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Influence of protodioscin content on digestibility and *in vitro* degradation kinetics in *Urochloa brizantha* cultivars

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Abstract. Five cultivars of *Urochloa brizantha* (Hochst. ex A.Rich.) R.D. Webster (syn. of *Brachiaria brizantha*) were evaluated for nutritional value and an anti-nutritional factor (protodioscin), in order to determine whether protodioscin content was correlated with reduced feed quality. We evaluated cvv. Arapoty, Paiaguas, Xaraes, Marandu and Piata and grouped the results into summer, autumn, winter and spring seasons. Protodioscin content and chemical composition of leaves, *in vitro* digestibility and cumulative gas production were analysed. Data were evaluated by analysis of variance as a completely randomised experimental design in a factorial arrangement (five cultivars × four seasons). There was no significant interaction between cultivar and season. All grasses showed highest protodioscin contents during autumn. Protodioscin contents ranged from 5.6 g kg^{-1} (spring) to 19.2 g kg^{-1} (autumn). Crude protein content varied significantly across seasons. No significant effect was detected for neutral detergent fibre (NDF) content among seasons. Cultivar Arapoty showed the highest NDF content in summer (669.3 g kg^{-1}) and the lowest in spring (601.9 g kg^{-1}). The best *in vitro* digestibility coefficients were observed in spring. The protodioscin content of *U. brizantha* cultivars can negatively affect their digestibility and some parameters of cumulative *in vitro* gas production.

Additional keywords: C_4 grasses, *in vitro* techniques, palisade grass, tropical crops.

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Introduction

In Brazil, grasses of the genus *Urochloa* represent ~90% of cultivated pastures (Moreira *et al.* 2009). *Urochloa brizantha* (Hochst. ex A.Rich.) R.D. Webster (syn. of *Brachiaria brizantha*; palisade grass) cv. Marandu is one of the main forages used in the Central-West region of the country (Moreira *et al.* 2009) owing to its high nutritional value.

Grasses of the genus *Urochloa* contain toxins, a primary one being protodioscin, a steroidal saponin that causes changes in the liver parenchyma and bile ducts, leading to disturbances in the mechanism of elimination of phylloerythrin, a photodynamic pigment (Brum *et al.* 2007), and eventually causing photodermatitis, considered the most obvious clinical manifestation of intoxication (Mustafa *et al.* 2012). According to de Melo *et al.* (2018), pastures comprising forages with low protodioscin contents (0.3–1.3%) may cause intoxication in suckling lambs. Supplementation with high-protein concentrates (21.9 g kg^{-1} body weight) in a creep-feeding system is a nutritional strategy that can reduce the effects of intoxication in suckling lambs raised on *Urochloa* pastures.

Grazing of *Urochloa decumbens* (Stapf) R.D. Webster (syn. of *Brachiaria decumbens*) is considered the main cause of hepatogenous photosensitivity (Lemos *et al.* 1998; Brum *et al.* 2007; Mendonça *et al.* 2008; Saturnino *et al.* 2010; Porto *et al.* 2013). However, cases of poisoning in buffaloes (Riet-Correa *et al.* 2010), sheep (Albernaz *et al.* 2010; Mustafa *et al.* 2012; Faccin *et al.* 2014) and cattle (Souza *et al.* 2010) have been reported to arise from overuse of *U. brizantha*.

Leal *et al.* (2016) observed a higher protodioscin content throughout the year in *U. decumbens* cv. Basilisk than in cv. D70 (31.4 vs 27.4 g kg^{-1}) and demonstrated a significant negative correlation (–0.22) between protodioscin content and lag time. Additionally, the authors reported a negative correlation (–0.25) between protodioscin content and total gas production.

Urochloa brizantha has been widely used because of its high nutritional quality (Euclides *et al.* 2009) and is considered less toxic than *U. decumbens* (Faccin *et al.* 2014) because of lower protodioscin content (Wina *et al.* 2005). However,

photodermatitis described in the literature in relation to *U. brizantha* mentions only the species, not the cultivar involved. Because cv. Marandu is one of the most widely used tropical grasses in Brazilian pasture areas (Euclides *et al.* 2009), those outbreaks are believed to be related to this cultivar. Studies on other *U. brizantha* cultivars are warranted to determine the anti-nutritional potential of these grasses.

