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In Vitro Digestibility of *Panicum maximum* Hay Associated with *Calliandra calothyrsus* or *Leucaena leucocephala* in the Presence or Absence of Polyethylene Glycol (PEG)

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ABSTRACT

The study of in vitro digestibility of Panicum maximum hay associated with Calliandra calothyrsus or Leucaena leucocephala in ruminants was conducted in May 2018 in the Animal Production and Nutrition Research Unit of the University of Dschang. Ruminal fluid (bovine), energy (Panicum maximum) and nitrogen sources (Calliandra calothyrsus or Leucaena leucocephala) with or without polyethylene glycol (PEG) were used in this study. At the beginning of the trial, sample of different rations based on Panicum maximum associated with 0; 30 and 50% of Calliandra calothyrsus or Leucaena leucocephala, in the presence or absence of polyethylene glycol was removed, dried and milled to determine the mineral, chemical composition and the evaluation of the in vitro digestibility. The results of this study showed that the addition of legumes increased the total nitrogen content (MAT) by 12.96, 16.67 and 17.05% DM, when Panicum maximum was associated with 0; 30 and 50% Calliandra calothyrsus and, (12.96; 13.77 and 14.03% DM) when Panicum maximum was associated with 0; 30 and 50% Leucaena leucocephala. The digestibility of organic matter (OM) (27.36; 36.02 and 33.59% DM), the content of digestible nitrogenous material (MAT) (10.49; 11.22 and 13.75 g / 100 g dOM), the feed unit milk (UFL) (0.74; 0.72 and 0.74 Kg DM) and the meat feed unit (UFV) (0.65; 0.74 and 0.71 Kg DM) also increased with the addition of Calliandra calothyrsus respectively in the proportions 0; 30 and 50% of the ration based on Panicum maximum hay. The parameters of in vitro digestibility evolved differently regardless of the level of incorporation of the different legumes into the diet. In fact, the in vitro digestibility parameters of Panicum maximum hay increased significantly ($p<0.05$) with the addition of legumes associated with PEG. The highest in vitro digestibility of organic matter (IVDOM) of the rations in the presence of PEG was obtained respectively with the rations P50C50 + PEG (50.38%) and P70L30 + PEG (51.49%) while, the lowest IVDOM was obtained with rations P70C30 + PEG (40.02%) and P70L30 + PEG (38.71) respectively. The highest gas (23.22 ml / 500 mg DM), volatile fatty acid (VFA) (0.50 mmol / 40 ml) and metabolizable energy (ME) (6.31 MJ / Kg DM) were obtained with the ration P50C50 + PEG. This study showed that adding legumes to Panicum hay maximizes nutrition and improves digestibility in ruminants.

Keywords: *In vitro digestibility, Calliandra calothyrsus, Leucaena leucocephala, Panicum maximum, PEG*

INTRODUCTION

Feed remains one of the principal constraints to the development of breeding in Sub-saharian Africa (Tendonkeng et al., 2011, Lemoufouet et al., 2014) because the quality of fodder is good only at the beginning of the raining season and became worst very earlier (Pamo et al., 2007). This deterioration of fodder quality is accompanied by a fall in digestibility Moreover, the natural pastures a major source of feed in ruminant production and as it is a case in Africa Can ensure a satisfactory production only for one relatively short period of the year, and vary according to the climatic zone (Riviere, 1991). During dry season quality and quantity of this pasture drop rapidly to avoid the fall in productions during the dry season.

It is necessary to consider an improvement of the feed value of fodder. Several solutions can help to achieve these goals (Riviere, 1991). Improvement of natural pastures is possible trough the introduction of improved plants, the constitution of good quality fodder reserves using high-output fodder crops accompanied by a suitable conservation method. Hay is the oldest, and remains the most significant

preserved fodder, in spite of its dependence on a favorable climate at the moment of harvest. It can be made with simple materials, manually or with mechanization, and many small farmers make hay to ensure animal feed for the periods of recession.

However, drying, like the other methods of conservation, reduce digestibility and ingestion which depend on the period during which the grass was exposed to scrubbing, on the losses occurring during haymaking, and on the stage of harvest of the fodder used for the hay (Sao et al., 2010). The feed value and consequently of the digestibility of fodder and especially of the hay is variable (Sao et al., 2010).

Several solutions have been proposed in other to improve the feed value of graminaceous fodder in general and the hay in particular. One of the solutions most easily achievable is the use of the fodder leguminous plants whose feed value is well established (Pamo et al., 2006; Boukila et al., 2009). Among these leguminous plants, there are *Calliandra calothyrsus*, *Leucaena leucocephala*, *Arachis glabrata* and *Desmodium intortum* (Pamo et al., 2006 Miégoué et al., 2016). The use of leguminous plants can also constitute an effective solution in the reduction of production cost of ruminants.

However the effectiveness of their use is not optimal in animals because of their high content in anti-nutritional substances, such as tanins Tanins are molecules naturally synthesized by plants, in response to the various abiotic and biotic stresses (Frutos et al., 2002) they can directly influence the ruminal microbiota and the enzymatic activity (Mc Sweeney et al., 2001), by reducing the availability of nutriments and performances of the animal.

To mitigate the harmful effect of the anti-nutritional factors contained in leguminous plants, several methods were used to decontaminate tanins and to make the nutriments of the ration more available. With this intention, polyethylene glycol (PEG) can be used. Pamo et al. (2004) showed that the supplementation of some graminaceous with *Calliandra calothyrsus* in the presence of the PEG improved the production of gases and volatile fatty acids by these graminaceous plants. Thus by associating *Calliandra calothyrsus* or *Leucaena leucocephala* to the PEG, one could improve the feed value and the digestive use of maximum hay and contribute to improve the productivity of ruminants during the harsh season. The objective of this work was to contribute to the valorization of tropical fodder in ruminants feed.

