2′′O and 530.7 m altitude), in Terenos, Mato Grosso do Sul.

Experimental Sites, Treatments, and Experimental Management

The trial was carried out at the Experimental Farm of the Federal University of Mato Grosso do Sul, Brazil (20º26′48.2′′S 54º50′39.2′′O and 530.7 m altitude), in Terenos, Mato Grosso do Sul. Meteorological data were obtained from a Monitoring Center for Weather, Climate, and Water Resources of Mato Grosso do Sul and at the experimental site (Figure 1).

The soil of the experimental site at the beginning of the study had the following characteristics: pH ($CaCl_2$)= 4.89 and (H_2O)= 5.49, OM= 34.38 g dm^{-3}, P= 3.50 mg.dm^{-3}, K= 0.23 cmol.dm^{-3}, Ca^2= 4.50 cmol.dm^{-3}, Mg^2= 2.55 cmolc. dm^{-3}, Ca + Mg= 7.05 cmolc.dm^{-3}, Al^3= 0.10 cmol.dm^{-3}, H + Al= 5.91 cmol.dm^{-3} exchange capacity= 13.19 cmolc.dm^{-3}, and base saturation= 55.19 %. All soil samples were analyzed following Embrapa (2006).

The plots were established in January 2015 by seeding marandu palisadegrass (*B. brizantha* cv. marandu). Fertilizer applications corresponded to 555.0 kg ha^{-1} of P_2O_5, 100.0 kg ha^{-1} of K_2O, and 1.0 t ha^{-1} of dolomitic limestone with a relative power of total neutralization 80% to increase base saturation to 60%.

In October 2015, a cut of 15 cm of canopy stubble height was carried out in order to uniform the plots. The

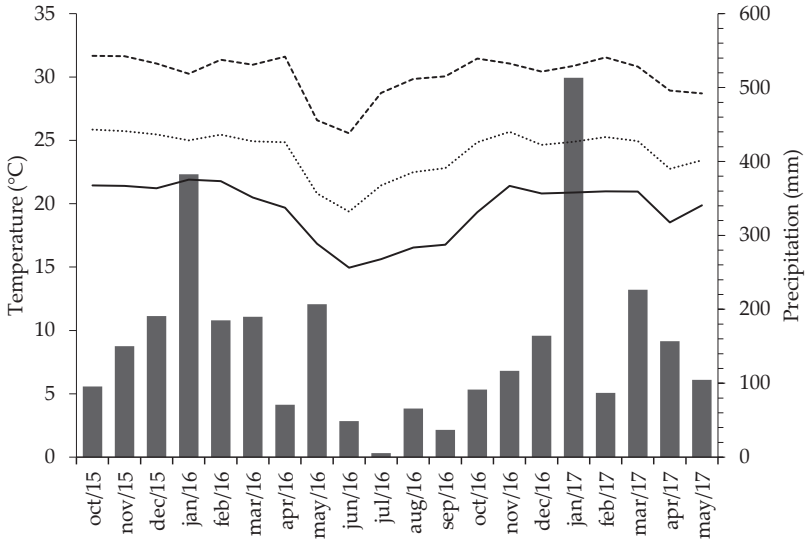

Figure 1. Accumulated monthly precipitation (mm) and minimum, average, and maximum temperatures (ºC) during the experimental period. ---- Maximum temperature; … Average temperature; ▬ Rainfall; ▬ Minimum temperature. The temperature and precipitation information were obtained from the weather database CEMTEC/MS-Semagro (available at URL: http://www.cemtec.ms.gov.br).

quantity of 100 kg ha^{-1} of nitrogen using urea (Heringer Fertlizers) was applied, divided into two applications. The first application (50 kg ha^{-1}) took place soon after the cut to uniform the plots, and the second one occurred after the first sampling period (50 kg ha^{-1}).

The trials were carried out during the rainy season between October 2015 and April 2016. The experiment was a completely randomized blocks design. The treatments were four days of regrowth (21, 35, 49, and 63 d of regrowth period up to forage harvest) of grass for haymaking, with four replications. Furthermore, the treatments were distributed in 16 plots of 5 x 10 m (50 m^2). To make the hay, the grass was harvested and dehydrated until it reached the content of 80% DM. Four rectangular bales of hay from each plot per cutting interval were made, with an average weight of approximately 20 kg per bale hay.

