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Ruminal degradability of tropical leguminous plants from eastern Amazonia

Degradabilidade ruminal de leguminosas tropicais da Amazônia Oriental

Ermino Braga¹; Ermino Braga Filho²; Jamile Andréa Rodrigues da Silva^{1*}; Cristian Faturi¹; Felipe Nogueira Domingues¹; José de Brito Lourenço Júnior³

Abstract

The aim of this study was to evaluate ruminal degradation of dry matter (DM), crude protein (CP), and neutral detergent fiber (NDF) of *Cratylia argentea*, *Flemingia macrophylla* (Willd.) Merrill, and *Stylosanthes guianensis* 'Campo Grande' (EMBRAPA), aged 55 and 75 days, in sheep with rumen cannulas, using the *in-situ* technique. A factorial design of 3 leguminous plants × 2 ages × 6 incubation times was used, totaling 36 experimental units. The experimental period included 14 days for adaptation to diet and 5 days for data collection. *Cratylia* at both ages and *Flemingia* aged 55 days showed the highest "a" fraction values for DM degradability. *Cratylia* aged 55 days and *Stylosanthes* at both ages showed the highest "a" fraction values for CP. The highest "b" fraction value for DM was 51.27% for *Stylosanthes* aged 55 days. This rate was lower for the other leguminous plants, especially for *Flemingia* at both ages. The "b" fraction values for NDF are considered low for *Flemingia*, what may be the result of lower ruminal degradation. The highest "c" fraction degradation rate for DM was observed in all leguminous plants studied aged 75 days. The highest "c" fraction value for CP was 14.84% for *Stylosanthes* aged 75 days. The highest Kd fraction values for DM, NDF, and CP were found in *Flemingia*. *Cratylia*, and *Stylosanthes* aged 75 days presented higher effective degradability (ED) values for DM at 2 and 5%/hour compared to *Flemingia* at both ages. *Cratylia* and *Stylosanthes* had the highest ED values for NDF, regardless of their passage rates. *Stylosanthes* presented higher ED values for CP at both ages, regardless of its passage rate. The highest DM, NDF, and CP potential degradability (PD) was obtained for *Cratylia* and *Stylosanthes*, at both ages. The leguminous plants *Cratylia* and *Stylosanthes* can be recommended for use as a protein bank and supplementation for ruminants, whereas, *Flemingia macrophylla* should not be used as a protein supplement for ruminants, because it contains high levels of fibrous fractions.

Key words: Degradation. Crude protein. Dry matter. Neutral detergent fiber.

Resumo

Objetivou-se avaliar os parâmetros relativos a degradação ruminal da matéria seca (MS), da proteína bruta (PB) e da fibra em detergente neutro (FDN) contidas nas amostras de leguminosas *Cratylia argentea*, *Flemingia macrophylla* (Willd) Merril e *Stylosanthes guianensis* cv Campo Grande (EMBRAPA), com idades de 55 e 75 dias, em ovinos com cânulas ruminais, pela técnica "in situ".

