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Article

Brachiaria grasses *in vitro* digestibility with bovine and ovine ruminal liquid as inoculum

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Abstract:

It was hypothesized that it is possible that inoculum from different ruminant species with different digestive abilities feeding from a certain forage may show different feed utilizations comparing to other ruminant species. Five *Brachiaria* grasses were evaluated: *B. decumbens* cv. Basilisk, *B. decumbens* access D70, *B. humidicola* cv. Tupi, *B.*

humidicola cv. Common, and *B. ruziziensis* access R124, at two regrowth ages (21 and 42 d). Production, bromatological content, *in vitro* dry matter digestibility (*iv*DMD) and *in vitro* neutral detergent fiber digestibility (*iv*NDFD) were analyzed using bovine or ovine inoculums. The experiment used a 5 × 2 × 2 factorial design and found significant effects for grass variety and regrowth age. In addition, significant interactions from grass × age on dry matter, crude protein, neutral detergent fiber and acid detergent fiber of total sample and leaf blade were found. There were significant effect of grass variety and grass age on forage mass, leaf blade/stem ratio, leaf blade, stem, senescent material and growth. *In vitro* digestibility assays of inoculum source showed significant effect in some varieties. Due to differences in *in vitro* assays, it was recommended the use of species-specific inoculums for feed evaluations according to the animal it is intended for. Also, *B. decumbens* cv. Basilisk presented the best *in vitro* digestibility (*iv*DMD and *iv*NDFD) in bovine inoculum, whereas *B. humidicola* cv. Tupi had better *in vitro* digestibility (*iv*DMD and *iv*NDFD) in ovine inoculum.

Key words: *Brachiaria decumbens*, *Brachiaria humidicola*, *Brachiaria ruziziensis*, digestibility, Rumen inoculum.

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Introduction

Brachiaria grasses are important because they enable ruminant production in acid soils of low fertility[1]. This genus, mainly from tropical and subtropical Africa, is comprised of approximately 100 species, including *B. decumbens*, *B. humidicola* and *B. ruziziensis*, which are widely used as forage sources in tropical America.

The evaluation and subsequent recommendation of a specific forage is determined by its ability to support grazing by certain animals of different species or categories and its nutritional value. One of the methods from which its nutritional value that can be inferred is to submit it to *in vitro* digestibility testing. *In vitro* is an alternative to *in vivo* and *in situ* techniques[2], requiring fewer animals, reducing costs and is a reliable method to evaluate feedstuff digestibility.

Nutrient components are closely correlated with the digestibility of forages[3]. Through bromatological analysis, it is possible to estimate nutrient components of forages, as well as the cellular content and structural components. These components include crude protein (CP), soluble content and neutral detergent fiber (NDF).

The *in vitro* digestibility technique has been widely used in the analysis of different types of feedstuffs provided to ruminants. However, it can be affected by the inoculum source, as well as the previous diet from the donor animal, the fasting time of animal before sampling, and occasionally by flaws in the execution of the technique[4].

It is possible that different ruminant species show different digestibility, and when being fed a certain forage they may show better-feed utilization than other ruminant species. Therefore, the aim of this research was to evaluate five *Brachiaria* grasses of two regrowth ages, submitted to *in vitro* digestibility assay using two different inoculums (bovine and ovine).

Material and methods

Ethical considerations

This study was carried out in strict accordance with the recommendations of the Guide for the National Council for Animal Experiments Control of Brazil. The experiment was approved by the Committee on Ethics of Animal Experiments of the Federal University of Mato Grosso do Sul, Mato Grosso do Sul State, Brazil (Protocol Number: 367/2011).

Location and experimental field of *Brachiaria* spp. cultivars

This study was carried out at the Federal University of Mato Grosso do Sul in partnership with the Biotechnology Laboratory Applied to Animal Nutrition at Dom Bosco Catholic University and Embrapa Beef Cattle. *Brachiaria* grasses were evaluated in experimental plots at Embrapa Beef Cattle (latitude 20°27'S, longitude 54°37'W and 530 m altitude, located in Campo Grande, MS, Brazil). The type of soil in the study area was Dystrophic Purple Latosol alic.

The climate according to Köppen & Geiger[5] classification is rainy tropical, AW subtype, characterized by a well-defined occurrence of a dry period during the colder months of the year (April–September) and a rainy season during the summer months (October–March) with an average annual rainfall of 1,469 mm and an average annual temperature of 23 °C.

