

## *In vitro* Digestibility of Indian Bamboo (*Bambusa vulgaris*) Leaves Associated with *Stylosanthes guianensis* in Ruminants

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### Abstract

The study of the *in vitro* digestibility of Indian bamboo (*Bambusa vulgaris*) leaves associated with *Stylosanthes guianensis* in ruminants was conducted in April 2019 in the Animal Production and Nutrition Research Unit of the University of Dschang. A bovine ruminal fluid, a source of energy (*B. vulgaris*) and a nitrogen source (*S.guianensis*) with or without polyethylene glycol (PEG) were used. A sample of rations based on *B. vulgaris* associated with 0; 20 and 30% of *S.guianensis*, with or without PEG was removed, dried and milled to determine the chemical composition and evaluation of *in vitro* digestibility. Results of this study showed that the addition of legume increased the total nitrogen content (MAT) (12.87, 13.03 and 13.56% DM) when the *B. vulgaris* was associated with 0; 20 and 30% *S.guianensis*. The digestibility of organic matter (DM) (89.89, 90.46 and 89.85% DM), also increased with the addition of *S.guianensis* respectively in proportions 0; 20 and 30% of the ration based on *B. vulgaris*. The *in vitro* digestibility parameters of *B. vulgaris* increased significantly ( $p < 0.05$ ) with the addition of legume associated with PEG. The highest *in vitro* digestibility of organic matter (IVDOM) in the presence of PEG was obtained respectively with the rations IB70 + Sg30 + PEG (47.60%) and IB80 + Sg20 + PEG (46.82%) while, the lowest IVDOM was obtained with rations IB70 + Sg30 (45.96%) and IB100 (40.60) respectively. The highest gas production (32.32 ml / 500 mgMS), volatile fatty acid (VFA) (0.71 mmol / 40 ml) and metabolizable energy (EM) (7.40 MJ / KgDM) were obtained with the ration IB80+ Sg20. This study showed that adding a legume to *B. vulgaris* improves digestibility in ruminants.

**Keywords:** *Bambusa vulgaris*; *in vitro* digestibility; PEG, ruminant; *Stylosanthes guianensis*

### Introduction

Livestock plays important socio-economic roles both at household and national levels in Africa [1]. The grazing stock play a complementary role of utilizing the vast natural pasturelands where crop production is not feasible besides providing the poor rural households with milk, meat, manure, power, and cash flow [2]. In order to obtain high yields of these products, it is advisable to produce livestock in confinement. Finishing lambs in confinement is a viable alternative to obtain higher-quality carcasses [2]. However, the production costs of confined animals are considered high due to factors as the great volume of grains and cereals used in the concentrate diets, especially in semiarid regions [3]. Most of the time, the low amount of forage during the dry period and the need to invest in irrigated systems for forage cultivation and/or to import supplies make adoption of this system unfeasible in these areas [3]. Hence, the use of agro-industrial by products and other alternative sources which are not used frequently should be adopted. This the case wherein we use Indian bamboo leaves as an alternative source of feed to replace other sources which have frequently used in animal production such as *Brachiaria ruziziensis*, *Panicum maximum*. This will be associated with other

sources considered as main proteins sources from the family of leguminous plants whose nutritive value has been established as complement of poor feeds [3,4]. These leguminous plants include; *Leucaena leucocephala*, *Arachis glabrata*, *Desmodium intortum* [3]. In fact, leguminous tree species have been used as protein supplements and as chemotherapeutics in ruminant animals [5,6]. When compared with forages from natural pastures, tree and shrub species contain enough minerals and nutrients to fulfill the nutritional requirements of ruminant animals [7]. However, the establishment of trees is slow and may take more than one season before the forage is available for use. On the other hand, shrub species grow relatively quickly compared with trees and thus fodders from these shrubs could alleviate the feed challenges that face smallholder farmers [8]. This is why *Stylosanthes guianensis* will be used as source of protein to supplement Indian bamboo leaves whose crude protein in fresh leaves vary according to the bamboo varieties which match with that of Food and Agricultural Organization's [9]. For example the crude protein content in fresh leaves of *Dendrocalamus strictus* a variety of Indian bamboo collected from India and Pakistan gave a range of 14.2 - 20.5% [9]. This value varies with season and the stage at which the plant is harvested given that bamboo is a gramineae plant. The presence of anti-nutritional factors such as phenols, tannins and condensed tannins in *Stylosanthes guianensis* especially condensed tannins with a value above 50g/kg DM becomes detrimental to the animal [10], leads to the use of Polyethylene glycol to ease the use of nitrogen by the microorganisms of the rumen. From books consulted, no work has been done in this context. Also, the fastest means of the evaluation of digestibility of a given diet is the *in vitro* digestibility method which deals with the microorganisms of the rumen such as bacteria, protozoans in an external medium. It has the advantage that it is faster, practicable and can help to digest many rations in a 24 hour period. This work aim to evaluate the chemical composition and nutrient content of the different rations; the digestibility of various nutritive components in Indian bamboo leaves; the effect of *Stylosanthes guianensis* associated with or without PEG 600 on the digestibility of Indian bamboo leaves and the effect of the rations on the rumen microbial population.

## Materials and Methods

### Plant material

The plant material was made up of Indian bamboo leaves (*Bambusa vulgaris*) and *Stylosanthes guianensis*.