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Utilizou-se um arranjo fatorial de 3 leguminosas x 2 idades x 6 tempos de incubação, totalizando 36 unidades experimentais. O período experimental foi de 14 dias de adaptação à dieta e cinco de coleta de dados. Para a degradabilidade da MS, a *Cratylia*, em ambas as idades e a *Flemingia*, com 55 dias, destacaram-se com maior fração “a”. Para a fração “a” da PB, as leguminosas *Cratylia* aos 55 dias e *Stylosanthes*, de ambas as idades, se destacaram com os maiores valores. O maior valor da fração “b” da MS foi encontrado para a *Stylosanthes* aos 55 dias, com 51,27%. Nas demais leguminosas, essa taxa foi menor, destacando-se a *Flemingia* em ambas as idades. Os valores de fração “b” da FDN da *Flemingia* são considerados baixos, deduzindo-se ser de menor degradação no rúmen. A maior taxa de degradação da fração “c” da MS foi observada em todas as leguminosas estudadas na idade de 75 dias. Para a fração “c” da PB, o maior valor foi de 14,84%, da leguminosa *Stylosanthes*, de 75 dias. Os maiores valores da fração “kd” da MS, da FDN e da PB foi da *Flemingia*. Quanto à degradabilidade efetiva (DE) da MS às 2 e 5%/hora, as leguminosas *Cratylia* e *Stylosanthes*, de 75 dias, apresentaram valores superiores em relação à *Flemingia*, em ambas as idades. Para a DE da FDN, as leguminosas *Cratylia* e *Stylosanthes* tiveram os maiores valores, independente da taxa de passagem. A leguminosa *Stylosanthes* de ambas as idades apresentou maiores valores de DE da PB, em todas as taxas de passagem apresentadas. A maior degradabilidade potencial da MS, FDN e PB obtida foi para a *Cratylia* e *Stylosanthes*, em ambas as idades. As leguminosas *Cratylia* e *Stylosanthes* podem ser recomendadas para formação de banco de proteína e suplementação alimentar de ruminantes. Já a *Flemingia macrophylla* (Willd.) Merrill, por apresentar elevados teores das frações fibrosas, deve ser evitada como fonte suplementar protéica na dieta de ruminantes.

Palavras-chave: Degradação. Proteína bruta. Matéria seca. Fibra em detergente neutro.

Introduction

Brazil is the 15th largest sheep producer in the world (FAO, 2014), with a herd of 18.43 million animals (IBGE, 2016). In the Amazon, this herd thrives because the region provides favorable conditions such as radiant energy supply and abundant rainfall, which allow a forage production of satisfactory nutritional value. However, depending on the climatic type, animals may suffer from nutrient deficiencies in pastures, and food supplementation is necessary to obtain acceptable animal performance levels.

The introduction of leguminous forage species in pasture is a feeding alternative for ruminants during the dry season. Leguminous forage plants produce more biomass than herbaceous plants - particularly in humid tropical areas - and are more tolerant to drought, besides being able to regrow and supply good fodder the whole year (MONTEIRO et al., 2009).

Cratylia argentea, *Flemingia macrophylla* (Willd.) Merrill, and *Stylosanthes guianensis* ‘Campo Grande’ (Embrapa) are leguminous plants with high forage potential. However, it is necessary

to investigate their use in animal nutrition, as well as their interaction in animal diets to improve metabolic processes in ruminant production systems. Thus, this study aims to evaluate the potential use of *Cratylia argentea*, *Flemingia macrophylla* (Willd.) Merrill, and *Stylosanthes guianensis* ‘Campo Grande’ (Embrapa), by assessing their potential and effective ruminal degradability.

Material and Methods

This experiment was conducted at the Goat and Sheep Research Center of Pará (CPCOP), Federal Rural University of Amazonia (UFRA), Belém, Pará (1°28’S 48°27’ W). In this region, the climate type is Afi (Köppen), comprising a rainier season between January and June, and a less rainy season from July to December, and an average annual precipitation of 3,001 mm. Local annual average temperature is 26.8°C, relative humidity is 83%, and sunshine duration is 2,279 hours per year (PACHÊCO et al., 2009).

Four adult male Santa Inês sheep, (mean weight =54 kg) were used in this study. Animals were

cannulated in the rumen and subjected to a basic diet to meet their maintenance requirements (Table 1). Their diet was composed of 60% elephant grass 'Napier' (*Pennisetum purpureum* Schum.) and 40% *Cratylia* (*C. argentea*), and was offered daily at 8:00 a.m. and 2:00 p.m. Mineral salt and drinking water were mixed and served *ad libitum* in individual troughs and drinking fountains. Animals were kept

in individual pens measuring 2.5 m × 3.0 m (7.5 m²) with 1.1 m fence during a total experimental period of 21 days, which included 14 days for adaptation to dietary changes and 5 days for data collection. We used the *in-situ* technique described by Mehrez and Orskov (1977) to determine nutrient degradability of *C. argentea*, *F. macrophylla*, and *. guianensis* 'Campo Grande' (Embrapa).