Structural Characteristics Evaluation

The forage mass (FM) was sampled in each cutting interval throughout the trial period. FM was harvested at 15 cm from the ground level using frames of 1 m^2 per plot, according to cutting intervals of 21, 35, 49, and 63 d. After harvesting the forage, two subsamples of fresh material were taken to evaluate dry matter (DM) concentration and morphological separations. To estimate the DM concentration of FM, one subsample was oven-dried at 55 °C for 72 h to a constant weight. The FM (kg ha^{-1}) was considered a whole-plant without dead material. Another subsample was separated into the stem (stem + sheath), leaf (leaf blade), and dead material to quantify the forage components, oven-dried at 55 °C for 72 h, weighed, and ground for analysis of chemical composition.

Forage accumulation rates (g kg-1) were calculated by dividing FM by days during the cutting intervals. The leaf accumulation rates (g kg^{-1}) were performed similarly, using the leaf proportion values. The canopy height, defined by Allen *et al.* (2011) as a surface height of an undisturbed canopy normally measured from ground level, of plots was determined by taking ten measurements randomly using a sward stick. The canopy height, forage mass, and pasture botanical composition were monitored throughout the trial period. The canopy height was recorded weekly in each treatment by taking 100 measurements randomly using a sward stick (Barthram, 1985). Forage mass was sampled by using six frames of 1 × 0.5 m per paddock, once for each period. After harvesting the forage, botanical separations were performed. Subsamples of approximately 250 g of fresh material were taken to evaluate dry matter concentration. Forage samples were then oven-dried at 55 °C for 72 h to a constant weight.

Determination of Chemical Composition and *In Vitro* Digestibility

Whole forage mass samples were utilized to determine the chemical composition and *in vitro* digestibility. After harvesting the forage, samples were dried at 55 °C for 72 h, weighed, and ground in a Cyclotec mill (Tecator, Herndon, VA) at 1 mm for further evaluations. The *in vitro* dry matter digestibility was determined using the DAISYII method at 48 h, followed by pepsin incubation for 24 hours (Ankom Technology Corp., Fairport, NY; Holden, 1999).

Rumen fluid was collected from two cannulated heifers fed a pasture of marandu palissadegrass. As much as 0.50g of sample was weighed directly into filter bag F57, except for the blank bag for correction factor. It used four digestion jars, which contained solution buffer and inoculum. In order to prepare the inoculum and perform the incubation, 2000 mL of rumen inoculum, including a fibrous mat, was collected and placed in a thermos. After 48 hours, 8 g of pepsin was diluted in 40 mL of 6N HCl, added to each jar, and left for 24 hours. Afterward, the bags were removed, washed, placed in an oven at 55 °C for 24 hours.

The *in vitro* dry matter (IVDMD), organic matter (IVOMD), neutral detergent fiber (IVNDFD), and acid detergent fiber (IVADFD) digestibilities of the leaf and stem of hay were evaluated. The DM was obtained by oven drying at 100 °C for 18 hours (method 934.01: AOAC 2005). The ash concentration was determined by incineration for 2 hours in a 600 °C muffle furnace (method 942.05: AOAC 2005). In addition, crude protein (CP) concentration was obtained from N concentration (CP= total N × 6.25), which was determined using the Kjeldahl procedure (method 920.87: AOAC 2005).

From the constituents of the fiber fraction, neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to Van Soest *et al*. (1991). Further, lignin was analyzed in ADF analysis sequence, according to the method 973.18D (AOAC 2005).

In Vitro Degradation Kinetics

The equipment Ankom RFGas Production System (Ankom Technology, NY, USA) was used to estimate the *in vitro* degradation kinetics. Kinetic parameters of gas production were obtained through the logistic model of dual-pool described by Schofield *et al*. (1993):

$$Y(t) = [vNFC / (1 + \exp(2-4kdNFC(t-L)))] + [vFC / (1 + \exp(2-4kdFC(t-L)))[$$

where 'Y' is the total volume of gas at 't' time (degradation extension), "vNFC" and "vFC" represent the volume of gas produced from the degradation of non-fiber and fiber carbohydrates, respectively; "kdNFC" and "kdFC" are the respective degradation rates of nonfiber and fiber carbohydrates; and "L" is the time of colonization (h) of the particle (lag).