### Harvest of Indian bamboo leaves

Indian bamboo leaves was harvested around the Laboratory of Animal Nutrition and Feeding of the Faculty of Agronomy and Agricultural Sciences of the University of Dschang and chopped to obtain sizes of 2-3 cm. This was dried in an oven at 60 °C and analyzed for proximate nutrient content to gather nutritional baseline data then used in the *in vitro* digestibility tests.

### Harvest of *Stylosanthes guianensis*

*S. guianensis* was harvested from the Application and Research Farm and was dried in an oven at 60 °C till a constant weight was obtained. This was ground so much so that the particle sizes ranged from 1-2 mm in diameter and kept in nylon papers for chemical analysis.

### Experimental rations

*S. guianensis* was harvested from the Application and Research Farm and was dried in an oven at 60 °C till a constant weight was obtained. This was ground so much so that the particle sizes ranged from 1-2 mm in diameter and kept in nylon papers for chemical analysis.

Five rations were used in this study thus:

R0: 100% Indian bamboo leaves;

R1: 80% Indian bamboo leaves 20% *S. guianensis*;

R2: 70% Indian bamboo leaves 30% *S. guianensis*;

R3: 80% Indian bamboo leaves 20% *S. guianensis* + 1g PEG;

R4: 70% Indian bamboo leaves 30% *S. guianensis*+1g PEG.

A sample of each ration (500 g) was kept for the chemical composition and *in vitro* digestibility analysis.

### Analysis of chemical composition of rations

The analysis of the chemical composition of *B. vulgaris* associated with *S. guianensis* were carried out in the Laboratory of Production and Animal Nutrition of the University of Dschang in order to determine the dry matter, ash, organic matter (OM), crude cellulose, lipids, cell walls (NDF) and total nitrogen matter (TNM)[11].

### *In vitro* digestibility

**Preparation of the samples and the standard solution:** For each ration, a sample (500 mg) was weighed in triples [12], and deposited at the bottom of syringes. One gram of polyethylene glycol 6000 was added to the rations containing 80% *B. vulgaris* and 20% *S. guianensis*, 70% *B. vulgaris* and 30% *S. guianensis* respectively and all of it covered with the piston previously embalmed with petroleum jelly to facilitate its movement. The reagent was prepared following the method and procedure described by [13].

**Conditioning and incubation of samples and the standard solution:** Samples and the freshly prepared standard solution following the procedure described above were placed in an incubator at 39 °C overnight. Also the bain-marie was put in place and the temperature controlled by two thermostats set at 39 °C. In the morning before collection of ruminal fluid, the standard solution was placed in a water bath at 39 °C. In this solution arrived continuously a stream of CO<sub>2</sub> from a gas bottle whose pressure was regulated at 4 bars. The sodium sulfite (417 mg) and the NaOH 6N (0.444 ml) were added to the reagent which goes from blue to colorless passing through the pink color.

**Collection of ruminal fluid:** Water was boiled at 100 °C and placed in a flask and well covered overnight. The next morning at 6:00 am this flask was carried to the Dschang municipal slaughter house. As the cow was killed and eviscerated, immediately the rumen was removed, the water in the flask was poured away and the rumen content was poured into the flask and covered. This was immediately carried to the laboratory of Animal Production. Once we arrived in the laboratory, part of the fluid was taken to the laboratory of physiology to identify and count the number of protozoans in it. This was treated with methylene blue formaldehyde solution. The formaldehyde helps to fix the microorganisms while the methylene blue colors the nucleus blue and this can be easily observed under a microscope at 40X according to the counting principle.

**Principle:** Counting is done on a Malassez cell which is a thick glass slide in which is found a counting chamber made up of 100 rectangles among which 25 are subdivided into 20 small squares to ease counting. The volume of a cell is equal to 1 µl which is equivalent to 0.01 µl per rectangle (Figure 3). This enables a quantitative and qualitative analysis of protozoans [12]. This same analysis is done on the liquid after *in vitro* digestibility.

The rest was placed on a transparent blind and the ruminal content was pressed to obtain ruminal fluid. This liquid was immediately filtered under a flux of CO<sub>2</sub> which arrived continuously from a gas bottle. For the preparation of 2100 ml of inoculum, 700 ml of this liquid was measured and introduced into the standard solution always under the flux of CO<sub>2</sub>. This mixture (inoculum) was homogenized for 10 minutes with the aid of a magnetic stirrer, and 40ml of this inoculum were collected and injected into each syringe with the aid of a distributor of precision of the Fortuna Optifix mark then, all was placed in the water bath for incubation.

The incubation lasted 24 hours and the volumes of gas produced were recorded after 3h, 6h, 9h, 12h, 18h and 24h. The gas production was calculated and corrected after the following formula [13]:

$$GP \text{ (ml/mg MS)} = \frac{(V_{24} - V_0 - GP_0) \times 200 \text{ mg} \times GP_h}{m \times MS}$$

Where:

V<sub>24</sub> = Volume of gases read after 24 hours of incubation;

V<sub>0</sub> = Volume of inoculum in the syringe at the start of incubation;

GP<sub>0</sub> = Volume of gases produced by the blank after 24 hours of incubation;

GP<sub>h</sub> = Volume of gases produced by the standard after 24 hours of incubation.