Table 1. Bromatological composition of the elephant grass cv purple Napier and the leguminous plant *Cratylia argentea*, used in the experiment as basic dietary foods.

Plant	Nutrient (%)			
	DM	CP	NDF	ADF
Elephant grass cv purple Napier	28.5	8.2	65.8	58.9
<i>Cratylia argentea</i>	32.8	21.9	72.5	63.6

DM: dry matter; CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber.

These plants were cut off at 0.3 m from the soil in rainy season and leaves were selected and pre-dried in a ventilated oven at 55°C for 72 h. Subsequently, they were ground to a particle size of 4 mm. Approximately 5 g of each tested leguminous plant aged 55 and 75 days were placed into nylon bags measuring 10 x 15 cm of free area with 50 microns porosity, achieving an approximate ratio of 20 mg/cm². These bags were incubated during 0, 12, 24, 36, 48, 72, and 96 h in triplicates per animal and incubation time, always in the morning (NOCEK, 1988).

Dry matter (DM), crude protein (CP), ethereal extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), ashes (ASH), and lignin contents of the incubated samples and residues were determined according to AOAC International (1995). Neutral detergent insoluble nitrogen (NDIN), acid detergent insoluble nitrogen (ADIN), and lignin indigestible in acid fiber (LIAF) were determined by nitrogen content analysis in residues from direct NDF and ADF determinations, according to the recommendations by Silva and Queiroz (2006) (Table 2).

Table 2. Bromatological composition of the tropical leguminous plants *Cratylia argentea*, *Flemingia macrophylla* (Willd.) Merrill, and *Stylosanthes guianensis* cv Campo Grande (EMBRAPA), aged 55 and 75 days.

Leguminous plant (age)	Nutrient (% in DM)								
	DM	CP	EE	NDF	ADF	LIAF	ASH	NIND	NIAD
<i>Cratylia argentea</i> (55 days)	19.32	27.62	4.64	63.3	55.61	15.01	1.27	17.13	11.22
<i>Cratylia argentea</i> (75 days)	22.65	22.42	3.07	65.92	56.59	19.97	1.86	19.01	12.51
<i>Flemingia macrophylla</i> (55 days)	23.66	19.59	3.14	68.78	62.05	35.99	1.93	14.25	12.54
<i>Flemingia macrophylla</i> (75 days)	26.3	16.81	3.02	71.9	67.45	39.41	1.84	17.01	14.89
<i>Stylosanthes</i> Campo Grande (55 days)	27.22	17.53	2.46	64.98	59.24	19.41	1.27	8.65	5.59
<i>Stylosanthes</i> Campo Grande (75 days)	29.75	14.12	2.02	68.62	61.42	27.77	1.36	7.55	6.47

DM: dry matter; CP: crude protein, EE: ethereal extract, NDF: neutral detergent fiber, ADF: acid detergent fiber; LIAF: lignin indigestible in acid fiber; ASH: ashes; NIND: nitrogen insoluble in neutral detergent; NIAD: nitrogen insoluble in acid detergent.

Weight loss at each incubation time was probably caused by nutrient disappearance. DM and CP ruminal degradation were adjusted using the degradability model suggested by Orskov and McDonald (1979), as follows: $DEG(t) = a + b(1 - e^{-ct})$, where DEG: accumulated degradability of the nutritional component, after some time t ; **a** = intercept of the degradability curve, when $t=0$, corresponding to the soluble fraction of the nutritional component; **b** = potential degradability of insoluble but potentially degradable fraction of the nutritional component; **c** = degradation rate by fermentative action of the fraction, and **t** = incubation time (h). Potential degradability (PD), which is the maximum degradability achieved if food remains in the rumen for indeterminate time, corresponds to the sum $a + b$. In this study, **Kd** values corresponds to the residual fraction found in the nylon bag after incubation period in the rumen (undegradable fraction).