Protodioscin Analyses

Stem (stem + sheath) and leaf (leaf blade) of marandu palisadegrass were used before haymaking and hay material for protodioscin analyses by High-Performance Liquid Chromatography (HPLC), according to Ganzera *et al*. (2001). One gram of the plant material finely ground was extracted with 3.0 mL of 50% aqueous acetonitrile by sonication and centri-

fuged. After centrifugation, the extracts were pored in a volumetric flask, and the volume was adjusted to 10 mL with the solvent used for extraction. Five milligrams of protodioscin standard were dissolved in 5.00 mL of 50% aqueous acetonitrile (stock solution). Protodioscin analysis by HPLC was conducted using a reversed-phase (RP-18) column equipped with an evaporative light scattering detector (ELSD), Shimadzu, and a water/acetonitrile gradient as the mobile phase.

Statistical Analysis

The applied design was a completely randomized blocks design with regrowth periods (21, 35, 49, and 63 d). The treatments (regrowth periods) were blocked according to the spatial difference of the experimental area. Data were submitted to analysis of variance and regression analysis for the regrowth periods. Data were analyzed by a one-way analysis of variance using the general linear model procedure of SAS 2000 PROC GLM (SAS Institute, Cary, NC, USA). Treatment averages were estimated and compared using the Tukey test with significance was declared at $p<0.05$. When a significant Tukey test was detected, treatment sum-of-squares was partitioned to provide linear contrasts (Kaps & Lamberson, 2017). The following model was used:

$$Yjk = \mu + Tj + Bk + \alpha jk$$

where Yjk was observation regrowth j, block k; μ was mean overall effect; Tj was the effect of regrowth age j, j was 21, 35, 49, and 63 d; Bk was the effect of block; and αjk was random error associated with each jk observation.

RESULTS

Structural Characteristics

Table 1 shows the data on structural and morphological characteristics. Total forage mass increased linearly along with the cutting interval (p= 0.0001; Table 1). The cutting interval of 63 d showed a forage mass of 65%, 33%, and 23% greater than 21, 35, and 49 d cutting interval, respectively. However, the forage accumulation rate was kept around 71.5 kg ha^1 DM and did not differ between treatments (p= 0.319; Table 1). In addition, there was an effect in the leaf accumulation rate, which was greater at 21 d of cutting interval than the other treatments (p= 0.0001; Table 1).

The cutting interval of 21 d showed greater leaf mass (599.3 kg ha^1 DM) and smaller stem mass (289.6 kg ha^1 DM) than the other treatments (p= 0.0117 and p= 0.0007, respectively; Table 1). Thus, the leaf : stem ratio of 21 d cutting interval was 34.6% greater than 35, 49, and 63 d (p= 0002; Table 1). Dead material was greater at 49 d and 63 d than 21 d and 35 d cutting intervals (p= 0.0197; Table 1). Canopy height increased linearly along with the increased cutting interval (p= 0.0001, Table 1).

Chemical Composition and *In Vitro* Digestibility

The chemical composition and *in vitro* digestibility of forage before haymaking are shown in Table 2. There was a positive linear effect with the increase of cutting interval on the DM of the whole plant (p= 0.0087). In addition, there was a linear effect with the increase of cutting interval on the contents of DM, OM, NDF, and lignin of leaf (p= 0.0001). In contrast, the opposite was noted on CP contents, decreasing while the cutting interval increased (p= 0.0001). The CP contents of leaf were 8.6%, 16.2%, and 28.0% greater at 21 d compared to 35, 49, and 63 d cutting intervals, respectively. Furthermore, there was a negative linear effect with the increase of cutting interval on the IVDMD and IVNDFD of the leaf (p= 0.0001 and p= 0.0049, respectively, Table 2).