Material and Method

Zone of Study

The study was conducted at the Experimental Farm of Dschang University in western Cameroon. The area is within the Sudano–Guinean zone of Central Africa (latitude 5–7°N, longitude 8–12°E; altitude 1400 m ASL). The annual temperature varies between 16 and 27°C with a relative humidity of 40–97%.

Plant Material

Panicum maximum was harvest at the Experimental Farm of Dschang University before flowering and sun dried (6 day) until the hay obtained. The hay obtained and the mown sheets of shrubby leguminous plants (*Calliandra calothyrsus* and *Leucaena leucocephala*) were finely crushed and preserved in plastic bags (to prevent the absorption of moisture) for the evaluation of the chemical composition and in vitro digestibility.

Preparation of Test Ingredients

Rations Formulation

After the harvest of the vegetable material, 500 g of each sample fodder was manually chopped using a machete into particles (2-5 cm) and dried at 60°C until constant weight in a ventilated drying oven (Gallemkamp). After drying, the samples were crushed using a hammer mill of sieve size 1 mm, then preserved in plastic sachets. Nine (09) rations were used in this study:

- R0:100% of *P. maximum* (P100);
- R1:70% of *P.maximum* and 30% *C. calothyrsus* (P70C30);
- R2: 50% of *P. maximum* and 50% *C. calothyrsus* (P50C50);
- R3: 70% of *P.maximum* and 30% *C. calothyrsus* + PEG (P70C30 + PEG);
- R4: 50% of *P.maximum* and 50% *C. calothyrsus* + PEG (P50C50 + PEG);
- R5: 70% of *P.maximum* and 30% *L. leucocephala* (P70L30);
- R6: 50% of *P.maximum* and 50% *L. leucocephala* (P50L50);
- R7: 70% of *P.maximum* and 30% *L. leucocephala* + PEG (P70L30 + PEG);

- R8:50% of *P.maximum* and 50% *L. leucocephala* + PEG (P50L50 + PEG).

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

Chemical Composition Analysis of the Rations

The analyses of the chemical composition of *P. maximum* associated with *C. calothyrsus or L. Leucocephala* with or without PEG were carried out at the laboratory of production and animal nutrition (LAPRONAN) of the University of Dschang in order to determine the nutritive value, according to the methods described by Van Soest et al. (1991) and AOAC (2002).

In Vitro Digestibility

Preparation of the Samples and Reagent

The evaluation of the quantity of gas produced and preparation of reagent were made according to the method and the procedure described by Menke et al. (1979). The rations (500 mg) were weighed in triple, using an electric balance of KERN 770 brand, with a range 210 g and sensitivity 0,0001g. The samples were deposited to the bottom of the syringes. One gram of polyethylene glycol 6000 was added with the rations containing 70% *P. maximum* and 30% *C calothyrsus*, 50% of *P. maximum* and 50% *C. calothyrsus*, 70% *P. maximum* and 30% of *L. leucocephala* and 50% of *P. maximum* and 50% of *L. leucocephala* respectively and covered with the piston previously embalmed with petroleum jelly to facilitate its movement. The reagent was prepared according to the method described by Menke et al. (1979). Similarly, a water bath was turned on and the temperature was maintained by two thermostats set at 39°C. The morning before the collection of rumen fluid, the reagent in which continually arrived a stream of gas (CO2) with a moderately pressure (4 Bars) was placed in a water bath at 39°C. Then, the sodium sulfide (417 mg) and 6N NaOH (0.444 ml) were added to this reagent. The ruminal fluid was obtained early in the morning before 7 AM just after slaughtering the adult bovines at the Dschang municipal slaughter-house and put in thermos flask heated containing boiling water then transported immediately to the laboratory. This liquid was immediately filtered under a CO2 flow. To prepare a 2100 ml inoculum, 700 ml of this liquid were taken and introduced into the standard solution always under the CO2 flow. This mixture (inoculum) was homogenized for 10 mm using a magnetic stirrer, and 40 ml of this inoculum were taken and injected into each syringe using a precision distributor (Fortuna Optifix).

Then, the unit was placed in the water bath for incubation for 24 hours. Volumes of gas produced were recorded after 3, 6, 9, 12, 18 and 24h. The production of gas was calculated and corrected according to the following formula (Menke and Steingrass, 1988):

$$\text{GP (ml/mg DM)} = \frac{(V_{24} - V_0 - GP_0)x200mgxGP_h}{mxMS}$$

Where, V24 = Volume of gas read after 24 hours of incubation;

V0 = Volume of incubation in the syringe at the beginning of incubation;

GP0 = Volume of gas produced by the control after 24 hours of incubation;

GPh = 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 with under soft fire for one hour and filtered in pre-damaged filter crucibles. These crucibles were dried at 103 ^{o}C overnight and weighed. This operation made it possible to withdraw the micro-organisms from more or less not degraded substrates. The IVDDM 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 (Van Soest and Robertson, 1985):

$$\text{IVDDM (\%)} = \frac{P_e - R}{P_e} x100$$

Where, Pe = 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 - 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 (Menke and Steingrass, 1988):

IVDOM (%) = 14.88 + 0.889GP + 0.45CP + 0.065C

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

CP = crude Proteins;

C = Ashes.