The model by Mertens and Loften (1980) was used for NDF ruminal degradation: $R_t = B \times e^{-ct} + I$, where R_t = fraction degraded in time t ; **B** = insoluble fraction potentially degradable, and **I** = indigestible fraction. After adjusting this equation for NDF degradation, fractions were standardized according to Waldo et al. (1972), using the equations: $B_p = B / (B + I) \times 100$; $I_p = I / (B + I) \times 100$, where: B_p = standardized potentially degradable fraction (%);

I_p = standardized indigestible fraction (%); **B** = insoluble fraction potentially degradable, and **I** = as defined above.

The equation proposed by Orskov and McDonald (1979) was used to calculate the effective DM and CP ruminal degradability of the nutritional component (ED): $ED = a + (b \times c / (c + k))$, where **a**, **b**, and **c** = as defined above; **k** = ruminal passage rate (% / h). Passage rates of 2, 5 and 8% per hour were adopted for this calculation, as suggested by AFRC (1993). The model by Waldo et al. (1972) was used to calculate ED for NDF, according to the equation $ED = B_p \times c / (c + k)$, where: B_p is the standardized potentially degradable fraction (%); **c** and **k** = as defined above. Data were expressed as means and standard deviation, and the statistical treatment was performed using SAS v.9.2 software (SAS Institute, Cary, NC, USA). Means were compared using the PROC GLM procedure and a Tukey's HSD test with 5% probability.

Results and Discussion

The “a” fraction represents the water-soluble fraction of food, estimated by the particles eliminated through a nylon bag mesh when immersed in ruminal liquid and then washed in running water (TONISSI et al., 2004). In the present study, *C. argentea* at both ages, and *F. macrophylla* aged 55 days

presented the highest “a” fraction for DM (Table 3). This could be attributed to higher CP levels in these plants, leading to a greater amount of nitrogen available in the rumen, which is quickly used by ruminal microorganisms to produce microbial protein. In addition, a high “a” fraction rate in these leguminous plants can show the presence of higher amounts of soluble carbohydrates such as starch and monosaccharides, which are present in plant cells. Notably, the soluble “a” fraction can contribute to ruminal synthesis of volatile fatty acids, considered the main source of energy for ruminants (SÁ et al., 2011). *Stylosanthes* aged 55 days presented a lower soluble “a” fraction than the other studied plants (13.26%). However, according to Oliveira et al. (2014), values above 10% are considered high.

As expected, all water-soluble fractions values for NDF were zero, since the NDF fraction is insoluble in water. These results differ from those found by Molina et al. (2003), who evaluated ruminal

degradability of sorghum silage and reported an “a” fraction value of 23% for NDF, probably due to the loss of minute particles when the nylon bags were washed at time zero.

Cratylia aged 55 days, and *Stylosanthes* at both ages, showed the highest “a” fraction values for soluble CP. These high rates obtained for the leguminous plants evaluated and the different “a” fraction degradability values agree with those from Broderick (1995), who reported that leguminous forage has high ruminal degradation rate for CP, and that there are different CP degradability levels among different leguminous plants. However, the lower degradability level results obtained for some plants are attributed (1) to tannin condensation, which has the property of binding to CP and carbohydrates; (2) to the protein complexed with lignocellulose, which affects cellulolytic microorganisms; and (3) to the protection against protein degradation by the ruminal microbiota (GODOY, 2007; ABDALLA et al., 2008; OLIVEIRA et al., 2009).

Table 3. Dry matter (DM), neutral detergent fiber (NDF), and crude protein (CP) ruminal degradation parameters of tropical legumes for 2, 5, and 8% passage rates and coefficient of variation (CV).