Our hypothesis was that the protodioscin content can reduce the digestibility and fermentation of leaves of tropical grasses. The present study was thus conducted to examine the anti-nutritional potential of protodioscin present in leaves of five cultivars of *U. brizantha* by evaluating digestibility and *in vitro* degradation kinetics.

Materials and methods

The experiment was performed at the Laboratory of Biotechnology and Animal Nutrition, Dom Bosco Catholic University, and at the Laboratory of Natural Product Chemistry, Department of Chemistry, Federal University of Mato Grosso do Sul, in Campo Grande, Brazil. Samples of green leaves of *U. brizantha* harvested from experimental plots belonging to the Brazilian Agricultural Research Corporation (Embrapa), Campo Grande, Brazil, were used in the experiment. The plants were harvested in the morning, every 30 days (monthly), from 20 flower beds. Four samples were collected per flower bed. Each bed measured 5 m by 5 m and contained one of five *U. brizantha* cultivars: Arapoty, Xaraes, Piatã, Marandu and Paiaguas.

For interpretation, comparison and presentation, the results of monthly samplings were grouped by season: summer (January–March), autumn (April–May), winter (June–August) and spring (September–October). Green-leaf samples were collected monthly as a simulation of the grazing activity performed by ruminants, following the methodology proposed by Moraes *et al*. (2005).

After collection, the samples were dried in a forced-air oven at 55°C for 96 h and ground through a 1-mm mesh screen. Dry matter (DM), organic matter (OM), crude protein (CP) and ether extract contents of the grasses were determined according to AOAC (2000) methods 930.15, 942.05, 976.05 and 920.39, respectively. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were determined without sodium sulfite or thermostable amylase.

The buffer solution was prepared in the same way for the two *in vitro* trials (i.e. digestibility and cumulative gas production). Solution A contained (per L) 10.0 g KH_2P0_4, 0.5 g $MgSO_4.7H_2O$, 0.5 g NaCl, 0.1 g $CaCl_2.2H_2O$ and 0.5 g urea. Solution B contained (per 100 mL) 5.0 g Na_2CO and 1.0 g $Na_2S.9H_2O$. Solutions A and B were mixed at a 1 : 5 ratio. The pH was 6.8 and temperature 39°C. *In vitro* digestibility (IVD), a technique developed by Tilley and Terry (1963), was adapted to the artificial rumen developed by ANKOM Technology (Macedon, NY, USA), as described by Holden (1999), using the methodology of the rumen fermenter (anaerobic incubator, MA443; Marconi Laboratory Equipment, Piracicaba SP, Brazil).

Samples (~0.5 g) were weighed in triplicate and placed in TNT (non-woven fabric) filter bags of dimensions 5.0 cm by 5.0 cm and density 100 g m^{-2}. Samples were placed in glass jars and kept in an incubator at a controlled temperature (39°C) after addition of the buffer solution (pH 6.8) and inoculum from three rumen-fistulated cows kept on *U. brizantha* pastures. After 72 h of incubation, the bags were washed in running water until they showed a clear appearance, rinsed at a pH 7 for drying, and then weighed.

After correction by using a blank bag, the IVD coefficients of DM, OM, NDF and ADF were calculated based on the difference between the amount of nutrients incubated and the amount in the residue: IVD = ((g incubated substrate – (g residual substrate – g blank))/g substrate incubated) × 100.

Protodioscin content was determined by high-performance liquid chromatography, using an evaporative light scattering detector (ELSD; Shimadzu, Kyoto), according to the technique of Ganzera *et al*. (2001).

For *in vitro* gas production, samples (0.5 g) were weighed in triplicate and incubated with buffer solution (pH 6.8) and inoculum from the three rumen-fistulated cows kept on *U. brizantha* pastures. Feed fermentation kinetics was evaluated for 72 h through a gas-production process, using a wireless computer system with a pressure transducer and communication by radio frequency (RF Gas Production System; ANKOM Technology). Pressure data (psi) were collected every 5 min and processed for cumulative gas produced (mL gas 100 mg^{-1} DM incubated). The parameters of gas-production kinetics were obtained via the two-compartment logistic model proposed by Schofield *et al*. (1994), as follows:

$$V_{\text{total}} = (V_{\text{rf}}/1 + \text{e}^{(2+4K_{\text{rf}}(t-L))}) + (V_{\text{sf}}/1 + e^{(2+4K_{\text{sf}}(t-L))})$$

