EFFECT OF THE CUTTING AGE OF *Brachiaria decumbens* ON RUMEN FUNCTIONS AND *in vitro* GAS PRODUCTION[†]

[EFECTO DE LA EDAD DE CORTE DE *Brachiaria decumbens* SOBRE LAS FUNCIONES DEL RUMINAL Y PRODUCCIÓN DE GAS *in vitro*]

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SUMMARY

The aim was to evaluate the effect of cut age on ruminal function and *in vitro* gas production of *Brachiaria decumbens* grass. Eighteen parcels established with *B. decumbens* were used. For each treatment, T1: 45, T2: 60 and T3: 75 (cut days), the chemical composition, rumen degradation, digestibility, pH, ammonia, volatile fatty acids (VFA) and gas production were analyzed. It was observed higher (P <0.05) effective degradation (58%; 0.02 k, 45%; 0.05 k and 38%; 0.08 k) and apparent digestibility (57%) at 45 days of cut. The ruminal pH was maintained between 6.99 to 7.51 from 6 h to 24 h post incubation between the different cut ages (P <0.05). With respect to NH_3-N it was higher at 45 and 60 days (27.3 and 25.6 mg/L respectively). The VFA did not show differences between the treatments evaluated (P> 0.05). The lowest (P = 0.0001) gas production was observed in the *B. decumbens* cut at 45 days (T1). It can be concluded that the best age for grazing of *B. decumbens* can be between 45 and 60 days of regrowth.
Keywords: gas production; rumen degradation; *Brachiaria decumbens.*

RESUMEN

El objetivo de esta investigación fue evaluar el efecto de la edad de corte sobre la función ruminal y producción de gas *in vitro* del pasto *Brachiaria decumbens*. Dieciocho parcelas establecidas con *B. decumbens* fueron usadas. De cada tratamiento, T1: 45, T2: 60 y T3: 75 (días de corte), se analizó la composición química, Degradación ruminal, digestibilidad, pH, nitrógeno amoniacal, ácidos grasos volátiles (AGVs) y producción de gas. Se observó mayor (P<0.05) degradación efectiva (58 %; 0.02 k, 45%; 0.05 k y 38 %; 0.08 k) y digestibilidad aparente (57 %) a los 45 días de corte. El pH ruminal se mantuvo entre 6.99 a 7.51 desde las 6h hasta las 24h post incubación entre las diferentes edades de corte (P<0.05). Con respecto al NH_3-N fue mayor a los 45 y 60 días (27.3 y 25.6 mg/L respectivamente). Los AGVs no mostraron diferencias entre los tratamientos evaluados (P>0.05). La menor (P=0.0001) producción de gas se observó en la *B. decumbens* cortada a los 45 días (T1). Se puede concluir que la mejor edad para pastoreo de la *B. decumbens* puede ser entre 45 y 60 días de rebrote.
Palabras clave: producción de gas; degradación ruminal; *Brachiaria decumbens.*

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INTRODUCTION

Ruminant production systems in tropical and subtropical regions of Ecuador are mainly based on grazing grasses of the genus Brachiaria spp (Garay *et al*., 2017), which are characterized by having ideal qualities for feeding ruminants under these conditions. Optimizing in this way the production systems in which they are implemented (Balseca et al., 2015), perhaps due to the great capacity they have to adapt and endure in poor quality soils, where the cultivation of other pastures is limited (Ramirez *et al*., 2009). In the humid subtropics of the Ecuadorian Amazon, rainfall can reach up to 4000mm/year, which affects the yield and quality of forages (González *et al*., 1997). However, the greater the physiological age of the forage, contributes to a low nutritional value and high fiber content, and with this, lower degradability and digestibility of nutrients at the ruminal level (Barros-Rodríguez *et al*., 2017a), minimizing the use of the protein and increases energy losses in the form of greenhouse gases in the animal (Barros-Rodríguez *et al., 2018*; Torres *et al*., 2018; Vallejo-Hernandez *et al*., 2018). Therefore, the optimal regrowth age for grazing in these regions is the factor that has the greatest impact on forage performance and quality and animal productivity. Based on the above, the objective of the present investigation was to evaluate the effect of the cutting age on the rumen degradation kinetics of nutrients, digestibility, rumen fermentation and in vitro gas production of *Brachiaria decumbens* grass

MATERIALS AND METHODS

Location

The field study (experimental plots) was carried out in the Amazon Postgraduate and Conservation Research Center (CIPCA: acronym in Spanish) of the Universidad Estatal Amazanica, Pastaza, Ecuador and in situ and in vitro tests in the Ruminology laboratory of the Faculty of Agricultural Sciences, from the Universidad Tecnica de Ambato, Ambato, Ecuador.