Forages were evaluated during two consecutive summers (December–February), which is the rainy season in the Brazilian Cerrado, due to the seasonality of the forages. The experimental area consisted of 20 plots (experimental units, four plots/cultivar),

measuring 4.0 × 4.0 m (16 m^2). The plots were cut at 5 cm above the ground in order to standardize them for evaluation.

Five cultivars of *Brachiaria* grasses were evaluated in each plot: *B. decumbens* cv. Basilisk; *B. decumbens* access D70; *B. humidicola* cv. Tupi; *B. humidicola* cv. Common and *B. ruziziensis* access R124. Each plot was divided into two parts, to be cut with 21 and 42 d of regrowth. Forage samples were collected by cutting at 5 cm from the ground inside a square 0.5 × 0.5 m (0.25 m^2) and sampled with five repetitions from the four plots of each cultivar, obtaining a composite sample from each forage.

Forage mass and growth determination

After forages were sampled, each was wrapped in a plastic bag and identified. In the laboratory they were weighed and divided into two parts: one to be processed as total sample and the other separated into leaf blade, stem and dead material[6]. Forage mass was estimated by the square method, with the quantification of forage, on a dry matter basis, sampled inside the 0.5 × 0.5 m square converted to metric ton (1,000 kg) per hectare (t ha^{-1}).

The leaf blade/stem ratio was obtained by dividing the mass of the leaf blades by the mass of the stems[7]. The vegetative canopy growth was determined at six different points in each experimental unit, which were marked for measurement according to the different growth ages evaluated[7].

Forage chemical composition

After sampling and separating into total sample, leaf blade, stem and dead material, materials were pre-dried at 55°C for 72 h and ground to 1 mm with a Wiley Mill (Tecnal, Piracicaba City, São Paulo, Brazil) then stored in hermetic plastic containers (ASS, Ribeirão Preto City, São Paulo, Brazil) until analysis. Dry matter (DM) content (Method 967.03, AOAC[8]) and CP content (Method 981.10, AOAC[8]) were determined. For the determination of the NDF and acid detergent fiber (ADF) content, the methodology of Van Soest *et al*[3] was used with modifications proposed in the ANKOM device manual (ANKOM Technology Corporation, Macedon, New York, USA).

In vitro dry matter digestibility (ivDMD) and *in vitro* neutral detergent fiber digestibility (ivNDFD)

The total samples (all canopy structures) of the different varieties of *Brachiaria* grasses of two regrowth ages (21 and 42 d) were submitted to *in vitro* tests, incubated with bovine or ovine rumen liquid (inoculum). The bovine inoculum was collected from three Nellore × Angus crossbred cattle and the ovine inoculum from five Dorper × Suffolk crossbred sheep, already fitted with ruminal silicon cannula and adapted to forage diet.

The *in vitro* digestibility of nutrients was determined according to the methodology of Tilley and Terry[9] adapted for the ANKOM Daisy system (ANKOM Technology Corp., Macedon, NY, USA) as described by Holden[10]. Bags of non-woven fabric containing samples of *Brachiaria* grasses were placed on jars (with a limit of 30 bags per jar, two of them blanks) containing approximately 1.6 L of buffer solution[11]. Bovine or ovine ruminal liquid (400 ml) was then added and purged CO_2 in the jars. The jars remained incubated with shaking at a constant temperature of 39 °C for 48 h, after which 40 ml of HCl (6 N) and 8 g of pepsin were added to each jar and left for another 24 h. When the incubation was finished, the jars were drained and the bags washed with distilled water and dried at 105 ºC for 16 h. They were then weighed to determine the post-incubation DM and submitted to NDF analysis[3] with adaptation of ANKOM device manual (ANKOM Technology Corp., Macedon, NY, USA). The *in vitro* digestibility (ivD) coefficients for DM (ivDMD) and NDF (ivNDFD) were obtained through the equation:
ivD (g/kg) = [(incubated nutrient, g) – (residual nutrient, g – blank, g)] / (incubated nutrient, g) × 1,000.

Statistical analysis

All data were analyzed using the statistical package SAS® (SAS® Inst. Inc., Cary, NC, USA), and the means were compared using Tukey's test with a significance level of $P<0.05$, and tendency considered at $P<0.10$.