**Evaluation of *In Vitro* Digestibility of Dry Matter (IVDDM):** At the end of incubation, the contents of the syringes were transferred in beakers of 600 ml. These syringes were then washed twice using 15 ml of Neutral Detergent Solution (NDS) and emptied in the corresponding beakers. The samples were boiled under soft fire for one hour and filtered in pre-damaged filter crucibles. These crucibles were dried at 103 °C overnight (12 hours) and weighed. This operation made it possible to withdraw the micro-organisms from more or less not degraded substrates. The IVDDM was calculated as the difference between the weight of the incubated substrate and the weight of non-degraded residue after treatment with NDS at the end of incubation, according to the following formula [14]:

$$IVDDM \text{ (\%)} = \frac{P_e - R}{P_e} \times 100$$

Where,

P<sub>e</sub> = Weight of the incubated sample;

R = Weight of the sample after incubation

**Evaluation of *in vitro* digestibility of organic matter (IVDOM) and metabolized energy (ME):** After 24 hour of incubation, the gases produced and corrected by gases of the pilot tubes were used to calculate the *in vitro* digestibility of organic matter (IVDOM), using the following regression equation [13]:

$$IVDOM \text{ (\%)} = 14.88 + 0.889GP + 0.45CP + 0.065C$$

Where,

GP = Quantity of gas produced after 24 hours of incubation;

CP = Crude Proteins;

C = Ashes.

Also, the content of the metabolizable energy (ME) was calculated according to the following equation [10]:

$$ME \text{ (MJ/kg DM)} = 2.20 + 0.136GP + 0.057CP$$

Where,

GP = Quantity of gas produces after 24 hours of incubation;

CP = Crude Proteins.

Determination of the partitioning factor (PF), microbial mass (MM) and volatile fatty acids (VFA): The partitioning factor (PF), which is the quantity of organic matter fermented to produce 1 ml of gas, was calculated using from the following formula [10]:

$$PF \text{ (mg / ml)} = \frac{MOD}{GP}$$

Where,

dOM (mg) = Degraded organic matter;

GP (ml) = Quantity of gas produces after 24 hours of incubation.

The microbial mass was calculated from the following formula [10]:

$$MM \text{ (mg)} = dOM - (GP \times SF)$$

Where,

dOM (mg) = Degraded organic Matter;

GP (ml) = Quantity of gas produces after 24 hours of incubation;

Stoichiometric Factor (SF) = 2.20 for fodder.

The volatile fatty acids (VFA) were obtained from the following formulas [10]:

$$VFA \text{ (mmol/ml)} = 0.0239GP - 0.0601 \text{ (In absence of PEG)}$$

$$VFA \text{ (mmol/ml)} = 0.0207GP + 0.0207 \text{ (In the presence of PEG)}$$

Where: GP (ml) = Gas produced after 24 hours of incubation.

## Calculated Parameters

The incubation of the various fodder samples made it possible to calculate the following parameters:

- Gas production (GP);
- Volatile fatty acids (VFA);
- Metabolisable energy (ME);
- Partitioning factor (PF);
- Microbial mass (MM);
- The *in vitro* digestibility of organic matter (IVDOM);
- The *in vitro* digestibility of dry matter (IVDDM).

## Statistical Analyses

The data on *in vitro* digestibility were subjected to the analysis of variance with one factor (rations), according to General Linear Model. The statistical model was as follows:

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

Where,

$Y_{ij}$  = Observation on the ration subjected to factors  $i$  and  $j$ ;

$\mu$  = general average;

$\alpha_i$  = effect of the type of leguminous plant  $i$ ;

$e_{ij}$  = residual error on the ruminal liquid subjected to factors  $i$  and  $j$ ;

When differences existed between the treatments, the averages were separated by the Waller Duncan test at 5% significance level [15]. The tests were carried out between the various parameters of *in vitro* digestibility. The statistics software used was the SPSS 20.0.

## Results

### Effect of *Stylosanthes guianensis* on the chemical composition and nutrient content *Bambusa vulgaris* based diets

There was a general increase in the chemical composition (DM, TNM) of the various rations with the graded level of *S. guianensis* (Table 1). Reversely, the crude cellulose decreased with the inclusion of *S. guianensis* in the various rations.

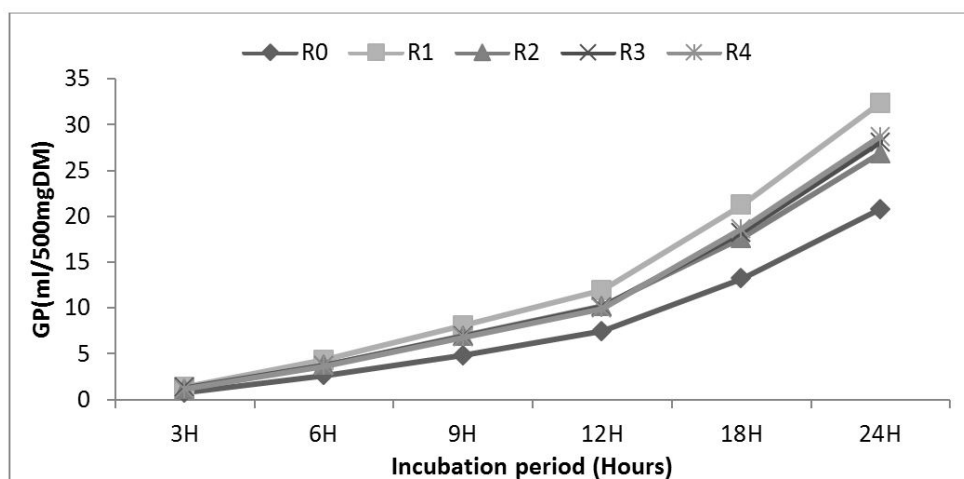
Chemical composition	Rations				
	R0	R1	R2	R3	R4
Dry matter (%DM)	92.48	92.43	93.1	92.43	93.1
Ash	10.10	9.53	10.14	9.53	10.14
Organic matter	89.89	90.46	89.85	90.46	89.46
Total nitrogenous matter	12.87	13.03	13.56	13.03	13.56
Crude cellulose	35.33	25.91	12.93	25.91	12.93
NDF	76.32	68.36	79.19	68.36	79.19

R0 = 100% *B. vulgaris*; R1 = 80% *B. vulgaris* and 20% *S. guianensis*; R2 = 70% *B. vulgaris* and 30% *S. guianensis*; R3 = 80% *B. vulgaris* and 20% *S. guianensis* + PEG; R4 = 70% *B. vulgaris* and 30% *S. guianensis* + PEG.