CP contents of stem showed a negative linear effect with the increase of cutting interval, which was 30.3%, 25.2%, and 45.9% greater at 21 d compared to 35, 49, and 63 d cutting interval, respectively (p= 0.0001, Table 2). However, the fiber components, NDF, ADF, and lignin increased linearly with the increased cutting interval (p = 0.0001, p = 0.0103, and p= 0.0023, respectively). In addition, IVDMD, IVOMD, and IVNDFD of stem decreased linearly as the cutting interval increased (p= 0.002, p= 0.0035, and p= 0.0007, respectively). There was a linear effect only on CP contents in dead material, which decreased with the increased cutting interval (p= 0.0001). The DM and NDF contents of dead material did not differ between treatments (p= 0.4658 and p= 0.4266, respectively).

Table 1. Structural and morphological characteristics of *Brachiaria brizantha* cv. marandu according to the cutting interval

Item	Days				SEM	P-*value*		Regression equations
	21	35	49	63		Linear	Quadratic	
Fresh forage mass (kg ha^{-1})	5713^c	10792^b	12249^b	14565^a	873.51	0.000	0.093	Y= 24426.13025 + 200.0903x (R^2=0.93)
Forage mass (kg ha^{-1} DM)	1481^c	2838^b	3270^b	4256^a	269.5	0.000	0.472	Y= 334.58813 + 62.5542x (R^2=0.96)
Forage accumulation rate (kg ha^{-1} DM)	70.55	81.1	66.76	67.56	5.791	0.319	0.350	NS
Leaf accumulation rate (g kg^{-1} DM)	28.54^a	14.59^b	10.32^c	7.78^d	0.465	0.000	0.000	Y= 40.0745 - 0.5797x (R^2=0.78)
Leaf (g kg^{-1} DM)	599.3^a	510.7^b	505.7^b	490.4^b	15.5	0.012	0.072	Y= 626.0418 – 2.3701x (R^2=0.76)
Stem (g kg^{-1} DM)	289.6^b	373.4^a	364.6^a	372.4^a	13.7	0.001	0.008	Y= 131.0930 + 9.8433x – 0.0968x^2 (R^2=88)
Dead material (g kg^{-1} DM)	111.1^b	115.9^b	129.8^a	137.2^a	8.855	0.020	0.868	Y= 95.8125 + 0.6589x (R^2=0.97)
Leaf:stem ratio	2.09^a	1.38^b	1.40^b	1.32^b	0.115	0.000	0.007	Y= 2.4552 - 0.0182x (R^2=0.85)
Height (cm)	26.50^c	38.87^b	42.39^b	55.79^a	1.596	0.000	0.717	Y= 13.4631 + 0.6530x (R^2=0.96)

Note: the means were compared by Tukey test at 5% significance. SEM: Standard error of median.

Table 2. Chemical composition of *Brachiaria brizantha* cv. marandu before haymaking according to cutting interval