The content of the Metabolizable energy (ME) was calculated according to the following equation (Makkar, 2002):

Where, ME (MJ/kg DM) = 2.20 + 0.136GP + 0.057CP

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 (Makkar, 2002):

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

Where, dOM (mg) = Degraded Organic Matter;

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

The microbial mass was calculated from thefollowing formula (Makkar, 2002):

MM (mg) = dOM - (GP X FS)

Where, dOM (mg) = Degraded organic Matter;

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

Stoechiometric Factor (SF) = 2.20 for fodder.

The Volatile Fatty Acids (VFA) was obtained from the following formulas (Makkar, 2002):VFA (mmol/ml) = 0.0239GP – 0.0601 (In absence of PEG)

VFA (mmol/ml) = 0.0207GP + 0.0207 (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 chemical composition and *in vitro* digestibility were subjected to the analysis of variance with 2 factors (*C. calothyrsus* + PEG) according to General Linear Model.

The statistical model was as follows:

$$Y\ ij = \mu + \alpha\ I + \beta j + (\alpha\ \beta)\ I\ J + E\ ij$$

Where, Y ij = Observation on the ration subjected to factors I and J;

μ = general average;

α I = effect of the type of leguminous plant I;

βj = effect of the PEG J;

eij = residual error on the ruminal liquid subjected to factors I and J;

(α β) I J = effect of the interaction between factors I and J.

When the differences existed between the treatments, the averages were separated by the Waller Duncan test at 5% threshold of significance (Steel and Torrie, 1980). The correlation tests were carried out between the various parameters of *in vitro* digestibility. The correlation tests were used to compare between the parameters of digestibility related to leguminous plant.

Results

Effect of *Calliandra calothyrsus* or of *Leucaena leucocephala* on the Chemical Composition and Nutriment Content of the Various Rations Containing *Panicum maximum*

Whatever the ration, the contents of DM, OM and total nitrogen and digestible nitrogen matter (DNM) significantly increased with the

incorporation of *Calliandra calothyrsus* or of *Leucaena leucocephala* (Table 2). On the other hand, the ash contents, crude fiber and neutral detergent fiber decreased with the inclusion of *Calliandra calothyrsus* or *Leucaena leucocephala* in the various rations. The contents of total lipid and carbohydrate remained comparable.

Table1. *Chemical composition and nutriments content of the various rations*

Chemical Composition	Rations				
	P100	P70C30	P50C50	P70L30	P50L50
Dry Matter (%) (% DM)	77.9	89.91	90.10	89.25	89.69
Ashes	10.40	9.99	9.04	9.69	9.14
Organic matter	89.60	90.01	90.91	90.31	90.86
total nitrogen content	12.96	13.77	16.67	14.03	17.05
Lipid	1.20	2.37	1.19	2.19	1.20
Crude fibre	33.07	28.94	30.10	30.80	25.30
NDF	75.20	69.69	60.80	65.40	61.60
Totals carbohydrates	75.44	73.87	73.04	74.08	72.62
Nutritive values					
dMO (%DM)	27.36	36.02	33.59	32.12	43.67
DNM (g/100gdOM)	10.49	11.22	13.75	11.47	14.08
MkFU/Kg DM	0.65	0.74	0.71	0.70	0.79
MtFU /Kg DM	0.54	0.64	0.60	0.60	0.71

P100 = 100% of P.maximum, P70L30 = 70% of P. maximum and 30% of L. leucocephala, P50L50 = 50% of P.maximum and 50% of L. leucocephala, P70L30+PEG = 70% of P.maximum and 30% of L. leucocephala + PEG, P50L50+PEG = 50% of P. maximum and 50% of L. leucocephala + PEG. leucocephala; NDF: Neutral Detergent Fiber; dOM = Digestible organic matter; DNM = Digestible nitrogen matter.

Production of Gases (GP) Compared to Those Obtained for That Obtained after Incubation of the Various Rations with Calliandra calothyrsus Associated or Not with PEG

Gas production of rations associated with PEG was significantly higher (P<0.05) than those of the rations without PEG (Figure 2).

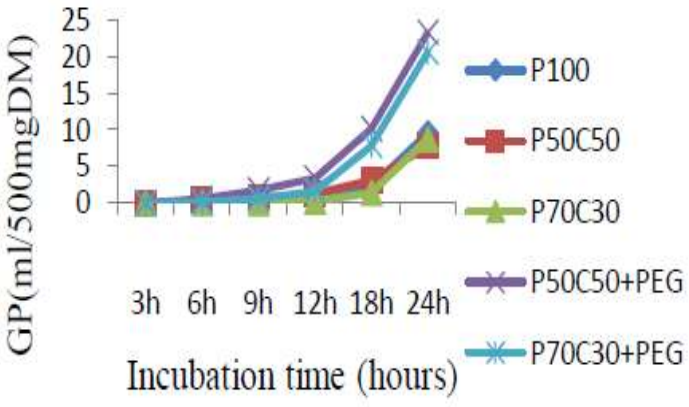

Figure2. *Gases production (GP) compared to those obtained after incubation of the various rations with Calliandra calothyrsus associated or not with the PEG*

P100 = 100% of P.maximum, P70L30 = 70% of P. maximum and 30% of L. leucocephala, P50L50 = 50% of P.maximum and 50% of L. leucocephala, P70L30+PEG = 70% of P.maximum and 30% of L. leucocephala + PEG, P50L50+PEG = 50% of P. maximum and 50% of L. leucocephala + PEG. leucocephala.

In presence of PEG, the production of gas increased with increasing level of *Calliandra calothyrsus* inclusion in the rations.

