



## Effect of Plantain (*Musa paradisiaca*) Leaves Ash Extract and the Source of Rumen Fluid on *in vitro* Digestibility of Rice Straw Complemented with *Calliandra calothyrsus* Leaves

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**Abstract** | This study was carried out to determine the effect of plantain leaves ash extract and the source of rumen fluid on *in vitro* digestibility of rice straw complemented with *Calliandra calothyrsus* leaves. At the beginning of the trial, the dry leaves of plantain harvested from vicinity of the University of Dschang Research Farm (UDRF), sundried and were ashed on concrete clean surfaces to obtain ash. Subsequently 50, 100 or 150 g of this ash were each dissolved in 1 liter of distilled water to obtain ash extracts solutions. Two sources of rumen fluid (sheep and goat) were collected separately and incubated with four diets (untreated or treated rice straw (70%) and *Calliandra calothyrsus* leaves (30%) (w/w basis)) each, in triplicate in a two-ways analysis of variance according to the test gas method. The results showed that with the exception of residual nitrogen (N-NDF) and microbial mass (MM) that were similar ( $p > 0.05$ ), all other parameters of the *in vitro* digestibility of rice straw were significantly ( $p < 0.05$ ) influenced by ash extract treatment irrespective of the rumen fluid source considered. The gas produced ( $32.06 \pm 0.6$  and  $32.90 \pm 0.09$  ml), volatile fatty acid ( $0.70 \pm 0.05$  and  $0.72 \pm 0.001$  mmol/ml), metabolisable energy ( $7.09 \pm 0.05$  and  $7.21 \pm 0.01$  MJ/kg/DM) and *in vitro* digestibility of organic matter ( $48.37 \pm 0.33$  and  $55.65 \pm 0.13\%$ ) obtained with rice straw treated with plantain ash extract from 50 and 100g ash mixed with *C. calothyrsus* diets respectively were significantly ( $p < 0.05$ ) higher with goat rumen fluid. In contrast, treatment of rice straw with ash extract ( $86.79 \pm 3.85$  and  $85.27 \pm 1.73$  mg) significantly ( $p < 0.05$ ) decreased microbial mass compared to untreated straw ( $103.94 \pm 1.64$  mg). From the above, therefore it can be concluded that treating rice straw with plantain leaves ash extract improved digestibility in small ruminants.

**Keywords** | Ash extract treatment, *In vitro* digestibility, Rice straw, Rumen fluid source

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## INTRODUCTION

The annual population growth in Central Africa is estimated at 2.9% and greater than the increase in agricultural production which is about 2.2% per year (FAO, 2004). This imbalance between the population growth and that of agriculture is responsible for the deficit in food products, especially animal protein. Indeed, the average

consumption of animal proteins origin, is 11.1g per inhabitant per day in Cameroon, almost one third of the 33 g per inhabitant per day recommended by WHO (FAO, 2012). This protein deficit is at the root of malnutrition, which is becoming common in some parts of the country and is forcing the government to import meat to meet home demand despite its significant production potential. In the livestock sector, small ruminants account for 17% of the