To evaluate bromatological composition, production and regrowth of grasses, the following statistical model was used:

$$\mathbf{Y_{ijkl}} = \mu + A_i + G_j + AG_k + e_{ijkl}$$

where
μ is the general average,
$\mathbf{I_i}$ is the effect of the *i*-th age (21, 42), G_j is the effect of the *j*-th grass (1,…, 5),
$\mathbf{IG_k}$ is the effect of interaction of *i*-th age with *j*-th grass,

$\mathbf{e_{ijkl}}$ is the random error.

To compare the values of ivD of DM and NDF carried out with different inoculums, the following statistical model was used:

$$\mathbf{Y_{ijkl}} = \mu + I_i + G_j + IG_k + e_{ijkl}$$

where
μ is the general average,
$\mathbf{I_i}$ is the effect of the *i*-th rumen inoculum (1, 2),
$\mathbf{G_j}$ is the effect of the *j*-th cultivar (1,…, 5),
$\mathbf{IG_k}$ is the effect of interaction of *i*-th rumen inoculum with *j*-th cultivar,
$\mathbf{e_{ijkl}}$ is the random error.

Results

Forage mass and growth determination

The forage mass and growth parameters are presented in Table 1. There was a significant effect ($P<0.05$) on forage mass from variety and age, leaf blade/stem ratio, leaf blade, stem, senescent material and growth. In addition, there was significant grass × age interaction ($P\leq0.0001$) for all variables. The variety that presented the highest forage mass at 21 d was *B. humidicola* cv. Common, and at 42 d it was *B. ruziziensis* access R124 (8.86 and 12.81 g kg^{-1}, respectively; $P=0.0001$).

A lower leaf blade/stem ratio was observed in *B. humidicola* cv. Common at 21 and 42 d (0.51 and 0.30, respectively; $P=0.0001$) with similarity among the other grasses. However, the highest leaf blade/stem ratio at 21 d was observed in *B. humidicola* cv. Tupi (1.21 ratio; $P=0.0001$), whereas *U. decumbens* access D70 presented the highest ratio at 42 days of regrowth (1.51; $P=0.0001$). For stem results, *B. humidicola* cv. Common had significantly high values at 21 and 42 d of age (448.8 and 656.8 g kg^{-1}, respectively; $P=0.0001$).

In the production of senescent material in the studied varieties, the highest amount was observed for *B. decumbens* access D70 with 42 d of regrowth (684.8 g kg^{-1}; $P=0.0001$), and at 21 d *B. decumbens* cv Basilisk had numerically the highest value, close to *B. decumbens* access D70 (389.0 and 385.7 g kg^{-1}, respectively). In contrast, *B. humidicola* cv. Common and *B. humidicola* cv. Tupi had numerically the lowest senescent material at 21 d (220.2 and 276.3 g kg^{-1}, respectively).

The growth parameter was significant (*P*=0.00001) with highest growth shown by *B. ruziziensis* access R124 at 42 d (28.2 cm) and the lowest by *B. humidicola* cv. Common at 21d (11.6 cm).

Forage chemical composition

In relation to chemical composition, there was significant effect (*P*<0.05) from variety of grass and growth age on DM, CP, NDF and ADF in all sample types (total sample, leaf blade and stem; Table 2). Also, there was a significant grass × age interaction (*P*<0.05) for DM, CP, NDF and ADF in all samples, except DM and NDF from stem material, which showed no significant interaction (*P*>0.05), but a trend for NDF (*P*= 0.0985).

Table 1: Forage mass, leaf blade/stem ratio (LB:S), stem, Senescent material and growth of different varieties of *Brachiaria* grasses at different cutting ages