where V_{total} (mL) is the total volume of gas produced *in vitro* at time t (h); V_{rf} (mL) is the total volume of gas produced *in vitro* from the degradation of the rapidly fermentable feed fraction of the Cornell System (A + B1); K_{rf} (h^{-1}) is the fractional rate of gas produced *in vitro* from the degradation of the rapidly fermentable feed fraction; V_{sf} (mL) is the total volume of gas produced *in vitro* from the degradation of the slowly degradable fraction (B2); K_{sf} (h^{-1}) is the fractional rate of gas produced *in vitro* from the degradation of the slowly fermentable feed fraction; and L (h) is the lag time before fermentation begins. The Gauss–Newton procedure was used in the cumulative gas production trial to estimate the fermentation parameters. Data were analysed as a completely randomised experimental design in a factorial arrangement of five cultivars × four seasons, using the MIXED procedure of SAS (SAS Institute, Cary, NC, USA).

Chemical composition and IVD data were analysed by one-way analysis of variance as a completely randomised experimental design in a factorial arrangement of five cultivars × four seasons, using the General Linear Models procedure of SAS. Significance was declared if $P < 0.05$. When significance was detected by the F-test, multiple comparisons were made by using Tukey's adjustment for probability. Pearson's correlation was used to determine the

strength of the correlations between variables analysed using the CORR procedure of SAS.

Data were analysed using the following model:

$$Yijk = \mu + Ci + Sj + C \times Sk + eijk$$

where Y*ijk* is the dependent variable; μ is the overall mean; C*i* is the effect of cultivar *i* (*i* = 1, . . ., 5); S*j* is the effect of season of the year *j* (*j* = 1 (summer, January–March), 2 (autumn, April–May), 3 (winter, June–August), or 4 (spring, September–October); C × S*k* is the interaction effect between cultivar and season; and e*ijk* is random error, NID assumption (0, σ2e).

Results

There was no significant cultivar × season of the year interaction effect for any of the evaluated variables. There was a significant difference in protodioscin content among all cultivars ($P < 0.001$) and across seasons ($P < 0.05$). All cultivars had higher protodioscin contents in autumn. Protodioscin contents ranged from 5.6 g kg^{-1}, which was found in cv. Xaraes in spring, to 19.2 g kg^{-1}, which was found in cv. Arapoty in autumn (Table 1).

There was a significant difference ($P < 0.01$) in leaf OM content across seasons for cvv. Arapoty, Xaraes, Marandu and Piata (Table 1). There were significant variations ($P < 0.05$) in CP content across seasons for Arapoty and Xaraes; for example, cv. Arapoty had 104.7 g CP kg^{-1} in summer and 130.6 g CP kg^{-1} in spring.

For cv. Arapoty, there were significant differences ($P < 0.01$) across seasons for NDF, with highest NDF content observed in summer (669.3 g kg^{-1}) and lowest in spring (601.9 g kg^{-1}) (Table 1). Arapoty and Xaraes showed significant variation ($P < 0.05$) across seasons for ADF content. In summer, Xaraes exhibited the highest ADF content (395.6 g kg^{-1}) and Marandu the lowest (265.5 g kg^{-1}) among cultivars ($P < 0.001$).

Among cultivars, Xaraes showed the highest IVD coefficients ($P = 0.0001$) for DM and NDF in all seasons. Paiaguas presented the highest IVD values for OM in autumn (761.0 g kg^{-1}) and spring (757.9 g kg^{-1}); cv. Arapoty showed the highest mean for this variable in winter (760 g kg^{-1}). Arapoty showed significant differences across seasons for IVD of DM ($P < 0.01$) and NDF ($P < 0.05$). Piata showed significant variation ($P < 0.05$) across seasons for IVD of OM (Table 2).

Table 1. Protodioscin content and chemical composition (g kg^{-1} DM) of leaves of five *Urochloa brizantha* cultivars
For each parameter, means followed by the same lowercase letter within columns, and uppercase letter within rows, are not significantly different by Tukey's test (at $P = 0.05$)