protein requirement in Cameroon. However, the decrease in the pastoral space on which these animals depend for the benefit of agriculture and competing activities poses a feed problem with negative consequences (Tendonkeng et al., 2013) on their production and productivity, especially during the dry season. At the same time, this agriculture generates large quantities of crop residues (corn stovers, rice straw, etc.) which can be used to compensate lack of fodder, especially during the dry season. In fact, the digestive characteristics of ruminants enable them to better valorize crop residues, which constitute a potential source of energy for them (Jami et al., 2014). This use will be more effective if the chemicals treatments processes are used. Indeed, the chemical treatment is effective in breaking down the lignin-polysaccharide membrane bonds contained in the lignified material and releases potentially available carbohydrates to microbial attack (Jami et al., 2014). For this purpose, several chemicals have often been used including sodium hydroxide, calcium hydroxide and ammonia. However, the unavailability, the high costs, the difficulties of handling and the environmental pollution that some of these substances cause lead to the search of alternative products which are less expensive and have no adverse effect on the environment, as the ashes extracts. Ash (powdery residue left after burning) is readily available in rural areas. It contains minerals that give it alkaline properties (Tiisekwa et al., 1999, Van Ryssen and Ndlovu, 2018). These alkaline properties suggest their potential to improve the digestibility of roughage through solubilization of silica and the weakening of the ester bonds between cellulose and lignin (Laswai et al., 2007). Moreover, Ramirez et al. (1992) showed that the dry matter digestibility of 20% ash-treated corn stovers increased by 20% in sheep compared to the control group. Hamed and Elimam (2008) also reported that the *in sacco* degradation of dry matter, organic matter, and crude proteins of sorghum stovers in Nubian goats receiving a basal ration of groundnut haulms increased with increasing levels (3, 5 and 8%) of Rabaa (*Trianthema pentandra*). Similarly, Kanyinji et al. (2014) showed that the treatment of *Chloris gayana* hay with 0, 20, 30 or 40 g of *Musa sapientum* ash per liter of solution improves its nutritive value in goats. Ingestions and optimal digestibilities were recorded when the hay was treated with 40 g of ash per liter of solution. However, the major shortcoming of crops residue treated with ash extract is their deficiencies in crude proteins (Abdulazeez et al., 2016) that is insufficient to meet microbial proteins needs. Thus, Protein complementation is necessary in addition to this treatment to optimize the digestibility of roughage fodder.

*Calliandra calothyrsus* adapts perfectly to the Cameroon pedoclimatic conditions, resists drought and is available all year round (Pamo et al., 2006). Its crude protein content is 28.6% dry matter (DM) (Pamo et al., 2005), and it could

be a good complement of rice straws based diets. Little work have been undertaken on the effect of ash extract level of plantain (*Musa paradisiaca*) leaves on the *in vitro* digestibility of rice straw complemented with leaves of *Calliandra calothyrsus* in small ruminants. The present study was therefore initiated to contribute to the valorization of crop residues in ruminants feeding. Its objective was to evaluate the *in vitro* digestibility of rice straw treated with plantain leaves ash extract complemented with leaves of *C. calothyrsus* incubated with goat or sheep rumen fluid.

## MATERIALS AND METHODS

### STUDY AREA

The experiment was carried out at the University of Dschang Research Farm (UDRF) and in the Animal Production and Nutrition Laboratory (LAPRONA) of the same university in West Cameroon. Animals used in this study were provided standardized housing and feeding conditions and their welfare was considered as per international standard.

### ANIMAL AND PLANT MATERIALS

One adult non-gestating West African Dwarf doe and one Djallonke ewe aged 18 month-old weighing 21 and 23 kg, respectively were used as donors of rumen fluid for this study. During the preliminary phase of the evaluation of the *in vitro* digestibility which lasted two weeks, donor animals received daily 801 g of diets composed of untreated (280 g) or treated rice straw (280 g) and *Calliandra calothyrsus* leaves (280 g) and water *ad libitum*.

A month before the study started, animals were administered a preventive drug of oxytetracycline (20%), repeated after two weeks. They were also dewormed with Ivermectine 1% (synthetic broad spectrum anthelmintic active against gastrointestinal nematods and pulmonary adults and larvae). The rumen fluids of ewe and goat were collected separately just after slaughter.

The plant material consisted of rice straw, variety NERICA (New Rice for Africa) low lands collected in the plots of the Institute of Agricultural Research for Development in Santchou (Western-Cameroon), transported to the UDRF, then chopped into pieces of 2-5 cm lengths about with a machete and sun dried before being stored in a shop. *Calliandra calothyrsus* leaves were harvested before flowering in the UDRF plots and wilted during 2 hours before being served. *In vitro* digestibility period lasted 24 hours.