**Table 1:** Chemical composition and nutrients content of different rations

## Gas production obtained after 24 hours of incubation of the different rations with *Stylosanthes guianensis* with or without PEG

All the diets led to an increase in the production of gas. The gas production was highest in the ration with 80% *Bambusa vulgaris* and 20% *S. guianensis*. The rations with PEG had significantly ( $p < 0.05$ ) high gas production compared to the rations without PEG (Figure 1). It can be seen from this figure that In the presence of PEG the gas production increased with the increasing level of inclusion of *S. guianensis* in the rations.



R0 = 100% *B. vulgaris*; R1 = 80% *B. vulgaris* and 20% *S. guianensis*; R2 = 70% *B. vulgaris* and 30% *S. guianensis*; R3 = 80% *B. vulgaris* and 20% *S. guianensis* + PEG; R4 = 70% *B. vulgaris* and 30% *S. guianensis* + PEG

**Figure 1:** Production of gas obtained after 24 hours of incubation of the different rations with *Stylosanthes guianensis* with or without PEG

## Parameters of *in vitro* digestibility of different rations incubated with *Stylosanthes guianensis* associated with or without PEG

**Table 2:** Parameters of *in vitro* digestibility of different rations compared after incubation of different rations with *Stylosanthes guianensis* associated with or without PEG

Rations	GP after 24h (ml/200mgDM)	ME (MJ/kgDM)	MM (mg)	PF (mg/ml)	VFA (mmol/40ml)	IVDDM (%)	IVDOM (%)	NDF-N (%)
R0	20.76 <sup>c</sup>	5.81 <sup>b</sup>	146.81 <sup>ab</sup>	2.28 <sup>b</sup>	0.43 <sup>b</sup>	37.71 <sup>b</sup>	40.26 <sup>b</sup>	12.87 <sup>b</sup>
R1	32.32 <sup>a</sup>	7.40 <sup>a</sup>	94.88 <sup>c</sup>	1.56 <sup>c</sup>	0.71 <sup>a</sup>	33.20 <sup>b</sup>	50.58 <sup>a</sup>	13.03 <sup>b</sup>
R2	26.84 <sup>b</sup>	6.68 <sup>a</sup>	145.93 <sup>b</sup>	2.14 <sup>b</sup>	0.58 <sup>ab</sup>	40.81 <sup>b</sup>	45.96 <sup>a</sup>	13.56 <sup>a</sup>
R3	28.09 <sup>ab</sup>	6.82 <sup>a</sup>	138.18 <sup>b</sup>	2.02 <sup>b</sup>	0.45 <sup>b</sup>	39.86 <sup>b</sup>	46.82 <sup>a</sup>	13.03 <sup>b</sup>
R4	28.69 <sup>ab</sup>	6.93 <sup>a</sup>	211.87 <sup>a</sup>	2.73 <sup>a</sup>	0.68 <sup>a</sup>	54.38 <sup>a</sup>	47.60 <sup>a</sup>	13.56 <sup>a</sup>
SE	1.17	2.21	1.05	0.16	0.11	11.31	0.93	0.08
P	0.006	0.006	0.007	0.007	0.002	0.002	0.008	0.005

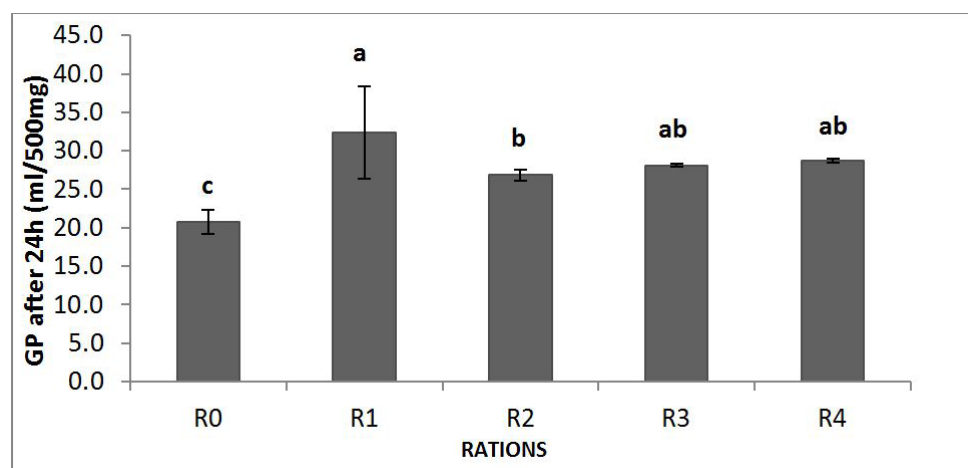
a,b,c: the means carrying the same letters in the same column are not significantly different ( $p > 0.05$ ). R0= 100% *B. vulgaris*; R1 = 80% *B. vulgaris* and 20% *S. guianensis*; R2 = 70% *B. vulgaris* and 30% *S. guianensis*; R3 = 80% *B. vulgaris* and 20% *S. guianensis* + PEG; R4 = 70% *B. vulgaris* and 30% *S. guianensis* + PEG; SE: Standard error; P: Probability; GP: gas produced; ME: metabolisable energy; MM: mineral matter; PF: partitioning factor; VFA: volatile fatty acids; IVDDM: *in vitro* digestibility of dry matter; IVDOM: *in vitro* digestibility of organic matter; NDF-N: residual nitrogen