	Cultivar					*P*-value
	Arapoty	Paiaguas	Xaraes	Marandu	Piata	
			Protodioscin			
Summer	11.3bA	8.2bcB	7.9abC	7.8bC	7.9bC	0.0001
Autumn	19.2aA	14.7aB	10.0aC	11.6aC	12.0aC	0.0001
Winter	12.8bA	12.8abA	8.5abB	10.6abB	9.6abB	0.0001
Spring	9.5bA	9.5cA	5.6bC	7.8abB	8.0bAB	0.0001
P-value	0.00491	0.00100	002861	0.02553	0.00439	
			Organic matter			
Summer	928.1aA	917.5B	911.1bB	937.3aA	931.2aA	0.0001
Autumn	910.9bD	917.9C	923.4aB	928.3bA	924.0bcB	0.0001
Winter	906.8bB	913.6AB	910.4bB	920.2cA	920.3cA	0.0001
Spring	911.1bC	914.4C	913.5bC	921.8dB	928.0abA	0.0001
P-value	0.0001	0.4088	0.0026	0.0001	0.0015	
			Crude protein			
Summer	109.0bB	114.4A	104.7aB	78.9D	87.9C	0.0001
Autumn	109.1bA	100.2A	81.1cB	77.4B	63.4D	0.0001
Winter	123.0abA	107.8B	92.9bC	74.7D	68.9D	0.0001
Spring	130.6aA	106.5B	97.0abC	71.7D	73.5D	0.0001
P-value	0.0128	0.2213	0.0001	0.4010	0.1188	
			Neutral detergent fibre			
Summer	669.3aA	676.3A	700.5A	492.4C	615.3B	0.0001
Autumn	626.2abB	651.2A	676.9A	570.3C	574.7C	0.0001
Winter	613.0bC	659.0B	684.6A	551.5D	569.3D	0.0001
Spring	601.9bC	659.9B	675.1A	550.6D	565.3D	0.0001
P-value	0.0028	0.4055	0.1412	0.1213	0.2426	
			Acid detergent fibre			
Summer	375.0aA	351.4B	395.6aA	265.5C	325.4B	0.0001
Autumn	345.5abB	355.0A	365.2bA	305.1C	295.5C	0.0001
Winter	339.0abB	355.7AB	367.2bA	293.4C	289.5C	0.0001
Spring	327.6bB	347.5B	373.6bA	284.7C	284.5C	0.0001
P-value	0.0469	0.4023	0.0022	0.2943	0.2351	

Table 2. Digestibility (g kg^{-1}) of leaves of *Urochloa brizantha* cultivars
For each parameter, means followed by the same lowercase letter within columns, and uppercase letter within rows, are not significantly different by Tukey's test (at $P = 0.05$)

	Cultivar					*P*-value
	Arapoty	Paiaguas	Xaraes	Marandu	Piata	
			In vitro *digestibility of dry matter*			
Summer	675.3bB	724.3B	794.6A	750.8AB	684.4B	0.0001
Autumn	771.7aB	760.0B	798.6A	748.0BC	708.2C	0.0001
Winter	770.6aB	737.3C	816.2A	728.1CD	714.3D	0.0001
Spring	771.8aAB	771.1AB	836.6A	762.5B	741.1B	0.0001
P-value	0.0074	0.1944	0.4075	0.4450	0.1765	
			In vitro *digestibility of organic matter*			
Summer	715.7A	707.0A	676.6B	657.5B	538.3bC	0.0001
Autumn	712.9B	761.0A	660.0C	696.4BC	588.3abD	0.0001
Winter	760.4A	721.8B	676.4C	712.0B	607.0aD	0.0001
Spring	757.4A	757.9A	705.1BC	727.6B	640.0aC	0.0001
P-value	0.3620	0.1657	0.3114	0.2815	0.0053	
			In vitro *digestibility of neutral detergent fibre*			
Summer	607.5bC	648.9B	754.3A	551.1D	529.1D	0.0001
Autumn	724.5aA	683.3B	752.3A	623.1BC	543.0C	0.0001
Winter	717.3aAB	655.1B	768.8A	568.7C	549.0C	0.0001
Spring	722.9aB	704.4B	791.6A	626.2C	582.0D	0.0001
P-value	0.0160	0.1548	0.4069	0.1317	0.2178	
			In vitro *digestibility of acid detergent fibre*			
Summer	462.3bD	648.4bB	712.0A	563.8C	554.3bC	0.0001
Autumn	681.2aB	704.0abA	686.6B	629.4BC	580.7abC	0.0001
Winter	675.1aB	677.6abB	709.0A	576.2C	578.1abC	0.0001
Spring	677.7aB	719.4aAB	753.5A	628.8BC	614.7aC	0.0001
P-value	0.0001	0.0379	0.4016	0.1273	0.0872	

In autumn, cv. Xaraes showed its highest seasonal protodioscin content (10.0 g kg^{-1}; Table 1) and the lowest total gas production among cultivars (V_{total}, 6.84 mL gas 100 mg^{-1} DM; Table 3). Fraction K_{rf}, which corresponds to the slowly degradable fraction, showed a negative correlation between protodioscin and fraction V_{sf} (B2). In winter, V_{total} averaged 10.84 mL 100 mg^{-1} DM over all cultivars, which was the highest seasonal average. By contrast, in spring, the V_{total} average was the lowest at 8.33 mL 100 mg^{-1} DM.