### PREPARATION OF PLANTAIN LEAVES ASH EXTRACT AND TREATMENT OF RICE STRAW

Plantains leaves (*Musa paradisiaca*) was ashed on a concrete clean surface at the University of Dschang Research

**Table 1:** Mineral composition of feeds ingredients

Feeds ingredients	Minerals contents (g/kg DM)				
	Ca	P	Mg	Na	K
UTRS	0.110	1.470	0.130	0.830	36.740
RSTP50	0.180	1.570	0.150	0.850	38.110
RSTP100	0.230	2.550	0.200	0.920	39.020
RSTP150	0.260	2.570	0.240	0.960	39.350
<i>Calliandra calothyrsus</i>	7.080	1.280	0.710	0.360	5.390

UTRS: Untreated rice straw; RSTP50: Rice straw treated with 50 g of plantains leaves ash extract; RSTP100: Rice straw treated with 100 g of of plantains leaves ash extract; RSTP150: Rice straw treated with 150 g of plantains leaves ash extract. Ca: Calcium; P: Phosphorous; Potassium; Mg: Magnesium; Na: Sodium

**Table 2:** Chemical composition of experimental diets

Chemical composition	Diets			
	UTRS+Cc	RSTPL50+Cc	RSTPL100+Cc	RSTPL150+Cc
DM (%)	97.73	96.21	95.88	95.91
OM (%DM)	88.81	86.51	84.09	81.98
Ash (%DM)	11.18	13.49	15.90	18.01
TN (%DM)	11.11	9.31	9.73	9.39
EE (%DM)	2.54	2.86	2.97	2.99
NDF (%DM)	68.74	66.80	66.36	63.60
ADF (%DM)	46.97	45.12	43.10	42.74
ADL (%DM)	32.24	32.11	31.95	28.93
DMO (%DM)	40.14	40.76	41.53	44.96

EE : Ether extract ; DM: Dry matter; OM:Organic Matter; NDF: Neutral Détergent Fiber; ADF:Acid Détergent Fiber; ADL: Acid Détergent Lignin ; dMO: digestibility of Organic matter; TN:Total Nitrogen; UTRS + Cc :Untreated rice straw + *Calliandra calothyrsus* leaves ; RSTPL50 + Cc : Rice straw treated with 50 g of plantains leaves ash extract + *C. Calothyrsus* leaves ; RSTPL100 + Cc: Rice straw treated with 100 g of of plantains leaves ash extract + *C. Calothyrsus* leaves RSTPL150 + Cc : Rice straw treated with 150 g of of plantains leaves ash extract + *C. Calothyrsus* leaves .

Farm (UDRF) in order to obtain ash. The solutions were prepared by dissolving 0, 50, 100 and 150 g of ash in 1 liter of distilled water and the mixtures were homogenized and allowed to stand for 48 hours. These solutions were filtered using a clean cotton fabric and the resulting filtrates constituted the ash extract solutions. For treatment, 100 g of previously chopped straw was weighed and then dipped into each of the ash extract solutions and kept soaked for 12 hours. At the end of this soaking, the straw was oven-dried at 60 °C up to constant weight, then packaged in envelopes and stored in a well-ventilated place to prevent the growth of mold. The pH of each solutions was measured using a portable EUTECH pH meter (01X244912 Fisher Scientific, France) before and after soaking.

**DIETS**

Both untreated and treated rice straw samples were mixed with *Calliandra calothyrsus* at 70: 30 % (w/w basis) to make up four diets, namely:

D0: Untreated rice straw + *Calliandra calothyrsus* leaves (UTRS + Cc)

D1: Rice straw treated with 50 g of plantains leaves ash

extract + *C. Calothyrsus* leaves (RSTPL50 + Cc)

D2: Rice straw treated with 100 g of plantains leaves ash extract + *C. Calothyrsus* leaves (RSTPL100 + Cc)

D3: Rice straw treated with 150 g of plantains leaves ash extract + *C. Calothyrsus* leaves (RSTPL150 + Cc)

After the preparation of these diets, 100 g were taken and stored in plastic bags for determination of chemical composition and *in vitro* digestibility assessment.