Item	Days				SEM	P-*value*		Regression equations
	21	35	49	63		Linear	Quadratic	
Whole plant								
Dry matter (g kg^{-1})	259.33^{b}	262.91^{b}	266.61^{b}	291.70^{a}	4.242	0.009	0.061	Y= 239.8901 + 0.7202x (R^2=0.79)
Leaf								
Dry matter (g kg^{-1})	250.70^{c}	263.10^{b}	263.53^{b}	283.68^{a}	3.997	0.000	0.285	Y= 235.4450 + 7.096x (R^2=0.88)
Organic matter (g kg^{-1} DM)	905.24^{c}	912.05^{b}	917.53^{a}	917.95^{a}	1.582	0.000	0.104	Y= 900.1153 + 0.3114x (R^2=0.90)
Crude protein (g kg^{-1} DM)	119.03^{a}	108.78^{b}	99.78^{c}	85.70^{d}	0.233	0.000	0.46	Y= 130.6113 – 0.6498x (R^2=0.69)
NDF (g kg^{-1} DM)	680.80^{c}	692.88^{c}	709.53^{b}	731.28^{a}	7.353	0.000	0.461	Y= 653.1963 + 1.2005x (R^2=0.98)
ADF (g kg^{-1} DM)	398.15	396.4	413.08	422.68	12.260	0.082	0.603	NS
Lignin (g kg^{-1} DM)	51.90^{b}	53.58^{b}	63.30^{a}	64.00^{a}	1.267	0.000	0.591	Y= 46.9163 + 0.8161x (R^2=0.80)
IVDMD (g kg^{-1} DM))	785.48^{a}	726.48^{c}	755.80^{b}	717.38^{c}	6.377	0.000	0.088	Y= 798.7738 – 1.2498x (R^2=0.54)
IVOMD (g kg^{-1} DM)	791.03	768.8	785.58	753.83	23.127	0.311	0.816	NS
IVNDFD (g kg^{-1} DM)	730.58^{a}	660.40^{c}	703.50^{b}	665.28^{c}	11.193	0.005	0.126	Y= 735.7775 – 1.0914x (R^2=0.35)
IVADFD (g kg^{-1} DM)	629.83	576.83	628.28	595	12.381	0.290	0.375	NS
Stem								
Dry matter (g kg^{-1})	222.9	224.05	228.03	238.43	5.320	0.073	0.059	NS
Organic matter (g kg^{-1} DM)	916.6	919.73	928.03	918.3	2.711	0.223	0.318	NS
Crude protein (g kg^{-1} DM)	74.33^{a}	51.80^{b}	55.63^{b}	40.18^{c}	2.236	0.000	0.093	Y= 85.0069 – 0.07045x (R^2=0.81)
NDF (g kg^{-1} DM)	778.38^{c}	785.38^{c}	807.70^{b}	834.68^{a}	6.710	0.000	0.110	Y= 744.1380 + 1.3659x (R^2=0.95);
ADF (g kg^{-1} DM)	493.93^{c}	509.05^{b}	527.30^{a}	532.80^{a}	5.539	0.010	0.121	Y= 478.6063 + 0.8848x (R^2=0.81)
Lignin (g kg^{-1} DM)	54.10^{c}	58.03^{b}	60.90^{b}	73.80^{a}	1.600	0.002	0.25	Y= 43.8713 + 0.4286x (R^2=0.86)
IVDMD (g kg^{-1} DM))	664.55^{a}	642.50^{b}	634.38^{b}	598.13^{c}	10.23	0.000	0.438	Y= 697.1050 – 1.4814x (R^2= 0.94);
IVOMD (g kg^{-1} DM)	685.72^{a}	673.50^{a}	646.18^{b}	649.73^{b}	9.658	0.004	0.364	Y= 704.3788 – 0.9666x (R^2=0.85);
IVNDFD (g kg^{-1} DM)	622.75^{a}	594.03^{a}	591.53^{b}	552.40^{b}	12.164	0.001	0.630	Y= 654.2400 – 1.5254x (R^2=0.91);
IVADFD (g kg^{-1} DM)	496.05^{b}	517.43^{a}	521.15^{a}	453.39^{c}	10.215	0.000	0.257	Y= 541.6554 - 1.0785 (R^2=0.87)
Dead material								
Dry matter (g kg^{-1})	464.26	461.43	412.51	464.98	16.032	0.466	0.070	NS
Crude protein (g kg^{-1} DM)	53.90^{d}	40.25^{c}	33.03^{b}	27.32^{a}	1.182	0.000	0.057	Y= 64.7150 – 0.6213x (R^2 = 0.96)
NDF (g kg^{-1} DM)	836.46	810.52	816.97	823.82	9.871	0.427	0.079	NS

Note: NDF= neutral detergent fiber; FDA= acid detergent fiber; IVDMD= *in vitro* dry matter digestibility; IVOMD= *in vitro* organic matter digestibility; IVNDFD= *in vitro* neutral detergent fiber digestibility; IVADFD= *in vitro* acid detergent fiber digestibility.The means were compared by Tukey test at 5% significance. SEM= Standard error of median.

The chemical composition and *in vitro* digestibility of hay are presented in Table 3. There was a linear effect of cutting interval on the DM and OM contents of hay (p= 0.0001 and p= 0.0002, respectively). The CP contents decreased with the increased cutting interval, greater at 21 d and 35 d compared to 49 d and 63 d (p= 0.0013). Nevertheless, the opposite was observed on lignin content, which increased with the increased cutting interval (p= 0.0001). The NDF content was greater at 49 d, intermediate at 35 d and 63 d, and smaller at 21 d (p= 0.0001). The IVDMD reduced with the increased cutting interval (p= 0.0001). In addition, IVNDFD and IVADFD were smaller at 63 d compared to 49, 35, and 21 d (p= 0.0001).