	B. decumbens **cv. Basilisk**		***B. decumbens*** **access D70**		***B. humidicola*** **cv. Common**		***B. humidicola*** **cv. Tupi**		***B. ruziziensis*** **access R124**		**CV (%)**	***P*-value**		
	21 d	**42 d**	**21 d**	**42 d**	**21 d**	**42 d**	**21 d**	**42 d**	**21 d**	**42 d**		**Grass**	**Age**	**G×A**
Mass, t ha^{-1}	6.18	4.58	6.13	5.75	8.86	8.21	7.69	5.21	7.31	12.81	36.54	0.0001	0.0001	0.0001
LB:S ratio	0.81	1.21	1.11	1.51	0.51	0.30	1.21	1.11	0.91	1.01	24.31	0.0001	0.0001	0.0001
Leaf blade, g kg^{-1}	238.1	364.9	319.3	198.4	238.5	131.1	409.7	406.5	306.3	215.0	21.32	0.0001	0.0001	0.0001
Stem, g kg^{-1}	270.4	294.3	303.3	125.0	448.8	656.8	343.9	325.6	334.4	212.3	29.17	0.0001	0.0001	0.0001
Senescent, g kg^{-1}	389.0	449.8	385.7	684.8	220.2	321.3	254.7	276.3	367.6	581.1	25.58	0.0001	0.0001	0.0001
Growth, cm	20.7	24.2	15.1	17.1	11.6	18.2	12.1	14.1	20.7	28.2	25.90	0.0001	0.0001	0.0001

CV = Coefficients of variation (%).

Table 2: Chemical composition of the total plant, leaf blade and steam samples of different *Brachiaria* grasses at different regrowth ages

	B. decumbens **cv. Basilisk**		***B. decumbens*** **access D70**		***B. humidicola*** **cv. Common**		***B. humidicola*** **cv. Tupi**		***B. ruziziensis*** **access R124**		**CV (%)**	***P*-value**		
	21 d	**42 d**	**21 d**	**42 d**	**21 d**	**42 d**	**21 d**	**42 d**	**21 d**	**42 d**		**Grass**	**Age**	**P G×A**
Total plant														
TDM	299.1	480.6	324.7	398.4	444.3	564.6	402.9	433.2	398.3	424.8	0.19	0.0001	0.0001	0.0001
OM	941.2	925.9	940.4	922.7	943.6	907.2	939.3	902.7	929.9	922.5	0.29	0.9431	0.0347	0.8609
CP	59.8	47.53	67.8	54.9	49.4	42.6	47.2	31.3	58.3	61.9	9.01	0.0001	0.0001	0.0001
NDF	676.3	743.8	702.3	798.0	669.3	772.5	701.9	769.4	572.8	710.4	9.20	0.0001	0.0001	0.0465
ADF	437.0	498.4	483.8	541.5	505.8	510.8	497.5	557.7	425.5	542.9	3.29	0.0001	0.0001	0.0001
Leaf blade														
DM	378.9	416.7	392.7	497.3	422.8	518.3	388.8	460.1	425.8	489.0	0.332	0.0001	0.0001	0.0009
OM	924.5	899.5	926.7	896.6	919.0	883.0	921.6	886.4	945.7	885.0	0.26	0.8885	0.0007	0.8312
CP	102.3	93.7	138.9	104.9	80.2	39.6	62.2	49.5	117.3	144.2	16.02	0.0001	0.0001	0.0001
NDF	513.0	608.0	600.0	668.4	580.3	624.3	726.9	810.9	652.2	700.7	3.71	0.0001	0.0001	0.0001
ADF	305.1	367.6	288.8	298.9	290.8	303.4	307.0	384.7	357.5	351.9	4.95	0.0001	0.0001	0.0001
Stem samples														
DM	353.4	402.8	421.0	464.1	383.8	449.1	336.1	415.8	406.2	463.0	0.33	0.0001	0.0001	0.1240
OM	934.1	924.7	931.5	926.2	948.0	934.9	939.0	932.0	924.7	921.4	0.34	0.8324	0.4687	0.9986
CP	37.6	36.9	55.5	44.6	31.3	48.7	31.2	31.7	50.1	49.5	5.08	0.0001	0.0214	0.0001
NDF	798.2	804.1	766.9	788.5	821.3	811.9	822.4	884.6	774.4	821.99	1.32	0.0001	0.0074	0.0985
ADF	463.0	457.1	412.9	442.7	445.3	442.1	432.1	476.7	484.4	486.1	2.69	0.0001	0.0119	0.0089

CV= Coefficients of variation (%).