In autumn, in addition to elevated seasonal protodioscin content in cv. Piata (12 g kg^{-1}), its lag time (L) of 9.63 h indicated a longer time for bacterial colonisation. In spring, Piata showed lower seasonal protodioscin content as well as its shortest seasonal lag time (4.69 h).

Protodioscin content was negatively correlated with leaf quality variables, which could interfere with the utilisation dynamics of NDF (–0.03), IVD of DM (–0.09) and lag time (–0.08). There was a significant ($P < 0.05$) negative correlation (–0.11) between NDF and IVD of DM. Conversely, there was a significant ($P < 0.05$) positive correlation between CP and IVD of DM (0.34), IVD of OM (0.60) and V_{total} (0.15), which suggests that the presence of CP positively influences the digestibility of DM and OM without markedly affecting total cumulative gas production (Table 4).

Five principal components (PC) were generated in the analysis of the variables. The results indicated that 84% of the variation in the dataset was explained by only two PCs (Fig. 1). Additionally, no similarity was observed between cultivars as evidenced by their equidistribution in the positive and negative quadrants. The first PC explained 54% of the total variation of the data and revealed that higher protodioscin contents meant lower IVD of DM, and that the protodioscin content is highly associated with cv. Arapoty.

Discussion

The protodioscin contents ranged from 5.6 to 19.8 g kg^{-1}, the latter value being considered toxic to farm animals. The highest protodioscin contents were found in autumn and winter, regardless of the evaluated cultivar, which shows that protodioscin content may be influenced by the environment and by the physiological state of the plant. Brum *et al*. (2009) evaluated *U. brizantha* at 56, 96, 141 and 218 days of age and found protodioscin contents of 0.5%, 1.0%, 0.8% and 2.1%, respectively. The literature describes cases of poisoning by *U. brizantha* (Albernaz *et al.* 2010; Souza *et al.* 2010; Faccin *et al.* 2014), regardless of season.

Leal *et al*. (2016) found different levels of protodioscin among the grasses *U. humidicola* (1.1 g kg^{-1}), *U. decumbens* (25.9 g kg^{-1}) and *B. ruziziensis* (8.5 g kg^{-1}). By comparing those results and the average obtained in the present study with *U. brizantha* (10.3 g kg^{-1}), we observe that intoxication by cultivars of *U. brizantha* is lower than the hepatic photosensitisation caused by *U. decumbens* cultivars. This statement is in line with the report by de Melo *et al*. (2018), who found a total protodioscin content of 7 g (± s.d. 3) kg^{-1} DM

Table 3. Parameters of *in vitro* degradation kinetics of *Urochloa brizantha* cultivars

V_{total}, Total volume of gas produced *in vitro* at time *t*; V_{rf}, total volume of gas produced *in vitro* from the degradation of the rapidly degradable fraction of the Cornell System (A + B_1); K_{rf}, fractional rate of gas produced *in vitro* from the degradation of the rapidly fermentable feed fraction; V_{sf}, total volume of gas produced *in vitro* from the degradation of the slowly degradable fraction (B_2); K_{sf}, fractional rate of gas produced *in vitro* from the degradation of the slowly fermentable feed fraction; *L*, lag time before fermentation begins; R^2, coefficient of determination. Within rows, means followed the same letter are not significantly different by Tukey's test (at $P = 0.05$)