Animals and accommodation

Six bulls of approximately two years with 450 21.2 kg of average live weight were used, provided with a fistula with a cannula in the rumen (Diamond Bar, Parma, Idaho, USA). The animals were housed in individual pens with zinc roof and cement floor and access a diet based in *Medicago sativa* and *Lolium perennial* and water *ad libitum*.

Vegetable samples and treatments

Eighteen plots established with *Brachiaria decumbens* were delimited and an equalization cut was made, to subsequently make the cuts according to the age corresponding to the treatments (45, 60 and 75 days). From each treatment and repetition (n = 6) 15 kg of green matter was collected, a subsample of 1 kg was taken to the laboratory and dried in a forced air oven at 60 ºC until constant weight to determine the dry matter. The remaining 14 kg of each sample were dehydrated under cover and subsequently processed in a hammer mill (CREMASCO DP2, Brazil) at a particle size of 2mm for later use in both *in vitro* and *in situ* tests.

In situ rumen degradation

It was evaluated by the technique of the nylon bag in the rumen described by Ørskov *et al*. (1980). Two bags containing 5 g DM of each treatment and repetition were placed in each animal and incubated for 0, 4, 8, 12, 24, 36, 48, 72 and 96 h. At the end of the incubation periods, the bags were removed, washed with running water and dried at 60 ° C. The bags used to measure the loss by washing (0 h) were not incubated in the rumen and only washed with running water. The residues were stored in polyethylene bags at -4 ° C. The disappearance of nutrients was calculated as a proportion of the incubated and residual material. The data were adjusted to the equation: $Y = a + b (1 - e^{-ct})$ and the effective degradation was adjusted using the equation $DE = a + [(b * c) / (c + k)]$ considering a rate of 2, 5 and 8% passage (Ørskov and McDonald, 1979).

Apparent digestibility and *in vitro* gas production

These tests were performed *in vitro*. For this, the rumen content (liquid and solid fractions) was obtained separately from each cannulated bull. The rumen content (1000 mL) was collected before providing the food in the morning and kept at 39 ° C in a sealed plastic container during transport to the laboratory. The analysis in the laboratory was done within the time of collection in a medium rich in nitrogen according to Menke and Steingass (1988). Gas production was determined according to the methodology described by Theodorou *et al*. (1994). Shortly, 0.5 g of DM of each treatment was placed in 100 mL glass bottles of nominal capacity, 60 mL of rumen inoculum (70:30 medium/rumen inoculum) was added under a constant CO2 flow. The bottles were sealed and incubated at 39-40 ° C. Gas pressure and volume were measured manually at 3, 6, 9, 12, 18, 24, 36, 48, 72 and 96 h after incubation with a pressure transducer model DELTA OHM DO 9704 (Delta OHM, Padova, Italy) and plastic syringes.

For each treatment six bottles (repetitions) were used for each incubation time and four additional bottles as blank. At the end of the incubation, *in vitro* digestibility (fermented DM) was estimated by

filtering the residues and corrected with the residual MS of the glass bottles used as blank. The total gas production was estimated by 0.5 g fermented DM, as well as the apparent digestibility of DM.

Ruminal pH, Ammoniacal Nitrogen (NH3-N) and Volatile Fatty Acids (VFA)

Under the same procedure mentioned above for gas production and digestibility, 72 glass bottles were prepared. From each treatment and each time (6, 12 and 24 h post incubation) the ruminal pH was measured with the help of a pH meter (BANTE-221 portable pH/ORP Meter). The NH3-N and VFA were determined only from the 6 hour post incubation. For this, 10 mL of ruminal content of each bottle (treatment and repetition) was filtered through gauze and mixed with 10 ml of 5% HCL in a bottle, then stored at 4 ° C, until further analysis of NH_3-N, under the same procedure, 10 mL of ruminal fluid was mixed with 2.5 ml of 25% metaphosphoric acid in a flask and stored at 4 ° C until further VFA analysis.