**SAMPLES ANALYSIS**

Mineral composition of untreated or treated rice straw and *Calliandra calothyrsus* leaves (Table 1) were carried out in the laboratory of Soil Chemistry and Environment of the University of Dschang for the determination mineral contents, following the methods described by Pauwels et al. (1992). Analysis of the chemical composition of experimental diets (Table 2) was done at LAPRONA to determine dry matter, ash, organic matter, crude fiber, cell wall, lipid and total nitrogen content by the methods described by AOAC (2000).



EVALUATION OF THE *IN VITRO* DIGESTIBILITY PREPARATION OF INOCULUM AND METHOD USED

The evaluation of the quantity of gas produced and preparation of reagent were done according to the method and procedure described by Menke et al. (1979) modified by Makkar (2002). The day before the test, the samples (weighed in triplicate and introduced into syringes) and two (one for goat rumen fluid and one for sheep rumen fluid) freshly prepared reagent (Menke et al., 1979) were placed in an incubator at 39 °C overnight. 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 of goat and sheep, the reagent in which continually arrived a stream of gas (CO<sub>2</sub>) with a moderately pressure (4 Bars) was placed in a water bath at 39 °C. Then, the Sodium sulfide (417 mg) and 6N NaOH(0.44 ml) were added to this reagent. The rumen fluid collected respectively from goat and sheep just after slaughter was immediately filtered under a stream of CO<sub>2</sub> and 700 ml of each rumen fluid were collected and introduced into each reagent still under CO<sub>2</sub> stream. This mixture (inoculum) was homogenized for 10 minutes using a magnetic rod and 40 ml of each ration with goat inoculum was put into the syringe using a precision dispenser and then placed in the water bath for incubation.

INCUBATION

After 24 hours of incubation, the gas products and corrected by the gas produced by the inoculum in control tubes were used to determine the organic matter digestible (OMD) using the regression equation proposed by Menke and Steingrass, (1988):

OMD (%) = 14.88 + 0.889GP + 0.45CP + 0.0651Ash, where: GP: Amount of gas produced after 24 hours of incubation, CP: Crude protein of the initial sample, Mean while, the content of metabolizable energy (ME), Partitioning Factor (PF) which is the amount of organic matter degraded to produce 1 ml of gas, the microbial mass (MM), the volatile fatty acid (VFA) were calculated using the equations proposed by Makkar (2002):

$$ME (MJ/kgDM) = 2.20 + 0.136GP + 0.057CP,$$

$$PF (mg/ml) = OMD/GP;$$

$$VFA (mmol/ml) = 0.0239GP - 0.0601;$$

$$MM (mg) = OMD - (GP \times SF);$$

OMD (mg) = Amount of organic matter degraded.

GP (ml) = Amount of gas produced after 24 hours incubation,

SF = Stochiometric factor (2.20 for fodder)

After 24 hours of incubation, the contents of each syringe was transferred into a 600 ml beaker and syringe washed twice with two portions of 15 ml Neutral Detergent Solution (NDS) (Van Soest et al., 1991) and emptied into the beaker. The samples were boiled gently for one hour and

filtered into pretared filter crucibles. These crucibles were dried at 103 °C overnight and weight of the substrate and the weight of the incubated residue after NDS treatment, at the end of the incubation. The residues obtained after treatment with NDS were used to determine the residual nitrogen (N-NDF) by Kjeldahl method.

STATISTICAL ANALYSIS

*In vitro* digestibility data was subjected to two-ways analysis of variance following General Linear Models using SPSS 20.0 software. When differences existed between treatments, the means were separated by the Duncan's test at 0.05 significance level (Steel and Torrie, 1980).

RESULTS

GAS PRODUCTION

When rice straw was treated with 50 or 100 g ash extract and incubated with sheep or goat rumen fluid, the quantity of gas produced from their incubation was similar to the untreated straw from the third to the 9<sup>th</sup> hour (Figure 1). However after the 9<sup>th</sup> hour until the end of the incubation (24 hours), gas produced from the treated straw was higher than that of untreated straw (UTRS+Cc) (Figure 2).