Production of Gases (GP) Compared to Those Obtained after Incubation of the Various Rations with *Leucaena leucocephala* Associated with the PEG or Not

The gas production in the rations associated with PEG was significantly higher (P<0.05) than those of the rations without PEG (Figure 3).

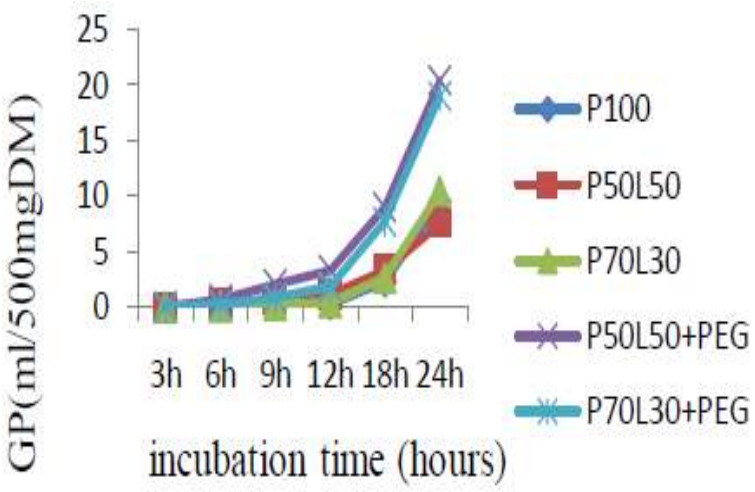

Figure3. *Production of gases (GP) compared to those obtained after incubation of various rations with Leucaena leucocephala associated with PEG or not*

P100 = 100% of P.maximum, P70L30 = 70% of P. maximum and 30% of L. leucocephala, P50L50 = 50% of P.maximum and 50% of L. leucocephala, P70L30+PEG = 70% of P.maximum and 30% of L. leucocephala + PEG, P50L50+PEG = 50% of P. maximum and 50% of L. leucocephala + PEG. leucocephala.

In presence of PEG, the production of gas increased with increasing level of *Leucaena leucocephala* inclusion in the rations. As a whole, it appears that associated with PEG, the production of gas significantly increased compared to the ration without PEG.

In Vitro Digestibility Parameters of Various Rations after Incubation with *Calliandra calothyrsus* or *Leucaena leucocephala* Associated with PEG or Not

The incorporation of *Leucaena leucocephala* to *Panicum maximum* in the presence or absence of PEG significantly ($p<0.05$) improved the various parameters of *in vitro* digestibility of rations (Table 3).

Table2. *In vitro digestibility parameters of the various rations from Panicum maximum associated Leucaena leucocephala in the presence or not of PEG*

Samples	GP (ml/200mg MS)	IVDDM (%)	IVDOM (%)	ME (MJ/kg MS)	PF (mg/ml)	MMmg)	VFA (mmol/ml)	NDF-N
P100	9.51^{b}	43.52^{c}	29.84^{c}	4.23^{b}	3.69^{a}	90.80^{bc}	0.17^{b}	1.55^{b}
P50L50	9.12^{b}	60.64^{a}	31.26^{c}	4.41^{b}	5.00^{a}	169.85^{a}	0.16^{b}	3.07^{a}
P70L30	$10,44^{b}$	55.02^{ab}	31.10^{c}	4.42^{b}	4.39^{a}	137.90^{ab}	0.19^{b}	2.01^{ab}
P50L50+PEG	20.40^{a}	50.54^{b}	41.29^{a}	5.95^{a}	3.17^{b}	79.20^{c}	0.44^{a}	2.16^{ab}
P70L30+PEG	18.99^{b}	51.49^{b}	38.71^{b}	5.58^{a}	3.38^{b}	93.60^{bc}	0.41^{a}	2.87^{a}
SEM	1.36	1.68	1.27	0.19	0.21	10.71	0.03	0.21
P	000	0,002	000	000	0.006	0.007	000	0.088

P100 = 100% of P.maximum, P70L30 = 70% of P. maximum and 30% of L. leucocephala, P50L50 = 50% of P.maximum and 50% of L. leucocephala, P70L30+PEG = 70% of P.maximum and 30% of L. leucocephala + PEG, P50L50+PEG = 50% of P. maximum and 50% of L. leucocephala + PEG. leucocephala; SEM: Standard error Mean; P: Probability; GP: Gas production; ME: Metabolizable energy; MM: Microbial mass; PF: Partitioning factor; VFA: Volatile fatty acids; IVDDM: In vitro digestibility of the dry matter; IVDOM: In vitro digestibility of the organic matter, NDF-N= Residual nitrogen.

Indeed, the gases produced (GP) after 24h of incubation, the ME, the residual nitrogen and the VFA of rations P70L30+PEG and P50L50+PEG were comparable ($p>0.05$) and significantly ($p<0.05$) higher than those of the other rations. The level of inclusion of *Leucaena leucocephala* to the *P.maximum* hay of significantly ($p<0.05$) improved IVDOM of ration P50L50+PEG.The best partitioning factor were obtained with the rations containing PEG. The *in vitro* digestibility parameters of the various rations from of the *Panicum maximum* hay associated with *Calliandra calothyrsus* in presence of PEG or not are presented in Table 4.