Parameter	Cultivar					*P*-value
	Arapoty	Paiaguas	Xaraes	Marandu	Piata	
			Summer			
V_{rf} (mL)	1.34b	1.52ab	1.87a	1.13b	1.48ab	0.0001
K_{rf} (h^{-1})	0.91ab	0.91ab	0.89b	0.92 a	0.89b	0.0001
L (h)	7.59b	6.20b	7.22b	6.60b	11.98a	0.0001
V_{sf} (mL)	7.65b	8.05b	10.24a	8.47b	9.14ab	0.0001
K_{sf} (h^{-1})	0.045ab	0.057a	0.027b	0.036b	0.034b	0.0001
V_{total} (mL)	9.00b	9.57b	12.11a	9.60b	10.62ab	0.0001
R^2	0.88	0.94	0.97	0.97	0.99	
			Autumn			
V_{rf} (mL)	1.99a	1.99a	0.44d	1.55b	0.93c	0.0001
K_{rf} (h^{-1})	0.99a	0.99a	0.94b	0.89c	0.93b	0.0001
L (h)	3.00c	3.00c	5.49b	8.25a	9.63a	0.0001
V_{sf} (mL)	11.46a	10.02b	6.40c	10.20b	6.67c	0.0001
K_{sf} (h^{-1})	0.050b	0.051b	0.054b	0.032c	0.099a	0.0001
V_{total} (mL)	13.46a	12.02b	6.84c	11.75b	7.60 c	0.0001
R^2	0.93	0.98	0.93	0.99	0.98	
			Winter			
V_{rf} (mL)	1.62b	0.28c	0.04c	1.99a	1.62b	0.0001
K_{rf} (h^{-1})	0.96b	0.92c	0.98a	0.99a	0.91c	0.0001
L (h)	4.46ab	5.65a	3.01b	3.00b	5.49a	0.0001
V_{sf} (mL)	11.19a	9.49b	9.78ab	8.56b	9.62b	0.0001
K_{sf} (h^{-1})	0.036a	0.035a	0.017b	0.030a	0.034a	0.0001
V_{total} (mL)	12.81a	9.77b	9.82b	10.56b	11.25ab	0.0001
R^2	0.95	0.99	0.94	0.99	0.94	
			Spring			
V_{rf} (mL)	1.99a	1.05c	1.45bc	1.74ab	1.63ab	0.0001
K_{rf} (h^{-1})	0.94b	0.99a	0.89c	0.94b	0.96ab	0.0001
L (h)	4.51ab	3.00b	4.92a	5.16a	4.69a	0.0002
V_{sf} (mL)	7.74a	5.72d	6.71bc	7.30ab	6.31cd	0.0001
K_{sf} (h^{-1})	0.044bc	0.049ab	0.023d	0.032cd	0.060a	0.0001
V_{total} (mL)	9.74a	6.78d	8.17bc	9.04ab	7.94c	0.0001
R^2	0.98	0.98	0.98	0.93	0.91	

Table 4. Pearson correlations between protodioscin, crude protein (CP) and neutral detergent fibre (NDF) contents, *in vitro* digestibility (IVD) of dry matter (DM) and organic matter (OM), lag time and total gas volume (V_{total})

*$P < 0.05$

	Protodioscin	CP	NDF	IVD of DM	IVD of OM	Lag time	V_{total}
Protodioscin	1.00	0.03	–0.03	–0.09*	0.03	–0.08*	0.35*
CP		1.00	0.22*	0.34*	0.60*	–0.14*	0.15*
NDF			1.00	–0.11*	0.04	–0.11*	–0.04
IVD of DM				1.00	0.44*	–0.24*	–0.05
IVD of OM					1.00	–0.36*	0.06*
Lag time						1.00	–0.01
V_{total}							1.00

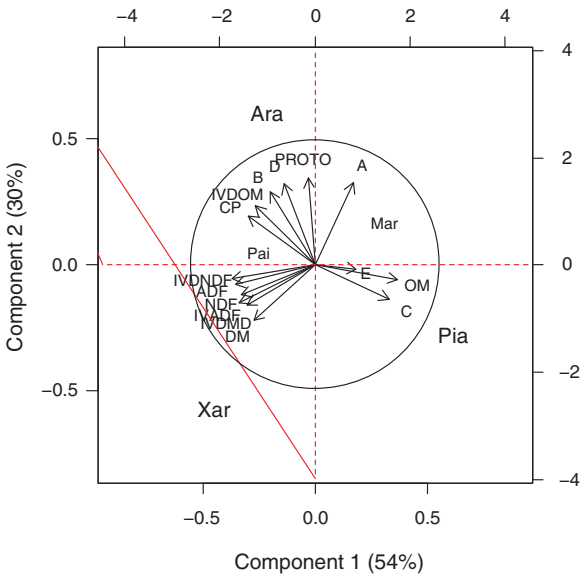

Fig. 1. Biplot of the first (*x*-axis) and second (*y*-axis) principal components. PROTO, Protodioscin; DM, dry matter; MM, mineral matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; IVDMD, *in vitro* dry matter digestibility; IVNDFD, *in vitro* neutral detergent fibre digestibility; IVADFD, *in vitro* acid detergent fibre digestibility; IVOMD, *in vitro* organic matter digestibility; Ara, Arapoty; Pai, Paiaguas; Xar, Xaraes; Mar, Marandu; Pia, Piata.