Legume/Age (days)	DM							
	Fraction (%)			ED (%/h)				
	a (%)	b (%)	c (%/h)	Kd (%)	PD	2 (%/h)	5 (%/h)	8 (%/h)
<i>Cratylia</i> /55	24.24a	35.74b	2.63b	40.03b	54.42a	42.89c	35.55b	32.44c
<i>Cratylia</i> /75	27.80a	37.18b	7.23a	35.03b	64.82a	56.59a	49.46a	45.21a
<i>Flemingia</i> /55	23.39a	13.13c	4.1b	63.49a	34.92c	31.26d	28.66b	27.41c
<i>Flemingia</i> /75	15.85bc	16.71c	5.7a	67.45a	33.46c	28.17d	24.71c	22.78a
<i>Stylosanthes</i> /55	13.26c	51.27a	3.8b	35.48b	62.76a	46.48bc	35.12b	29.56c
<i>Stylosanthes</i> /75	18.66b	42.22b	7.5a	40.12b	59.54a	51.29ab	43.46a	38.68b
CV	10.02	8.67	32.5	6.01	5.62	7.33	8.49	8.32
	NDF							
	0	53.79 b	2.54a	46.21bc	48.82a	29.94a	18.03a	12.91ab
	0	59.11a	4.85a	40.91c	46.42a	38.98a	26.68a	20.55a
	0	29.42c	3.54a	70.58a	25.54b	17.13b	11.27b	8.48b
	0	30.95c	2.38a	69.05a	26.92b	16.41b	9.78b	6.99b

continue

continuation

<i>Stylosanthes</i> /55	0	58.71a	4.28a	41.29c	56.74a	38.95a	26.22a	19.85a
<i>Stylosanthes</i> /75	0	50.85b	4.49a	49.15b	49.85a	34.84a	23.79a	18.09a
CV	-	5.73	53.74	5.11	8.98	17.29	27.91	34.14
CP								
<i>Cratylia</i> /55	55.07ab	28.74a	3.62b	15.09c	82.81a	73.63b	67.40b	64.47b
<i>Cratylia</i> /75	54.95b	27.53a	4.53b	17.53c	81.42a	73.35b	67.51b	64.55b
<i>Flemingia</i> /55	42.71c	14.94bc	1.53b	42.35b	54.02b	49.09c	46.18c	45.09c
<i>Flemingia</i> /75	26.34d	13.33c	2.23b	60.34a	37.89c	33.24d	30.39d	29.21d
<i>Stylosanthes</i> /55	65.66a	19.15b	5.54b	15.18c	84.07a	78.85a	74.97a	72.93a
<i>Stylosanthes</i> /75	58.76ab	24.84a	14.84a	16.41c	83.44a	77.16ab	72.56a	69.18ab
CV	9.22	11.49	48.13	8.87	3.29	3.79	4.11	3.83

Means followed by different letters in the same line differ among themselves by the Tukey test at 5% probability.

a = soluble fraction of the nutritional component; b = potential degradability of the insoluble but potentially degradable fraction of the nutritional component; c = degradation rate; Kd = undegradable fraction; PD = potential degradability; ED = effective degradability.

The “b” fraction corresponds to the insoluble but potentially degradable portion of the nutritional component. The highest value of “b” for DM was 51.27% in *Stylosanthes* ‘Campo Grande’ (Embrapa) aged 55 days, which shows that this plant has good nutritional potential. The other plants presented lower values for this fraction, with *Flemingia* showing only 13.13 and 16.71% aged 55 and 75 days, respectively. This is probably due to the high contents of NDF, ADF, NIND, NIAD, and LIAF fibrous fractions, which can interfere in its degradability. *Flemingia* also showed high lignin in acid fiber (LIAF) levels at 55 (35.99%) and 75 (39.41%) days. These values are higher than those for *Cratylia* (19.97% and 27.77%) at the same ages.

C. argentea at 75 days and *Stylosanthes* at 55 days showed a higher percentage of potentially degradable “b” fraction for NDF, with 59.11 and 59.71%, respectively. The insoluble “b” fraction values for NDF in *Flemingia* were 29.42% and 30.95%, at 55 and 75 days, respectively. These values are considered low when compared to *Cratylia* and *Stylosanthes* aged 55 and 75 days, probably because of lower ruminal degradation, showing that these plants’ cell wall components compromise insoluble

“b” fraction degradation.