Table3. *In vitro digestibility parameters of the various rations from the of P. maximum hay associated with Calliandra calothyrsus in presence with the PEG or not*

Sample	GPGP (ML/200 mg MS)	IVDDM (%)	IVDOM (%)	ME (MJ/kg Ms)	PF (mg/ml)	MM (Mg)	VFA (mmol/ml)	Ndf-n
P100	9.5^{c}	43.52^{c}	29.84^{c}	4.23^{c}	3.69^{b}	90.80^{c}	0.17^{c}	1.5^{a}
P50C50	7.70^{c}	56.3^{a}	298^{c}	4.20^{c}	5.0^{a}	166.20^{a}	0.12^{c}	2.3^{a}
P70C30	8.70^{c}	51.46^{b}	29.46^{c}	4.17^{c}	4.3^{a}	131.90^{b}	0.15^{c}	1.80^{a}
P50C50+PEG	23.2^{a}	50.38^{b}	43.61^{a}	6.31^{a}	$3.04b^{c}$	72.70^{c}	0.50^{a}	2.09^{a}
P70C30+PEG	20.57^{b}	42.29^{c}	40.02^{b}	5.78^{b}	2.66^{c}	36.90^{d}	0.45^{b}	1.70^{a}
SEM	1.78	1.51	1.63	0.25	0.25	12.73	0.04	0.14
P	000	000	000	000	000	000	000	0.472

P100 = 100% of P.maximum, P70L30 = 70% of P. maximum and 30% of L. leucocephala, P50L50 = 50% of P.maximum and 50% of L. leucocephala, P70L30+PEG = 70% of P.maximum and 30% of L. leucocephala + PEG, P50L50+PEG = 50% of P. maximum and 50% of L. leucocephala + PEG. leucocephala. SEM: Standard error Mean; P: Probability; GP: Gas production; ME: Metabolizable energy; MM: Microbial mass; PF: Partitioning factor; VFA: Volatile fatty acids; IVDDM: In vitro digestibility of the dry matter; IVDOM: In vitro digestibility of the organic matter, NDF-N= Residual nitrogen.

Table 3 showed that the incorporation of *Calliandra calothyrsus* to *Panicum maximum* hay in the presence or absence of PEG significantly ($p<0.05$) improved various parameters of the *in vitro* digestibility of the rations. The effect of gases produced (GP) after 24h of incubation, the ME, the IVDOM and the VFA of ration P50C50+PEG were significantly ($p<0.05$) higher than those of the other rations which were also comparable ($p>0.05$). The inclusion level of *Calliandra calothyrsus* to *Panicum maximum* hay significantly ($p<0.05$) improved the PF of ration P70C30+PEG.The microbial highest mass was obtained with ration P50C50.

Gases Production (GP) After Incubation of Various Rations with *Calliandra calothyrsus* or *Leucaena leucocephala* Associated with PEG or Not

The production of gas compared of the rations containing 0; 50 or 70% of *Panicum maximum* associated with 0; 50 or 30 % of leguminous plants (*Calliandra calothyrsus* or *Leucaena leucocephala)* in the presence with the PEG or not showed that the gases production is comparable for the rations containing 50 and 70% of *Panicum maximun* non associated to PEG. (Figure 4)

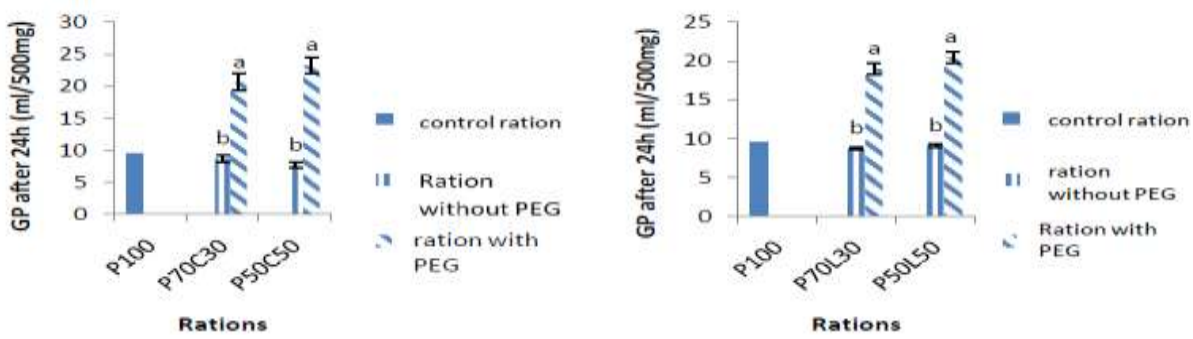

Figure4. *Gases production (GP) obtained after incubation of the various rations with Calliandra calothyrsus or Leucaena leucocephala associated or not with the PEG*

a,b: mean on the same band with the same ration are not significantly different (p>0,05). P100 = 100% of P.maximum, P70L30 = 70% of P. maximum and 30% of L. leucocephala, P50L50 = 50% of P.maximum and 50% of L. leucocephala, P70L30+PEG = 70% of P.maximum and 30% of L. leucocephala + PEG, P50L50+PEG = 50% of P. maximum and 50% of L. leucocephala + PEG. leucocephala.

On the other hand, in the presence of PEG, the production of gases of the rations associated with *Calliandra calothyrsus* were significantly ($P<0.05$) higher than those associated with *Leucaena leucocephala.*

In Vitro Digestibility of Dry Matter (IVDDM) Compared after Incubation of the Various Rations with *Calliandra calothyrsus* or *Leucaena leucocephala* Associated with PEG or Not

Whatever the ration, *the in vitro* digestibility of the dry matter (IVDDM) of various rations remained comparable (p>0.05) between the two leguminous plants, except ration P70+PEG where the ration containing *Leucaena leucocephala* showed values significantly (P<0.05) higher than that containing *Calliandra calothyrsus* (Figure 5).