The incorporation of *S. guianensis* to *Bambusa vulgaris* in the presence or absence of PEG has significantly ( $p < 0.05$ ) increased the *in vitro* digestibility parameters of the rations. In fact, the gas produced (GP) after 24h of incubation, the ME, the IVDOM and the VFA in the ration IB80Sg20 were significantly ( $p < 0.05$ ) higher than those of the other rations which were otherwise comparable ( $p > 0.05$ ). The level of inclusion of *S. guianensis* to *Bambusa vulgaris* significantly ( $p < 0.05$ ) improved the PF of the ration IB70+Sg30+PEG. The microbial mass which was the highest was obtained with the ration IB70+Sg30+PEG (Table 2).

### Gas production (GP) obtained after incubation of the different rations with *Stylosanthes guianensis* with or without PEG

The gas production was comparable between the rations containing PEG and are significantly ( $p < 0.05$ ) greater than the ration containing 30% *S. guianensis* and 70% *Bambusa vulgaris* but is less than the ration containing 20% *S. guianensis* and 80% *Bambusa vulgaris* which has the highest gas production (Figure 2).

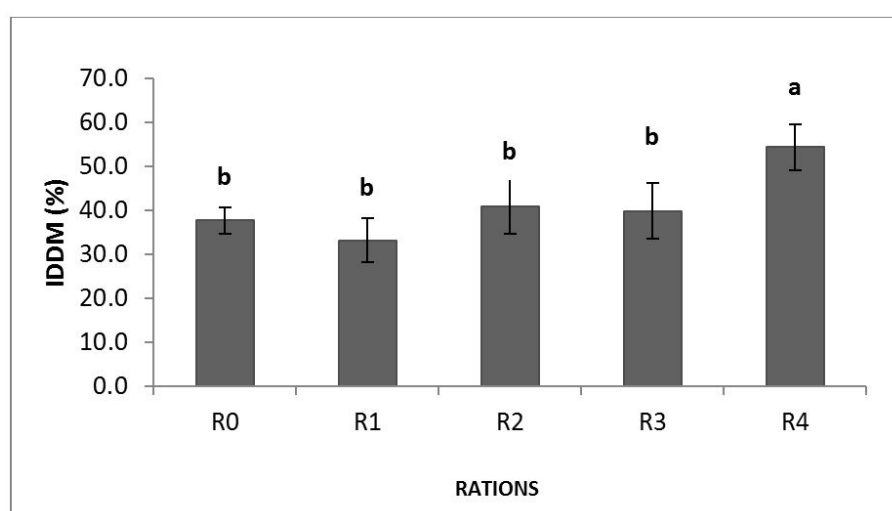


a,b,c: the means carrying the same letters in the same column are not significantly different ( $p > 0.05$ ). R0 = 100% *B. vulgaris*; R1 = 80% *B. vulgaris* and 20% *S. guianensis*; R2 = 70% *B. vulgaris* and 30% *S. guianensis*; R3 = 80% *B. vulgaris* and 20% *S. guianensis* + PEG; R4 = 70% *B. vulgaris* and 30% *S. guianensis* + PEG

Figure 2: Gas production obtained after incubation of the different rations with *Stylosanthes guianensis* with or without PEG

### *In vitro* digestibility of dry matter compared after incubation of different rations with *Stylosanthes guianensis* with or without PEG

Independently on the ration, the *in vitro* digestibility of dry matter (IVDDM) the different rations remain comparable ( $p > 0.05$ ) between these rations except the ration containing 70% *B. vulgaris* and 30% *S. guianensis* + PEG which showed a significantly ( $p < 0.05$ ) higher value than the others (Figure 3).



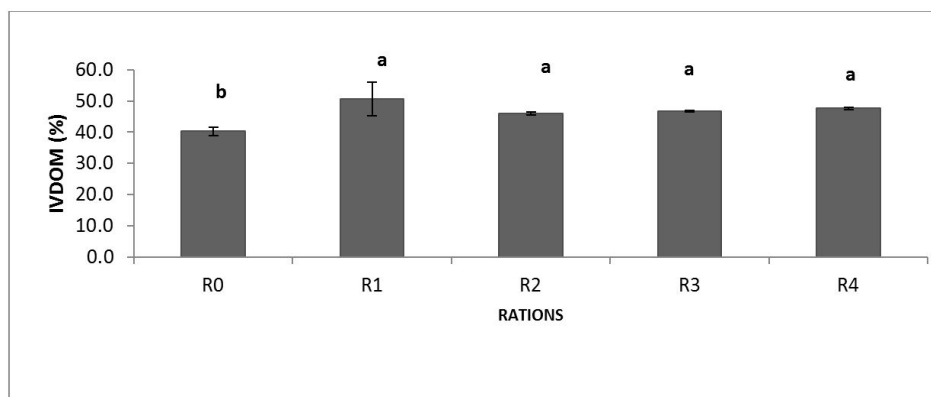
a,b: The means carrying the same letter in the same column are not significantly different ( $p > 0.05$ ).