The highest “b” fraction values for CP degradability kinetics were found in *C. argentea*, at both ages, with 28.74% and 27.53%, and *Stylosanthes* at 75 days, with 24.84% of potentially degradable fraction. It can be inferred that *Stylosanthes* presents a high “b” fraction degradation rate for DM, NDF, and CP, being potentially degradable in the rumen and able to supply energy, hence providing structural carbohydrates for the cellulolytic bacteria and for the animal.

The highest “c” fraction degradation rate for DM was observed in all three leguminous plants at 75 days. There was no significant difference between the NDF degradation rate and the “c” fraction for CP, detecting the highest value (14.84%) for *Stylosanthes* at 75 days (see Table 3). For all plants studied here, DM, CP, and NDF degradation rates agreed with those from NRC (2001), which consider good degradability rates values from 2.0 to 9.2% / h. However, our results differ for *Flemingia* at 55 days, which has a “c” fraction below 2% / h, and for *Stylosanthes* at 75 days, which showed a “c” fraction higher than 9.2% / h. This low degradation

rate for *Flemingia* could be related to high content of fibrous fractions in cell wall.

Flemingia showed the highest percentages of Kd undegradable fraction for DM and NDF at both ages, and for CP at 75 days, suggesting that ruminal degradation is difficult in this plant. *Flemingia* has high values of fibrous fractions and lignification in its chemical composition, what may explain this result. The lower undegradable fraction values for DM, NDF, and CP in *Cratylia* and *Stylosanthes* at both ages suggest that these plants are easily digestible in the rumen, and can contribute to microbial protein synthesis.

The highest PD values for DM, NDF, and CP were found in *Cratylia* and *Stylosanthes* at both ages, showing that these plants achieve maximum degradability if food remains in the rumen for indeterminate time. Low PD values for *Flemingia* can be associated with high fiber content in its bromatological composition, limiting its potential degradation in the rumen. These chemical composition and degradation characteristics restrict the use of *Flemingia* as a protein bank and supplement in the diet of ruminants.

Depending on the size of food particles after rumination, they can enter the posterior compartment before being fully degraded ("passage rate"). PD was calculated by the equation proposed by Orskov and McDonald (1979), and was estimated without considering the passage rate, which can lead to an overestimated ruminal degradability (PIRES et al., 2006). Therefore, it was necessary to calculate ED, considering passage rates of 2, 5, and 8% per hour. ED is related to the energy produced in the rumen, thus, higher ED for DM, NDF, and CP provide higher energy content for ruminal microorganisms (AROEIRA, 1996).

In this study, *Cratylia* and *Stylosanthes* aged 75 days showed similar and higher ED values for DM at 2 and 5%/hour, compared to *Flemingia* at both ages, showing that, with degradation rates of 2 and 5%/h, these plants are more rapidly used by

rumen microorganisms to produce energy. Ladeira et al. (2002) reported a similar ED for DM at 5%/h passage rate with *S. guianensis* hay. ED at 8%/hour was higher for *Cratylia* and *Flemingia* at 75 days, 45.21 and 22.78%/hour, respectively. ED for NDF in *Cratylia* and *Stylosanthes* at both ages had the highest values at all passage rates. *Stylosanthes* stood out at both ages, with higher ED for CP, regardless of the studied passage rates.

Despite of the relatively high fibrous fraction values for NDF, ADF, NIND, NIAD, and LIAF in *Cratylia argentea* and *S. guianensis*, these plants do not seem to interfere negatively in potential DM, CP, and NDF degradability values. These values tend to present decreasing results due to different plant ages, with consequent higher LIAF content. Santos et al. (2009) studied *Cratylia* with high lignified content (30%), and showed that it did not interfere in consumption and DM, CP, and NDF digestibility.

Conclusions

Leguminous plants *Cratylia* and *Stylosanthes* have high degradation rates in "a" and "b" fractions of CP, and "b" fraction of NDF, and they can be recommended as a protein bank and supplement for ruminants. However, *F. macrophylla* (Willd.) Merrill should be avoided as a supplementary protein source for ruminants because of its high values of fibrous fractions, with difficult DM, CP, and NDF degradation.

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