Chemical analysis

Dry matter (DM) (# 7.007) and ash (# 7.009) were determined according to the AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by method 12 and 13 respectively, ANKOM2000 fiber analyzer (ANKOM Technology, Macedon, NY, USA). The CP was determined by elemental analysis (N) using a LECO CHN 628 (LECO Corporation). The VFA were determined by a gas chromatograph (Clarus 400, PerkinElmer, Shelton, CT, USA) following the methodology described by Ryan (1980). Ammoniacal nitrogen was determined by a colorimetric technique using a spectrophotometer (Lambda 25, PerkinElmer, Waltham, MA, USA.

Experimental design and statistical analysis

A completely randomized design with 3 treatments and 6 repetitions was used. The results of all the variables were subjected to an analysis of variance using the PROC GLM of SAS (SAS, 2002). The averages were evaluated using the Tukey test. Additionally, a transformation of orthogonal polynomials was performed to observe the linear or quadratic response of the treatments.

RESULTS

The rumen degradation kinetics of the MS showed no differences (P>0.05) between treatments in the soluble fraction (a). However, in the insoluble but potentially degradable fraction (b), the degradation rate in % per hour and the potential for rumen degradation (a+b) was higher (P<0.05) at 45 d (61%), 75 d (0.055) and 45 d (77%) respectively (Table 1). With respect to the effective degradation (SD), it was greater than 45 d for all passage rates, observing a linear and quadratic effect with respect to the cutoff days of *B. decumbens*.

In vitro rumen digestibility showed a downward linear effect (P=0.0059) to the treatments. The rumen pH at different hours after incubation was within the normal pH range for this type of diet, however, the highest pH was observed in the *B. decumbens* cut at 60 d (P<0.05). The highest (P=0.0001) concentration of NH_3-N was at 45 and 60 d (27.3 and 25.6 mg/L respectively) cut, observing a linear response (P=0.0001) decreasing with increasing days to cut. Regarding the molar proportion of VFA, no differences (P>0.05) were observed between the treatments (Table 2).

Table 1. *In situ* degradation parameters of the DM (%) of *B. decumbens* at three cutting ages.

	Days cut			SEM	*P*	Contrasts	
	45 d	60 d	75 d			linear	quadratic
a	16.26	16.31	10.24	1.980	0.0885	0.0613	0.2151
b	61.26a	51.38b	58.38ba	2.273	0.0257	0.4119	0.0099
c	0.045ab	0.039b	0.055a	0.0033	0.0169	0.0687	0.0168
a+b	77.29a	67.69b	68.77b	0.904	0.0001	0.0001	0.0004
ED							
0,02 *k*	58.36a	50.24b	53.18b	0.954	0.0002	0.0024	0.0005
0,05 *k*	45.12a	38.86b	40.96b	0.998	0.0026	0.0122	0.0051
0,08 *k*	38.24a	33.14b	34.14b	1.030	0.0102	0.0157	0.0325

[abc] Means with different letters between rows differ significantly (p <0.05). a: fraction soluble; b: insoluble but potentially degradable fraction; *c*: degradation rate in % per hour; a+b: degradation potential DE: effective degradation at different passage rates.

The parameters gas production (PG) and kinetic show differences between the days of cut, obtaining the lowest (P=0.0001) gas production at day 45, with a difference of 135 mL gas / 0.500 g of fermented DM with respect to those obtained in *B. decumbens* harvested at 75 d (Table 3 and Figure 1).

DISCUSSION

The results obtained in the rumen degradation kinetics and digestibility were possibly due to the lower fiber content of *B. decumbens* at 45 days. With regard to the lower digestion observed at a higher cutting age, it was probably due to the aging of the plant, which may be related to the reduction of the synthesis of protein compounds, when compared to the younger stages. In addition, at an older age the amount of leaves decreases, the synthesis of structural carbohydrates (cellulose and hemicellulose) increases and the quality of the grass decreases, mainly due to an increase in the lignification of the cell wall (Herrera, 2004; Valles de la Mora *et al*., 2016). Which decreases the enzymatic action of rumen microorganisms (Vallejo-Hernández *et al*., 2018). These results are consistent with those reported by Barros-Rodríguez *et al*. (2017b) who mention that increasing fiber in forages reduces the action of microorganisms towards fiber digestion. As well as those reported by Bolívar and Ibrahim (2005) who report similar values of digestibility in rainy periods (54.4%), for a species of the genus Brachiaria (humidicola), although in their studies it is important to highlight that rainfall exceeded 2000 mm in the year and that, in the dry season, digestibility values were not higher than 38%.