R0 = 100% *B. vulgaris*; R1 = 80% *B. vulgaris* and 20% *S. guianensis*; R2 = 70% *B. vulgaris* and 30% *S. guianensis*; R3 = 80% *B. vulgaris* and 20% *S. guianensis* + PEG; R4 = 70% *B. vulgaris* and 30% *S. guianensis* + PEG.

Figure 3: *In vitro* digestibility of dry matter compared after incubation of different rations with *Stylosanthes guianensis* with or without PEG

### *In vitro* digestibility of organic matter compared after incubation of different rations with *Stylosanthes guianensis* with or without PEG

No matter the ration, the *in vitro* digestibility of organic matter (IVDOM) of different rations is comparable ( $p < 0.05$ ) except the ration R0 containing 100% *Bambusa vulgaris* which is the smallest (Figure 4).

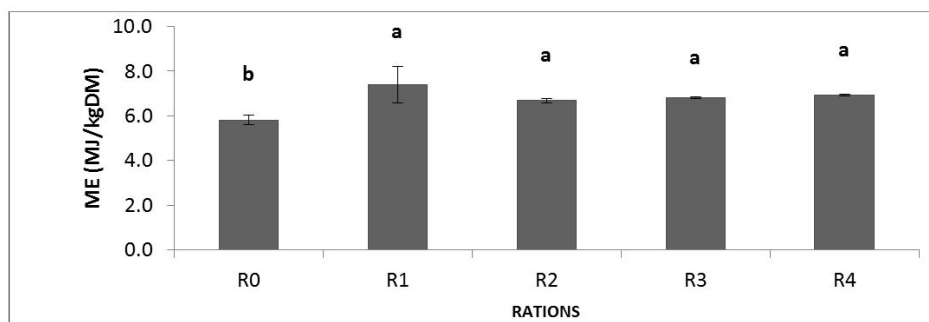


*a, b*: the means carrying the same letter in the same column are not significantly different ( $p < 0.05$ ). R0 = 100% *B. vulgaris*; R1 = 80% *B. vulgaris* and 20% *S. guianensis*; R2 = 70% *B. vulgaris* and 30% *S. guianensis*; R3 = 80% *B. vulgaris* and 20% *S. guianensis* + PEG; R4 = 70% *B. vulgaris* and 30% *S. guianensis* + PEG

**Figure 4:** *In vitro* digestibility of organic matter compared after incubation of different rations with *Stylosanthes guianensis* with or without PEG

### Metabolisable energy (ME) content after incubation with *Stylosanthes guianensis* with or without PEG

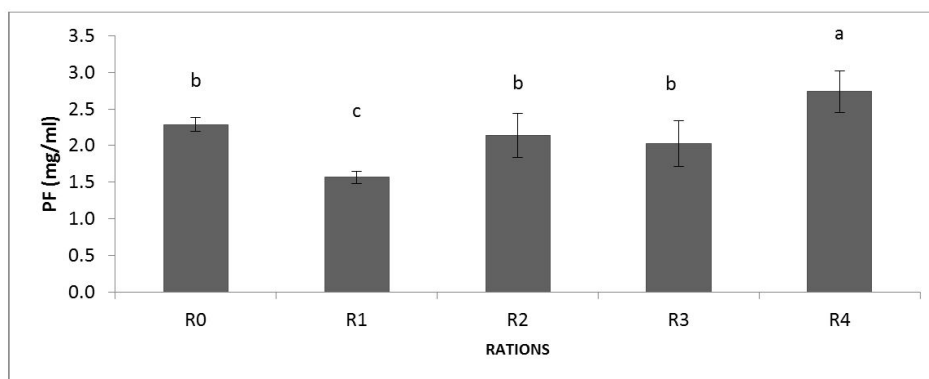
No matter the ration, the metabolisable energy of different rations was comparable ( $p > 0.05$ ) between the rations except for the ration containing 100% *Bambusa vulgaris* which recorded the lowest value (Figure 5).



*a, b*: the means carrying the same letter in the same column are not significantly different ( $p < 0.05$ ). R0 = 100% *B. vulgaris*; R1 = 80% *B. vulgaris* and 20% *S. guianensis*; R2 = 70% *B. vulgaris* and 30% *S. guianensis*; R3 = 80% *B. vulgaris* and 20% *S. guianensis* + PEG; R4 = 70% *B. vulgaris* and 30% *S. guianensis* + PEG

**Figure 5:** Metabolisable energy (ME) compared after incubation with *Stylosanthes guianensis* with or without PEG

### Partitioning factor compared after incubation of different rations with *Stylosanthes guianensis* with or without PEG