In Vitro Degradation Kinetics

The "lag time" (Figure 2) of the leaf and stem of grass material did not show any effect with cutting interval (p>0.05). Nevertheless, there was a quadratic effect on the lag time of grass hay with the increase of cutting interval (p>0.05).

There was no effect of cutting interval on accumulated gas production from leaf (p>0.05), whereas there was a linear increase in the stem with the increased cutting interval (p<0.05). The hay showed a linear reduction in gas production with the increased cutting intervals where the minimum values were reached with the highest cutting interval (p<0.05; Figure 3).

Protodioscin

There was a linear increase in protodioscin concentration in the leaf of the grass material and hay with the increased cutting interval (p<0.05, Figure 4). Protodioscin concentration in hay increased with the increased cutting interval, which in 63 d cutting interval were 7%, 29%, and 43.5% greater than 49 d, 35 d, and 21 d. There was no effect of the protodioscin concentration on the stem of the grass material with the increase in the cutting interval (p>0.05, Figure 4).

DISCUSSION

The regrowth period is related to the dry matter production, nutritive value, and longevity of pastures (Lemaire *et al.*, 2011). Canopies managed with a greater cutting interval (63 d) resulted in higher forage mass (FM, Table 1), with a lower proportion of leaves and senescence of basal leaves, due to the greater renewal of plant tissues. This process responds to stem elongation, leading to increased canopy height and light competition on the basal canopy strata (Da Silva *et al.*, 2015).

Table 3. Chemical composition of *Brachiaria brizantha* cv. marandu hay according to cutting interval

Item	Days				SEM	P-*value*		Regression equations
	21	35	49	63		Linear	Quadratic	
Dry matter (g kg^{-1} DM)	802.48^{c}	796.48^{c}	846.13^{a}	829.08^{b}	3.450	0.101	0.088	NS
Organic matter (g kg^{-1} DM)	911.63^{b}	907.85^{b}	913.28^{b}	923.25^{a}	1.952	0.456	0.562	NS
Crude protein (g kg^{-1} DM)	91.98^{a}	88.59^{a}	72.43^{b}	68.86^{b}	3.550	0.001	0.383	Y= 98.8013 – 0.4109x (R^2= 0.47)
NDF (g kg^{-1} DM)	731.50^{c}	790.60^{b}	812.83^{a}	789.20^{b}	7.395	0.002	0.115	Y= 693.6554+1.8185x (R^2= 0,84)
ADF (g kg^{-1} DM)	461.98	466.85	476.05	460.58	6.462	0.845	0.094	NS
Lignin (g kg^{-1} DM)	44.65^{c}	58.20^{b}	59.92^{b}	69.21^{a}	1.467	0.000	0.119	Y= 35.3782 + 0.5385x (R^2= 0.92)
IVDMD (g kg^{-1} DM))	684.08^{a}	661.43^{b}	653.03^{bc}	640.00^{c}	6.322	0.000	0.397	Y= 701.8186 – 1.0045x (R^2= 0.96)
IVOMD (g kg^{-1} DM)	705.75	724.23	692.1	709.4	6.662	0.427	0.920	NS
IVNDFD (g kg^{-1} DM)	623.20^{a}	609.48^{a}	611.70^{a}	490.10^{b}	9.974	0.000	0.254	Y= 692.6554-3.2985x (R^2= 0.94)
IVADFD (g kg^{-1} DM)	596.18^{a}	539.98^{a}	565.38^{a}	403.48^{b}	22.423	0.000	0.019	Y= 665.6554-3.2985x (R^2= 0.97)

Note: NDF= neutral detergent fiber; FDA= acid detergent fiber; IVDMD= *in vitro* dry matter digestibility; IVOMD= *in vitro* organic matter digestibility; IVNDFD= *in vitro* neutral detergent fiber digestibility; IVADFD= *in vitro* acid detergent fiber digestibility.The means were compared by Tukey test at 5% significance. NS= not significant; SEM= Standard error of median.