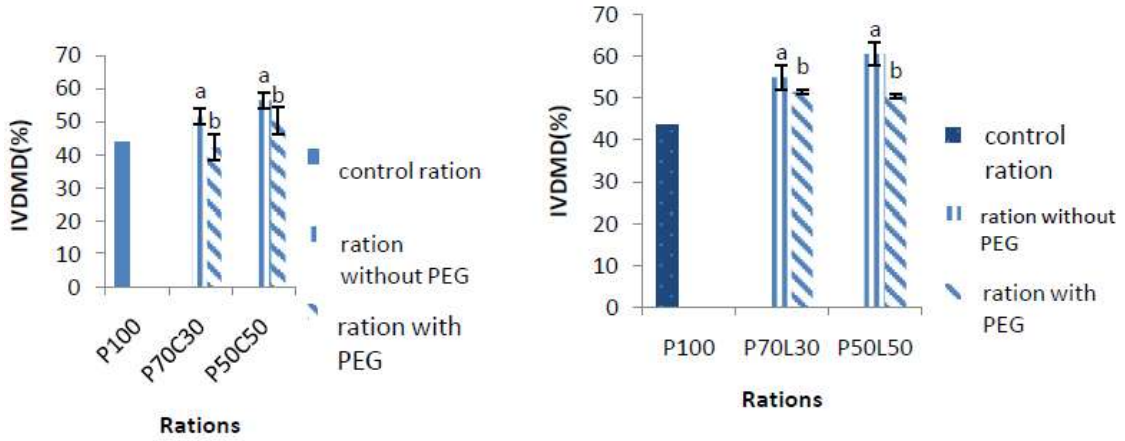

Figure5. *In vitro digestibility of dry matter (IVDDM) compared obtained after incubation of the various rations with Calliandra calothyrsus or Leucaena leucocephala associated or not with PEG*

a,b: mean on the same band with the same ration are not significantly different (p>0,05). P100 = 100% of P.maximum, P70L30 = 70% of P. maximum and 30% of L. leucocephala, P50L50 = 50% of P.maximum and 50% of L. leucocephala, P70L30+PEG = 70% of P.maximum and 30% of L. leucocephala + PEG, P50L50+PEG = 50% of P. maximum and 50% of L. leucocephala + PEG.

In Vitro Digestibility of Organic Matter (*Ivdom*) After Incubation of the Various Rations with *Calliandra calothyrsus* or *Leucaena leucocephala* Associated with the Peg or Not

Whatever the ration, the *in vitro* digestibility of the organic matter (IVDOM) of various rations was comparable (p>0.05). It appears however that the PEG improved IVDOM.(Figure 6)

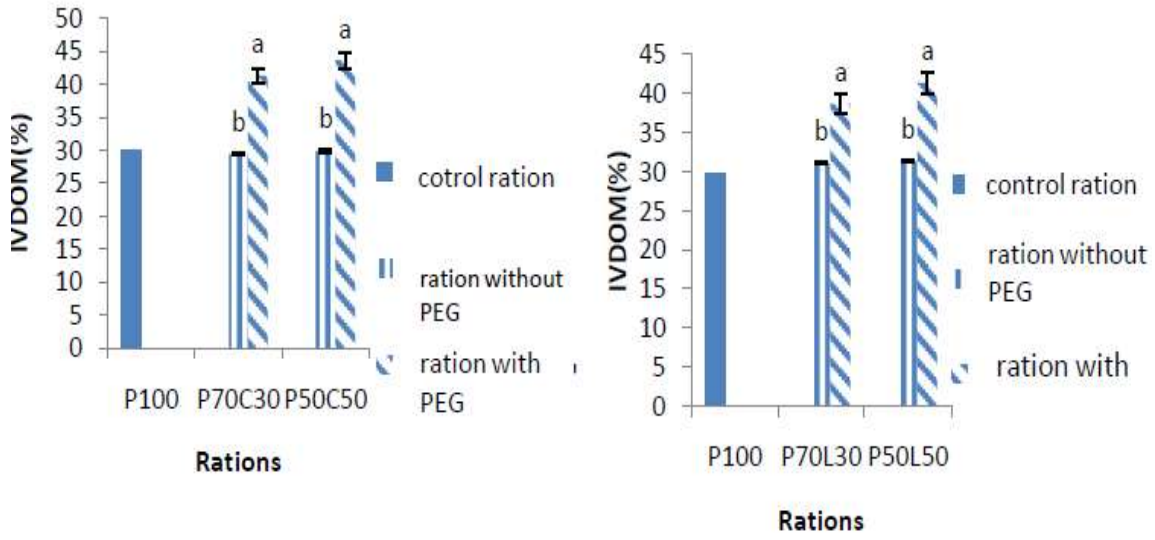

Figure6. *In vitro digestibility of organic matter (IVDOM) obtained after incubation of the various rations with Calliandra calothyrsus or Leucaena leucocephala associated with PEG or not*

a,b: mean on the same band with the same ration are not significantly different (p>0,05). P100 = 100% of P.maximum, P70L30 = 70% of P. maximum and 30% of L. leucocephala, P50L50 = 50% of P.maximum and 50% of L. leucocephala, P70L30+PEG = 70% of P.maximum and 30% of L. leucocephala + PEG, P50L50+PEG = 50% of P. maximum and 50% of L. leucocephala + PEG.

Metabolizable Energy Compared after Incubation of the Various Rations with *Calliandra calothyrsus* or *Leucaena leucocephala* Associated or Not with PEG

Whatever the ration, metabolizable energy of the various rations was comparable (p>0.05) between the two leguminous plants. The ration P50C50+PEG was significantly higher (P<0.05) than the ration containing *Leucaena leucocephala* (Figure 7).