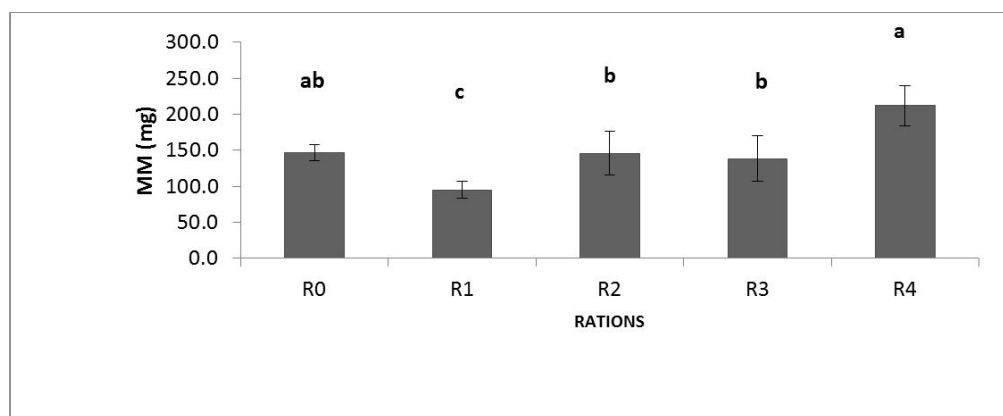
*a, b, c*: The means carrying the same letters in the same column are not significantly different ( $p > 0.05$ ). R0 = 100% *B. vulgaris*; R1 = 80% *B. vulgaris* and 20% *S. guianensis*; R2 = 70% *B. vulgaris* and 30% *S. guianensis*; R3 = 80% *B. vulgaris* and 20% *S. guianensis* + PEG; R4 = 70% *B. vulgaris* and 30% *S. guianensis* + PEG

**Figure 6:** Partitioning factor compared after incubation of different rations with *Stylosanthes guianensis* with or without PEG

The partitioning factor of the ration 70% *B. vulgaris* and 30% *S. guianensis* + PEG is significantly ( $p < 0.05$ ) greater than that of the other rations and that among the rations 100% *B. vulgaris* 70% *B. vulgaris* and 30% *S. guianensis*; 80% *B. vulgaris* and 20% *S. guianensis* + PEG are comparable ( $p > 0.05$ ) and the ration 80% *B. vulgaris* and 20% *S. guianensis* is the lowest (Figure 6).

### Microbial mass compared after incubation of different rations with *Stylosanthes guianensis* with or without PEG

The microbial mass of the ration R3 is comparable ( $p > 0.05$ ) with the ration R4 as well as with the rations R0 and R2. So there is no significant difference between them. The microbial mass of the ration R1 on the other hand is significantly lower to all the other rations (Figure 7).



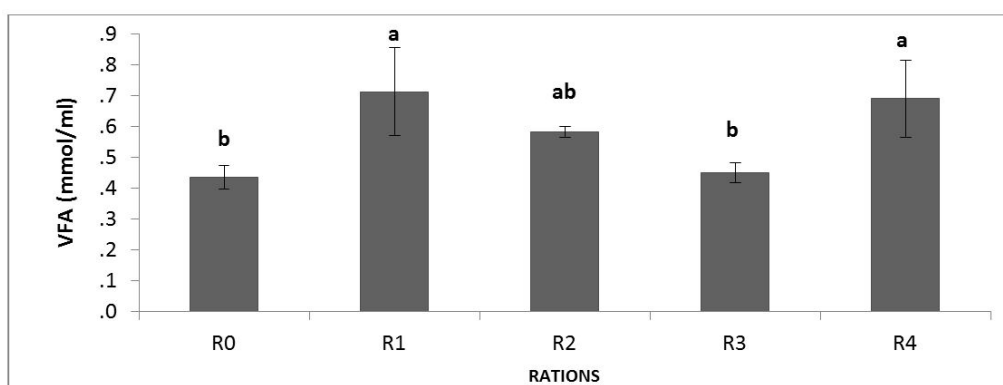
a,b,c: The means carrying the same letters in the same column are not significantly different ( $p > 0.05$ ).

R0 = 100% *B. vulgaris*; R1 = 80% *B. vulgaris* and 20% *S. guianensis*; R2 = 70% *B. vulgaris* and 30% *S. guianensis*; R3 = 80% *B. vulgaris* and 20% *S. guianensis* + PEG; R4 = 70% *B. vulgaris* and 30% *S. guianensis* + PEG

Figure 7: Microbial mass compared after incubation of different rations with *Stylosanthes guianensis* with or without PEG

### VFA compared after incubation of different rations with *Stylosanthes guianensis* with or without PEG

The production of VFA in the rations R1 and R2 were comparable and that R2 and R3 were comparable and R0. There was a significant ( $p < 0.05$ ) increase in R4 and R1 (Figure 8).



a,b: The means carrying the same letters in the same column are not significantly different ( $p > 0.05$ ).

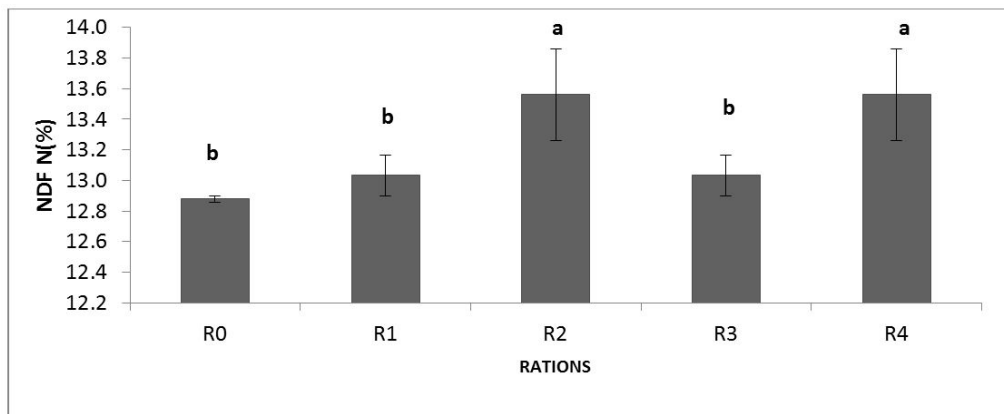
R0 = 100% *B. vulgaris*; R1 = 80% *B. vulgaris* and 20% *S. guianensis*; R2 = 70% *B. vulgaris* and 30% *S. guianensis*; R3 = 80% *B. vulgaris* and 20% *S. guianensis* + PEG; R4 = 70% *B. vulgaris* and 30% *S. guianensis* + PEG