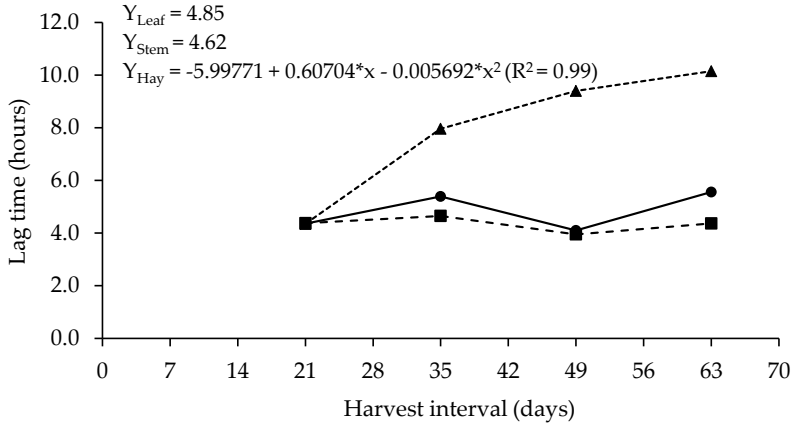

Figure 2. *In vitro* degradation kinetics parameters of grass before haymaking and hay of *Brachiaria brizantha* cv. marandu as a function of the cutting interval. —•— Leaf; --■--Stem; --▲-- Hay.

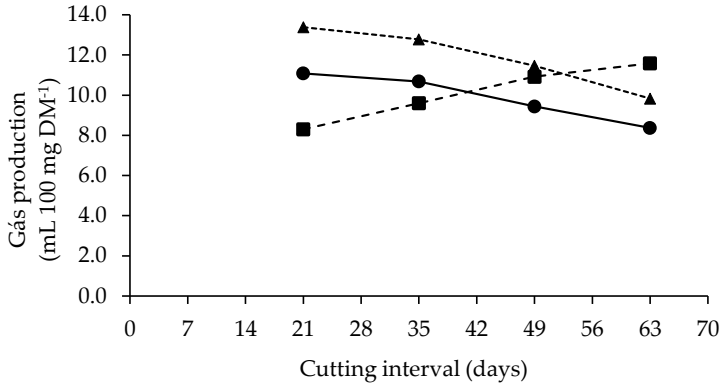

Figure 3. Gas production of grass before hay making and hay of *Brachiaria brizantha* cv. marandu as a function of the cutting interval. —•— Leaf; --■--Stem; --▲-- Hay.

To produce forage and use it more efficiently, it is essential to determine strategies that contemplate appropriate structural characteristics of the canopy, allowing greater participation of leaves and reduction in the processes of stem elongation, resulting in the increase of L:S ratio and decrease of senescence. In our study, the cutting interval of 21 d showed a higher L:S ratio in relation to the other treatments (Table 1).

Despite the lower FM observed in the 21 d cutting interval, which was 60% lower than the 63 d treatment, there was no difference in forage accumulation rate (Table 1). Lower cutting interval leads to a greater

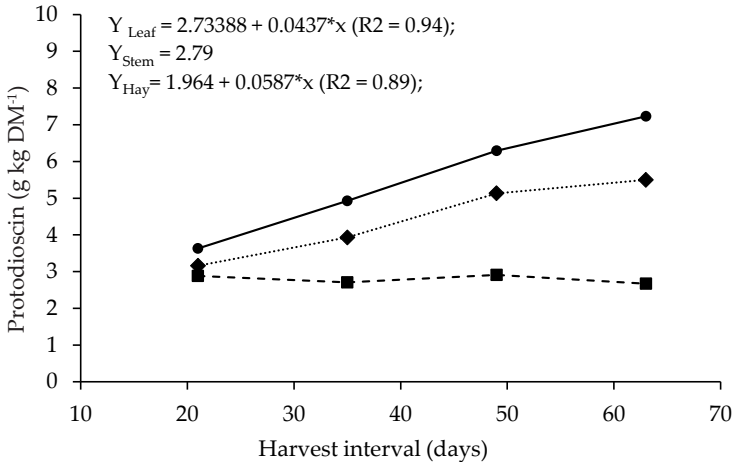

Figure 4. Protodioscin levels of grass before haymaking and hay of *Brachiaria brizantha* cv. marandu as a function of the cutting interval. —•— Leaf; --■--Stem; --▲-- Hay.

renewal of the tissues, thus resulting in more tillering and greater accumulation of forage through the largest amount of leaves produced (Sbrissia *et al.*, 2018). The dynamics of forage accumulation, which directly responds to both pasture and animal productivity, is one of the essential processes for understanding the relationship between defoliation and plant responses (Sbrissia *et al.*, 2018).