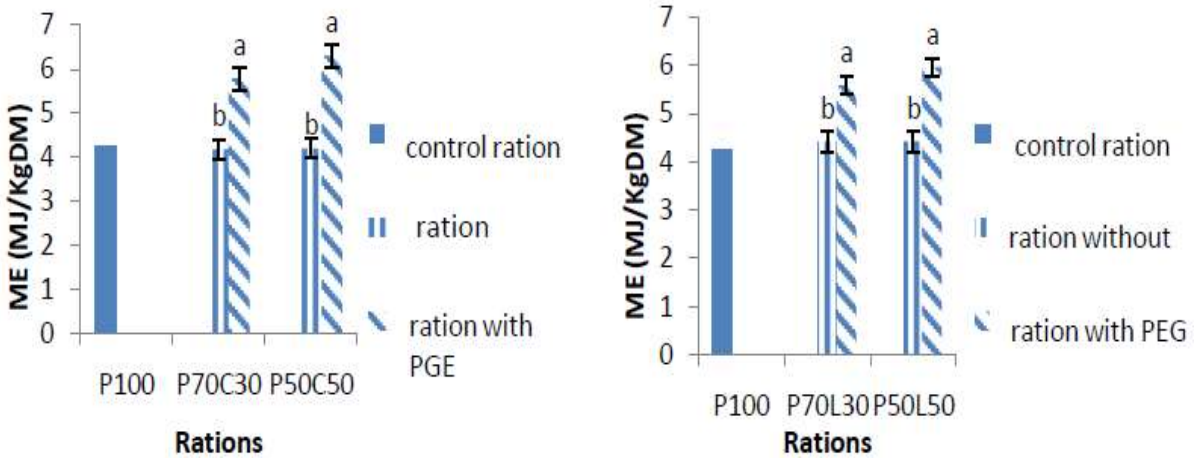

Figure7. *Metabolizable energy obtained after incubation of the various rations with Calliandra calothyrsus or Leucaena leucocephala associated or not with the PEG*

a,b: mean on the same band with the same ration are not significantly different (p>0,05). P100 = 100% of P.maximum, P70L30 = 70% of P. maximum and 30% of L. leucocephala, P50L50 = 50% of P.maximum and 50% of L. leucocephala, P70L30+PEG = 70% of P.maximum and 30% of L. leucocephala + PEG, P50L50+PEG = 50% of P. maximum and 50% of L. leucocephala + PEG.

Partitioning Factor after Incubation of the Various Rations with *Calliandra calothyrsus* or *Leucaena leucocephala* Associated with PEG or Not

The addition of leguminous plant did not significantly (p>0.05) affected the partitioning factor whatever the ration (Figure 8)

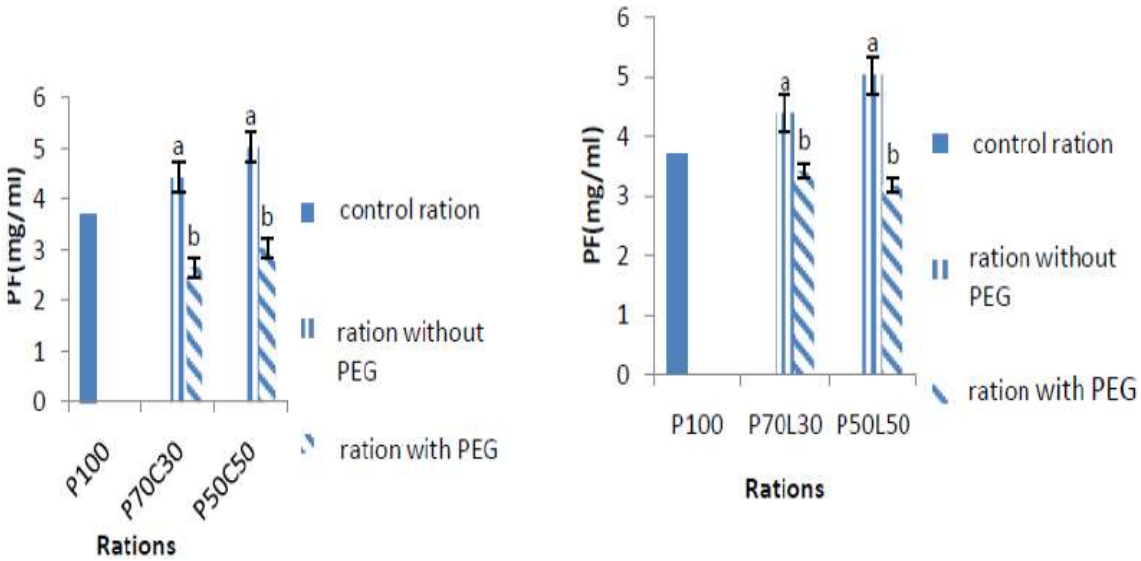

Figure8. *Partitioning factor obtained after incubation of various rations with Calliandra calothyrsus or Leucaena leucocephala associated with the PEG or not*

a,b: mean on the same band with the same ration are not significantly different (p>0,05). P100 = 100% of P.maximum, P70L30 = 70% of P. maximum and 30% of L. leucocephala, P50L50 = 50% of P.Maximum and 50% of L. leucocephala, P70L30+PEG = 70% of P.maximum and 30% of L. leucocephala + PEG, P50L50+PEG = 50% of P.maximum and 50% of L. leucocephala + PEG.