With regard to pH of rumen, despite showing differences are found in normal values for this type of diet. These results are consistent with those reported by Barros-Rodríguez *et al*. (2013). The reported values for NH3-N were possibly due to the higher synthesis of the protein compounds that occur in the physiological stages before flowering. The results obtained in the fermentation pattern may be related to the type of structure of the diet, the same type of carbohydrates (cellulose and hemicellulose) (Barros-Rodríguez *et al*., 2015). The linear effect observed in the production of gas could be related to a greater use of proteins in the rumen, which leads to an increase in the synthesis of microbial protein and a greater bypass protein to the lower parts of the gastrointestinal tract. As well as, the lower fiber content in the B. decumbens that he had at 45 days (Blummel *et al*., 1997). These results are consistent to those reported by Barros-Rodríguez *et al*. (2018); Torres *et al*. (2018).

Table 2. Parameters of *in vitro* ruminal fermentation of *B. decumbens* at three cutting ages

	Days cut			SEM	*P*	Contrasts	
	45 d	60 d	75 d			linear	quadratic
IVDDM	57.16a	51.03ab	47.90b	1.738	0.0059	0.0019	0.4919
pH hours post incubation							
6 h	6.99b	7.03a	7.00ab	0.010	0.0401	0.2582	0.0210
12 h	7.01	7.05	6.02	0.575	0.3779	0.2385	0.4613
24 h	7.20b	7.46a	7.51a	0.058	0.0042	0.0021	0.1402
post incubation (6 h)							
NH_3 – N (mg/L)	27.3a	25.6a	21.8b	0.89	0.0001	0.0001	0.3418
VFA (molar %)							
Acetic (A)	72.42	73.59	73.73	1.324	0.0657	0.0725	0.0612
Propionic (P)	17.3	16.76	16.67	0.932	0.1430	0.0901	0.0780
Butyric	7.63	7.14	7.11	0.984	0.2814	0.1470	0.0987
Isobutyric	0.92	0.87	0.88	0.092	0.1027	0.0941	0.0894
Isovaleric	1.12	1.1	1.1	0.154	0.0951	0.2587	0.1842
Valeric	0.61	0.54	0.51	0.083	0.0810	0.1080	0.9541
Ratio: A/P	4.19	4.39	4.42	0.751	0.1807	0.2530	0.1871

[abc] Means with different letters between rows differ significantly ($p<0.05$). IVDDM: in vitro digestibility of dry matter

Table 3. *In vitro* gas production (mL gas/0.500 g fermented DM) of *B. decumbens* at three cutting ages.

	Days cut			SEM	*P*	Contrasts	
	45 d	60 d	75 d			linear	quadratic
GP	393.47c	460.87b	528.10a	8.080	0.0001	0.0001	0.9934
B	31.62b	39.59a	44.04a	1.615	0.0002	0.0001	0.3885
c	1.318a	1.183b	1.140b	0.0189	0.0001	0.0001	0.0684

[abc] Means with different letters between rows differ significantly ($p<0.05$). GP: gas production. B: gas production asymptote, *c*: gas production rate per hour

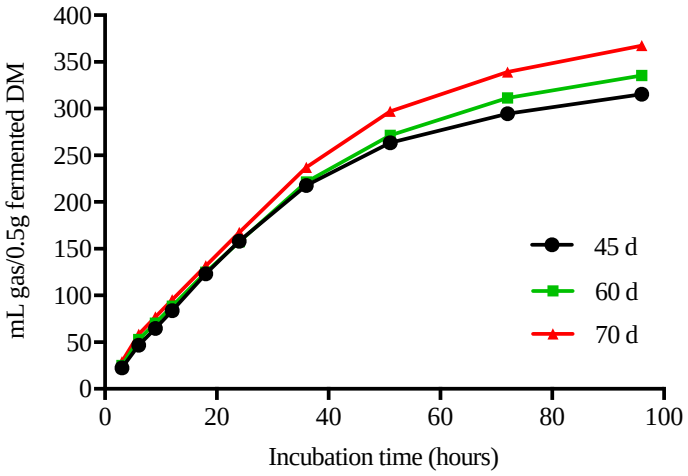

Figure 1. *In vitro* gas production kinetic (mL gas/0.5 g fermented DM) of *B. decumbens* at three cutting ages.

CONCLUSIONS

Under the conditions of this study, it can be concluded that the best age for grazing of B. decumbens can be between 45 and 60 days of regrowth since it has better digestion and favors the reduction of enteric gas production in the rumen.

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