The nutritive value of forage is dependent on canopy management (Nave *et al.*, 2013, Coblentz *et al.*, 2017, Habermann *et al.*, 2019). Canopies with a high cutting interval and a longer rest period are characterized by older plants. As physiological maturity advances, the production of potentially digestible components in tillers decreases (Moore *et al.*, 2020). These factors are directly related to the nutritional value of forage found in Table 2. Thus, canopies of 63d had higher NDF, ADF, and lignin levels and lower content of CP, IVDMD, and IVNDFD in leaves and stem before haymaking (Table 2).

The nutritional value of hay follows the same pattern as the fresh material before haymaking. With a higher proportion of stem and dead material in the canopies with longer cutting intervals, the CP content was lower than 21d, negatively influencing the nutritional value of forage.

When comparing the treatments of 21 d (minimum) and 63 d (maximum) cutting intervals, there is a decrease of 6.4%, 21.4%, and 32.3% in IVDMD, IVNDFD, and IVADFD of hay, respectively. This decrease was due to the higher contents of NDF, ADF, and lignin, reducing the proportion of potentially digestible nutrients, resulting in a sharp drop in digestibility.

The greatest gas production of hay occurred in the cutting interval of 21 d, as well as IVDMD, contributing to a greater potential for degradation. This happened due to the high amount of soluble material since the soluble fractions in feed contribute to the production of volatile fatty acids, the main source of energy for ruminants. In the longer cutting intervals, the opposite condition occurred; this can be explained by NDF level and ADF and lignin contents that affected degradability (Tables 2 and 3). The volume of gas produced depends on the composition of the food. Therefore, the greater the fiber amount, the lower gas production (Miranda-Romero *et al.*, 2020).

The increase in the cutting interval allows a longer time for bacteria to attach and colonize the feed (Lag time) in the marandu palisadegrass hay (Figure 2). Lag time indicates the time involved between the beginning of the incubation and the microbial action on the sample; therefore, the greater the presence of readily fermentable soluble substances, the shorter the microbial colonization time (Schofield *et al.*, 1994).

The highest concentrations of protodioscin in the leaf and hay (Figure 4) were observed in 63 d. Brum *et al*. (2007) reported that the period of the highest concentration of protodioscin was during the final phase of the life cycle of marandu palisadegrass and *B. decumbens*. Protodioscin concentrations in hay increased as the cutting intervals advanced. The protodioscin concentration is normally higher in rainy periods, yet outbreaks may occur throughout the year, probably due to unknown processes (Riet-Correa *et al.*, 2011). Leal *et al.* (2020), evaluating five cultivars of *B. brizantha*, found that all grasses showed higher protodioscin content during the autumn. The protodioscin contents varied from 5.6 g kg^{-1} (spring) to 19.2 g kg^{-1} (autumn).

According to Leal *et al*. (2020), protodioscin contents may vary between species and within the same species, depending on the age of the plant and the collection site. However, it is known that the concentration of saponins evaluated only in the laboratory does not correlate with cases of poisoning. They can be subjected to different variables, such as the time of year, resistance, individual sensitivity, type and category of animals, age of the forage, period, and storage conditions (Riet-Correa *et al.*, 2011).

Liver toxicity from protodioscin in ruminants grazing on some Brachiaria grass pastures is problematic. However, the use of haymaking may reduce the protodioscin levels in forage (Lima *et al.*, 2015). The levels of protodioscin before haymaking were lower in the hay

with fresh material (Figure 4). Protodioscin levels in fresh leaves, which are the most consumed by grazing animals, were higher in relation to hay, with an average of 4.57 $g.kg^{-1}$ and 4.43 $g.kg^{-1}$, respectively. The reduction of saponin during haymaking was observed in the literature (Lima *et al.*, 2015), yet it was not well explained until now.

CONCLUSION

Based on marandu palisadegrass hay, our study demonstrated better conditions to develop a productive canopy associated with better nutritive value and lower protodioscin levels when forage was harvested at 21 d cutting interval the shorter harvest cutting intervals.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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