Microbial Mass after Incubation of Various Rations with *Calliandra calothyrsus* or *Leucaena leucocephala*

Whatever the ration, microbial mass of various rations were comparable (p>0.05) between the two leguminous plants, except the ration P70 + PEG associated *Leucaena leucocephala* which was significantly higher (P<0.05) than that containing *Calliandra calothyrsus* (Figure 9).

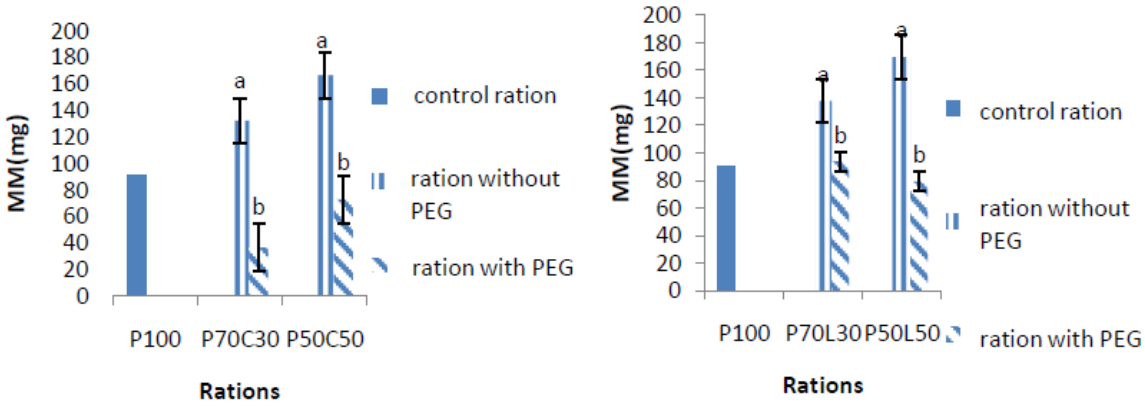

Figure9. *Microbial mass compared after incubation of various rations with Calliandra calothyrsus or Leucaena leucocephala*

a,b: mean on the same band with the same ration are not significantly different (p>0,05). P100 = 100% of P.Maximum, P70L30 = 70% of P. maximum and 30% of L. leucocephala, P50L50 = 50% of P.Maximum and 50% of L. leucocephala, P70L30+PEG = 70% of P.Maximum and 30% of L. leucocephala + PEG, P50L50+PEG = 50% of P.Maximum and 50% of L. leucocephala + PEG.

DISCUSSION

The chemical composition (DM, OM, total nitrogen and crude fiber) of the various rations from *Panicum maximum* hay was influenced by the addition of *Leuceana leucocephala* and *Calliandra calothyrsus.* Contradictory results were observed by Matumuini et *al.* (2013) and Lemoufouet et *al.* (2014). These differences could be due to the method of ground processing, the time of harvest, the treatment methods of coarse fodder and, of the incorporation level of molasses in the various rations used.

The *in vitro* digestibility parameters were improved by the addition of PEG in this study. Similar observations were reported by Jetana et *al.* (2011) and Getachew et *al.* (2000) in animals nourished with the rice straw associated with *Leucaena leucocephala* in the presence of PEG (0.46%). These results confirm the affinity of polyethylen glycol fixing on tannins sites where the proteins should have been fixed to become inalienable with the micro-organisms of the rumen and the metabolic activities. Gas production after 24 hours significantly ((P<0.05) dropped with the incubation of various levels of *Calliandra calothyrsus* or *Leucaena leucocephala* for the rations non-associated with PEG. As observed by Menke et *al.* (1979), fodder having an increased in gas production, have a low microbial mass. The micro-organisms may use the energy brought by the molasses on the one hand and ammonia produced by the accelerated fermentation of food proteins on the other hand (Pathoummalangsy and Preston, 2008) for their growth and their development in order to effectively degrade the ration in the rumen (Getachew et *al.*, 2000; Matumuini et *al.*, 2014).

However, the contribution of *Calliandra calothyrsus* or *Leucaena leucocephala* associated with PEG makes it possible to raise the production of gases of various rations. The partitioning factor and the microbial mass were improved by the addition of *Calliandra calothyrsus* or *Leucaena leucocephala* in the presence of PEG to the ration made up of *Panicum maximum* hay.

Indeed, Blümmel et *al.* (1997) and Getachew et *al.* (2000) reported that associated to PEG, the partitioning factor of animals fed with *Calliandra calothyrsus* is located between 3.1 and 16 mg/ml respectively. The PF of this study in the presence of PEG corresponds to that what was obtained by these authors in absence of PEG. These results could be explained by the fact that the treatment restored the effect of PEG on PF by increasing the microbial mass on the 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 prevent with the PEG in the presence of *Panicum maximum* hay.

CONCLUSION

At the end of this study, these conclusions can be withdrawn. The leguminous plants associated with PEG improve the chemical composition of various rations. The contents of organic matter, total nitrogen matter, total lipids and carbohydrate increased the rations contrary to the contents of cellular walls, ashes and crude fiber. The digestible organic matter (dOM), the digestible nitrogen matter, the fodder unit milk (UFL) and the fodder unit meat (UFV) also increased with the addition of *Calliandra calothyrsus* and *Leucaena leucocephala* in the various rations.

The addition of *Calliandra calothyrsus* made it possible to increased the content of total matter nitrogenized (MAT) of the *Panicum maximum* hay. The addition of *Calliandra calothyrsus* and *Leucaena leucocephala* associated PEG6000 made it possible to significantly increase all the parameters (production of gas, IVDDM, IVDOM) of the *in vitro* digestibility of *Panicum maximum* hay.

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