Figure 8: Volatile fatty acids (VFA) compared after incubation of different rations with *Stylosanthes guianensis* with or without PEG

### Residual nitrogen (NDF-N) compared after incubation of different rations with *Stylosanthes guianensis* with or without PEG

The different rations had residual nitrogen which was comparable between R0, R1 and R3 meanwhile R2 and R4 were equally comparable and were significantly higher than the other rations (Figure 9).





a,b: the means carrying the same letters in the same column are not significantly different ( $p > 0.05$ ).

R0 = 100% *B. vulgaris*; R1 = 80% *B. vulgaris* and 20% *S. guianensis*; R2 = 70% *B. vulgaris* and 30% *S. guianensis*; R3 = 80% *B. vulgaris* and 20% *S. guianensis* + PEG; R4 = 70% *B. vulgaris* and 30% *S. guianensis* + PEG

**Figure 9:** Residual nitrogen (NDF-N) compared after incubation of different rations with *Stylosanthes guianensis* with or without PEG

### Effect of different rations on the population of protozoans

The observation of the ruminal fluid under a light microscope after coloration of the liquid with methyl blue formaline saline solution before the *in vitro* digestibility showed the presence of *Balantidium coli* and trophozoites of *Isospora* as showed on Figure 10 respectively. After the *in vitro* digestibility test, none of these protozoans be it *B. coli* or *Isospora*, was observed in the rations. They were not present in the control ration as well.



**Figure 10:** *Balantidium coli* and Trophozoid of *Isospora*

### Discussion

The chemical composition (DM, OM, total nitrogenous matter TNM and crude fiber) of the various rations was influenced by the addition of *S. guianensis*. Contradictory results were reported by [16] who studied digestibility of *Bambusa vulgaris* with the associative effect of *S. guianensis* with king grass silage. These differences could be due to the method of ground processing, the time of harvest, the treatment methods of fodder and, of the incorporation level of *S. guianensis* in the various rations used. The *in vitro* digestibilities tend to be improved by the addition of PEG in this study. Similar results were reported in animals fed with the rice straw associated with *L.leucocephala* in the presence of PEG (0.46%). This result confirm the affinity of polyethylene glycol fixing on tannins sites where the proteins should have been fixed to become unavailable to the micro-organisms of the rumen and the metabolic activities.

Gas production after 24 hours significantly ( $P > 0.05$ ) increased with the incubation of various levels of *S. guianensis* for the rations non-associated with PEG. As observed by [13], fodders having an increase in gas production have low microbial mass which is what was observed. The microorganisms may use the energy brought by the molasses on the one hand and ammonia produced by the accelerated fermentation of feed proteins on the other hand [17] for their growth and their development in order to effectively degrade the ration in the rumen [18]. However, the contribution of *S. guianensis* associated with PEG allows increase of the production of gases of various rations.

The partitioning factor and the microbial mass were improved by the addition of *S. guianensis* in the presence or absence of PEG except for one, to the ration made up of *B. vulgaris*. The value of the partitioning factor (2.73) obtained in this study with Indian bamboo leaves associated with *S. guianensis* with PEG is less than PF (3.04) obtained by [19] that did the nutritional evaluation of

bamboo cultivars. The PF of this study in the presence of PEG is less than what was obtained by these authors in absence of legumes and PEG [20]. Noted that values between 2.7 and 4.41 mg/ml do correspond to ATP yield from 10 to 32mg, and a yield of 32mg is considered to be maximum microbial efficiency. These results could be explained by the fact that the addition of *Stylosanthes* and PEG to the bamboo increased the PF by increasing the microbial mass on one hand. On the other hand, the result could be explained by the depressive effect of tannin on the degradation of the OM which could be prevented with the PEG in the presence of *B. vulgaris*.

The protozoan population was influenced by the various ration based on *B. vulgaris*. This is because the protozoans which were found in the ruminal fluid before the digestibility trials were absent at the end. This could have been because of the presence of tannins in *S. guianensis* which inhibited the protozoa. This was explained by [12] who studied this effect in neem leaves which had as consequence the improvement of feed utilization by increasing fermentability [21].

## Conclusion

At the end of this study, concerning the *In vitro* digestibility of Indian bamboo (*Bambusa vulgaris*) leaves associated with *Stylosanthes guianensis* in ruminants, it appears that:

- The addition of legume increased the total nitrogen content (MAT) when the *Bambusa vulgaris* was associated with 0; 20 and 30% *Stylosanthes guianensis*;
- The digestibility of organic matter (DM) increased with the addition of *Stylosanthes guianensis* respectively in proportions 0; 20 and 30% of the ration based on *Bambusa vulgaris*;
- This study showed that adding a legume to *Bambusa vulgaris* improves digestibility in ruminants; the parameters of *in vitro* digestibility increased significantly ( $p < 0.05$ ) with the addition of legume associated with PEG; the highest *in vitro* digestibility of organic matter (IVDOM) was obtained with the rations IB70 + Sg30 + PEG and IB80 + Sg20 + PEG;
- The protozoan population was influenced by rations based on *B. vulgaris*.

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