Comparing regrowth ages of total sample, forages at 21 d of regrowth showed lower values of DM, NDF and ADF (P<0.05); however, they presented higher CP values. As expected, CP from leaf blades was higher than from stem samples. The CP of leaf blades of *B. ruziziensis* R124 at 42 d of regrowth showed the highest amount (144.2 g kg^{-1}; P<0.05)

B. humidicola cv. Tupi presented higher NDF and ADF values for leaf blades at 42 d of regrowth (769.4 and 557.7 g kg^{-1}, respectively; P<0.05). The highest stem NDF values were also presented by *B. humidicola* cv. Tupi (884.6 g kg^{-1}; P<0.05). High ADF values were also observed for *B. humidicola* cv. Tupi and *B. decumbens* cv. Basilisk (384.7 and 367.6 g kg^{-1}, respectively; P<0.05).

In vitro dry matter digestibility (*iv*DMD) and *in vitro* neutral detergent fiber digestibility (*iv*NDFD)

There was no significant interaction (P>0.05) between inoculum and grasses. It was observed that ovine inoculum *vs* bovine inoculum resulted in higher *iv*DMD values for *B. decumbens* cv. Basilisk at 21 d (611.2 *vs* 571.9 g kg^{-1}; P=0.0200), *B. humidicola* cv. Tupi at 21 d (631.8 *vs* 568.4 g kg^{-1}; P=0.0370), and *B. ruziziensis* access R124 at 21 d (548.8 *vs* 613.4 g kg^{-1}; P=0.0420; Table 3). When evaluating only bovine inoculum, *B. humidicola* cv. Tupi and *B. ruziziensis* access R124 at 42 d of regrowth showed the lowest *iv*DMD values (544.3 and 542.0 g kg^{-1}, respectively; P=0.0078). When evaluating only ovine inoculum, *B. ruziziensis* access R124 at 42 d also presented the lowest *iv*DMD (535.0 g kg^{-1}; P=0.0184).

Table 3: *In vitro* dry matter digestibly (*iv*DMD) of different varieties of *Brachiaria* grasses at different regrowth ages incubated with bovine or ovine rumen liquid (g kg^{-1})

	Age	Rumen inoculum		CV (%)	*P*-value
		Bovine	Ovine		
B. decumbens cv. Basilisk	21	571.9 ^{ABb}	611.2 ^{ABa}	0.96	0.0200
	42	612.9 ^{AB}	575.5 ^{ABC}	1.48	0.0520
B. decumbens access D70	21	583.4 ^{AB}	599.2 ^{AB}	2.85	0.1235
	42	558.6 ^{BC}	550.7 ^{BC}	2.12	0.1354
B. humidicola cv. Common	21	515.9 ^{B}	574.3 ^{B}	2.86	0.0650
	42	593.2 ^{ABC}	598.8 ^{AB}	1.30	0.1845
B. humidicola cv. Tupi	21	568.4 ^{ABb}	631.8 ^{ABa}	2.09	0.0370
	42	544.3 ^{C}	577.6 ^{ABC}	1.81	0.0820
B. ruziziensis access R124	21	548.8 ^{ABb}	613.4 ^{ABa}	3.30	0.0420
	42	542.0 ^{C}	535.0 ^{C}	0.07	0.2002
CV, %		2.564	1.972		
P-value		0.0078	0.0184		

Mean values with different capital letters in the same column or lowercase letters superscript differ (P<0.05) based on Tukey's test.
CV= Coefficients of variation.

Regarding the *iv*NDFD, as presented on Table 4, ovine resulted in higher values than bovine inoculum on *B. humidicola* cv. Common at 21 d of regrowth (420.3 *vs* 369.5 g kg^{-1}; *P*=0.0040), and *B. humidicola* cv. Tupi at 42 d (490.8 *vs* 452.4 g kg^{-1}; *P*=0.0270). However, on *B. decumbens* cv. Basilisk at 42 d, the opposite was seen: ovine inoculum resulted in lower *iv*NDFD values than bovine (413.9 *vs* 472.4 g kg^{-1}; *P*=0.0150).