in paddocks of *Brachiaria* spp. The protodioscin contents in the pasture during clinical cases of intoxication were 3 and 13 g kg^{-1} in April and June, respectively (de Melo *et al.* 2018). Those contents are lower than the contents we observed in autumn for cvv. Arapoty and Paiaguas. According to de Melo *et al.* (2018), cases of intoxication occurred both when the protodioscin contents increased and after they decreased. The results characterise the cumulative effect of protodioscin as a mechanism of adaptation of the animal to the main toxin (Faccin *et al.* 2014; Pupin *et al.* 2016).

However, previous studies have shown that *U. decumbens* and *U. brizantha* may have similar protodioscin contents and be equally toxic to animals. These factors vary according to plant physiological stage, time of the year (Brum *et al.* 2007) and sensitivity of species (Riet-Correa *et al.* 2011), as well as the individual's sensitivity to intoxication (Pupin *et al.* 2016).

In *U. decumbens* pastures, cases of intoxication by protodioscin have been reported in sheep at protodioscin contents ranging from 4.4 to 23.6 g kg^{-1} (Brum *et al.* 2007; Porto *et al.* 2013; Pupin *et al.* 2016). Cases of poisoning have also been reported in sheep raised in *U. brizantha* pastures at protodioscin contents ranging from 3.3 to 25.8 g kg^{-1} (Albernaz *et al.* 2010; Mustafa *et al.* 2012; Faccin *et al.* 2014).

In both the summer and spring, the grasses had lower leaf protodioscin contents than in autumn and winter, demonstrating an inverse relationship whereby lower protodioscin content meant higher leaf protein contents and vice-versa (Table 1). This reveals a need for providing concentrate feeds, because ADF does not contain partially digestible polysaccharides, and grasses have a lower proportion of better quality fractions (leaves) than forage

legumes. An increase in ADF content implies a reduction in forage digestibility due to the presence of structural tissues. Protodioscin contents may vary according to several factors such as time of the year, hardiness, individual sensitivity and type of pasture, animal category and species, as well as the age of the plant material, storage time and storage conditions (Riet-Correa *et al.* 2011).

The lower gas production of fraction V_{rf} (A + B1) occurs because this fraction is rapidly degraded and is associated with soluble sugars and starch. Grasses are composed mostly of cellulose hemicellulose, which correspond to fraction D (slowly degradable) (V_{sf}, Table 3). According to Cabral *et al.* (2000), in tropical grasses, especially at a stage of advanced maturity, the NDF fraction contributes to increasing the proportion of gases produced during incubation. The *in vitro* gas-production method is more efficient than the *in situ* method for evaluating the effects of anti-nutritional factors. During the *in situ* method, these factors are diluted in the rumen after being released through the nylon bags, thus not significantly affecting ruminal fermentation (El-Waziry *et al.* 2007). Castro *et al*. (2007) evaluated the degradation kinetics and ruminal fermentation of cv. Marandu at different stages of maturity and observed that the best harvest time is at 28–56 days.

When the first two PCs explain >60% of the variation of the data, it is recommended to discard the other generated components (Da Silva and Sbrissia 2010). The main function of principal component analysis (PCA) is to reduce the dimensionality of the dataset by retaining as much information as possible in a smaller number of PCs. Therefore, although there were a few differences between cultivars according to univariate statistical techniques, PCA can detect differences through combinations of variables that explain the total data variation (Da Silva and Sbrissia 2010). It is important to note that, in PCA, the isolated effect of blocks is not taken into account. The conclusions drawn with PCA are similar to those obtained with conventional univariate techniques.

All cultivars presented higher protodioscin contents in autumn. There was a negative correlation between protodioscin content and degradability, which reduces the quality of the grasses. Cultivar Arapoty showed high levels of the saponin protodioscin. Cultivar Xaraes is a high-quality grass, based on the IVD results. The protodioscin content of *U. brizantha* cultivars can negatively affect IVD, extending the time for bacterial colonisation.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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