Table 4: *In vitro* neutral detergent fiber digestibility (*iv*NDFD) of different varieties of *Brachiaria* grasses at different regrowth ages incubated with bovine or ovine rumen liquid (g kg^{-1})

	Age	Rumen inoculum		CV(%)	*P*-value
		Bovine	Ovine		
B. decumbens cv. Basilisk	21	436.8 ^{A}	439.1	9.75	0.3542
	42	472.4 ^{Aa}	413.9 ^{b}	1.64	0.0150
B. decumbens access D70	21	482.7 ^{A}	487.7	5.76	0.1423
	42	445.7 ^{A}	412.5	4.87	0.2540
B. humidicola cv. Common	21	369.5 ^{ABb}	420.3 ^{a}	0.84	0.0040
	42	443.4 ^{A}	458.7	1.30	0.1210
B. humidicola cv. Tupi	21	458.4 ^{A}	510.3	3.62	0.0970
	42	452.4 ^{Ab}	490.8^{a}	1.35	0.0270
B. ruziziensis access R124	21	212.2 ^{B}	455.7	70.20	0.4080
	42	427.6 ^{A}	424.0	15.53	0.0870
CV, %		25.333	4.622		
P-value		0.0044	0.1562		

Mean values with different capital letters in the same column or lowercase letters superscript differ (*P*<0.05) based on Tukey's test.
CV= Coefficients of variation.

There was no effect (*P*>0.05) on *iv*NDFD from cultivar and regrowth age when the samples were incubated with ovine inoculum; however, the highest values were observed for *B. humidicola* cv. Tupi at 21 and 42 d (510.3 and 490.8 g kg^{-1}, respectively). When using bovine inoculum only the variety *B. ruziziensis* access R124 at 21 d presented *iv*NDFD values below the others (*P*=0.0044). At 21 d of regrowth the varieties that presented the higher values (*P*=0.0044) were *B. decumbens* cv. Basilisk, B. decumbens access D70, *B. humidicola* cv. Common and *B. humidicola* cv Tupi, *B. decumbens* access D70 being highlighted numerically, with the highest mean value. At 42 d *B. decumbens* cv. Basilisk, B. decumbens access D70, *B. humidicola* cv. Common, *B. humidicola* cv. Tupi and *B. ruziziensis* access R124 had statistically the highest values (*P*=0.0044), highlighting *B. decumbens* cv. Basilisk numerically with the highest mean.

Discussion

The stoloniferous habit of *B. humidicola*, with strong nodes branching into new plants, favors a high residue during the standard cut of forage for regrowth evaluation[12]. The large root system results in more carbohydrate reserves for more vigorous regrowth, as observed at this study in the higher weight of leaf blade in *B. humidicola* cv. Tupi and stems in *B. humidicola* cv. Common (Table 1). It is possible that higher regrowth of leaves is due to the intense turnover of nutrients and an increase in CP in young leaves related to a thinner cell wall[13]. However, high stem development in tropical grasses is also due to two other main factors: the low frequency of defoliation and flowering[14]. In response to a need to expose the younger leaves to the upper canopy, where light is most abundant, a competition for light may occur between the tillers forcing them to elongate their stems[15,16].

B. humidicola grasses presented less senescence material (Table 1), favoring the positive assessment of green leaves, which are of great importance in the nutritional value of a forage. The leaf blades of *B. humidicola* cultivars are morphologically thinner (0.5–0.8 cm width) than those of *B. decumbens* (average 1.5 cm width) and *B. ruziziensis* (1.0–1.5 cm width), providing less shade (less than 65 %). Therefore, they have lower senescence and/or death of the young tillers and old leaves, as previous reported in other studies[6,16,17]. The expanding leaf blades, especially those intermediate in the tiller, run a higher path between their connection point with the meristematic region and the end of pseudo-stems and, consequently, reach full size[6]. Comparing the ages of regrowth, the sampling dates did not affect significantly the vegetative growth (Table 1).

Regarding forage production, it is possible to suggest that *B. humidicola* cv. Tupi showed the best performance, despite *B. decumbens* cv. Basilisk having an increased production of leaf blade, stem and senescent material but fewer leaf blades and higher senescent material than the others ($P<0.05$), which may interfere with nutrient content.

Assessing chemical compositions of *B. decumbens* grasses, this study presented higher CP content at 21 d of regrowth, but at 42 d *B. ruziziensis* had high CP content (Table 2). Findings of a study[18] evaluating *B. decumbens* cv. Basilisk and *B. ruziziensis* cv. Kennedy reported higher CP content, but this may have been due to a different cutting height (10 cm *vs* 5 cm); when cutting closer to the ground more stem and senescent material may be present in the samples,– but, may also have been due to differences of soil fertility, and others edaphoclimatic conditions where plants were grown.

Concerning the fibrous portion of the material, due to NDF consisting of cellulose, hemicellulose, lignin and silica and ADF being the fraction composed of cellulose, lignin and silica, even a slight change in those compounds will alter the NDF and ADF values[19]. The NDF and ADF of the total sample from *B. decumbens* varieties found in this study were lower than observed in another study[20], which quoted values at 30 d of regrowth

of 832.4 g kg^{-1} NDF and 462.1 g kg^{-1} ADF. A study with *Brachiaria decumbens*[13] obtained mean of 809.0 g kg^{-1} NDF and 475.0 g kg^{-1} ADF. These results are closer to our findings with the two *B. decumbens* varieties at 42 d of regrowth (*B. decumbens* cv Basilisk 743.8 g kg^{-1} NDF and 498.4 g kg^{-1} ADF; *B. decumbens* access D70 798.0 g kg^{-1} NDF and 541.5 g kg^{-1} ADF; Table 2). Plants with fewer structural carbohydrates (waste FDA) are more efficient at nutrient cycling and have beneficial effects on crop yields[21].

Taking all bromatological composition parameters into account, at 21 d of regrowth *B. ruziziensis* access R124 showed the best combination of parameters, with less NDF and ADF (P<0.05) and one of highest values of CP content. However, at 42 d, the variety that had the best bromatological content combination was *B. decumbens* cv. Basilisk; even with not such a high CP value, it had lower ADF and NDF content and a lower proportion of ADF inside the NDF, which interferes directly with the digestion of a feedstuff and consequently its nutritional value.

In vitro digestibility techniques using ovine and bovine inoculums have advantages for a rapid evaluation of feedstuffs, such as the physical and chemical uniformity of the fermentative containers and the convenience of keeping fewer fistulated animals; although they do not perfectly reproduce the process of digestion as do live animals. This could be observed when we compared the results from this study with others that analyzed the same forage *in situ* or *in vivo*[12,22,23,24]. Furthermore, the ovine ruminal digestibility presented percentages that were 10 to 15 % higher than bovine ruminal fluid (Table 3). However, the prediction ability and applicability of *in vitro* techniques can depend on the degree of similarity between the technical and ruminant digestive process. *In vitro* systems use rumen fluid and a standard solution to simulate the anaerobic process of ruminal fermentation[25]; the standard solution is typically a buffer solution simulating the saliva of ruminants[11].

All grasses presented values higher than the 500 g kg^{-1} *iv*DMD, indicated by authors[26] to be a minimum value to qualify them as forage of good nutritional quality and to not compromise animal performance, even given the expected drop in microbial colonization of 0.1 to 0.2 % per day with the increase in the physiological age of the plant[13]. As expected, the mean *iv*NDFD was lower than that of *iv*DMD (Tables 3 and 4). However, finding no significant difference (P>0.05) between regrowth ages in a few *Brachiaria* variety contradicts the results of Paciullo *et al*[27], who found that with the development of the plant during the rest period, metabolites arise from photosynthesis and are converted into structural components. Solubilization of hemicellulose may occur, expansion of the fiber possibly increasing the availability of fermentable substrates, thereby providing suitable conditions for microbial growth and consequently NDF digestibility. The reduction of intermolecular hydrogen bonds and the type of ester bond between the lignin and hemicellulose allows for its release and exposure to attack by rumen bacteria, aside from the possible presence of elevated, readily fermentable carbohydrate content[19].

Finally, when analyzing the results of *iv*DMD and *iv*NDFD and considering the two inoculums, it was possible to observe that in bovine inoculum *B. decumbens* cv. Basilisk presented the best digestibility at both regrowth ages, while in ovine inoculum *B. humidicola* cv. Tupi had the best digestibility, also for both ages.

Conclusions and implications

The source animal species for inoculum has an effect on *in vitro* digestibility tests. Therefore, is highly recommended to use a specific inoculum for grass evaluations according to the target species (cattle or sheep). From the obtained results, *B. decumbens* cv. Basilisk presented the best *in vitro* digestibility (*iv*DMD and *iv*NDFD) in bovine inoculum and also a good combination of nutrient content and increasing production of all its canopy components, whereas *B. humidicola* cv. Tupi had better *in vitro* digestibility (*iv*DMD and *iv*NDFD) in ovine inoculum and the best production.

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