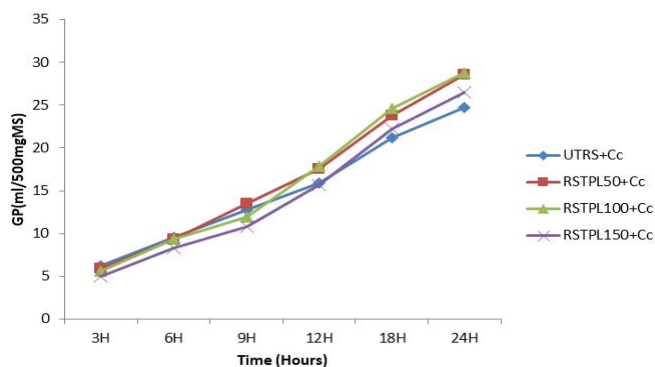


Figure 1: Evolution of the gas produced of different diets incubated with the sheep rumen fluid

UTRS + Cc : Untreated rice straw + *Calliandra calothyrsus* leaves ; RSTPL50 + Cc : Rice straw treated with 50 g of plantains leaves ash extract + *C. Calothyrsus* leaves ; RSTPL100 + Cc: Rice straw treated with 100 g of plantains leaves ash extract + *C. Calothyrsus* leaves RSTPL150 + Cc : Rice straw treated with 150 g of plantains leaves ash extract + *C. Calothyrsus* leaves

IN VITRO DIGESTIBILITY

The amount of gas produced after 24 hours of incubation (R<sup>2</sup>=0.65), the VFA production (R<sup>2</sup>=0.62), ME (R<sup>2</sup>=0.63), *in vitro* digestibility of dry matter (IVDDM) (R<sup>2</sup>=0.631) and *in vitro* digestibility of organic matter (IVDOM) (R<sup>2</sup>=0.67) obtained with sheep rumen fluid were significantly (p<0.05) increased with ash extract treatment level (Table 3). In contrast, the MM (R<sup>2</sup>=0.83) values was

**Table 3:** *In vitro* digestibility parameters of various diets incubated with sheep or goat rumen fluid.

Diets	Rumen fluid source	Parameters							
		GP (ml)	MM (mg)	ME (MJ/kg/DM)	VFA (mmol/ml)	PF (mg/ml)	IVDDM (%)	IVDOM (%)	N-NDF (mg)
UTRS+Cc	Sheep	24.71± 0.82 <sup>cB</sup>	113.13± 2.09 <sup>aA</sup>	6.19± 1.11 <sup>cB</sup>	0.53± 0.01 <sup>cB</sup>	3.79± 0.44 <sup>aA</sup>	43.802± 1.96 <sup>cA</sup>	42.57± 0.73 <sup>bB</sup>	0.78± 0.09 <sup>aB</sup>
	Goat	28.58± 0.50 <sup>bA</sup>	103.94± 1.64 <sup>bB</sup>	6.72± 0.06 <sup>bA</sup>	0.62± 0.01 <sup>bA</sup>	3.50± 0.29 <sup>aA</sup>	41.50± 4.45 <sup>cA</sup>	46.02± 0.44 <sup>bA</sup>	0.87± 0.22 <sup>aA</sup>
RSTPL50+Cc	Sheep	28.60± 0.35 <sup>bB</sup>	94.39± 2.25 <sup>bA</sup>	6.62± 0.04 <sup>aB</sup>	0.62± 0.001 <sup>aB</sup>	3.69± 0.24 <sup>aA</sup>	50.277± 0.99 <sup>bA</sup>	45.30± 0.31 <sup>aB</sup>	0.58± 0.09 <sup>aB</sup>
	Goat	32.06± 0.37 <sup>aA</sup>	86.79± 3.85 <sup>bB</sup>	7.09± 0.05 <sup>aA</sup>	0.70± 0.001 <sup>aA</sup>	3.29± 0.28 <sup>aA</sup>	45.52± 1.04 <sup>bA</sup>	48.37± 0.33 <sup>aA</sup>	1.34± 0.39 <sup>aA</sup>
RSTPL100+Cc	Sheep	28.73± 0.41 <sup>bB</sup>	94.45± 1.11 <sup>bA</sup>	6.64± 0.05 <sup>aB</sup>	0.62± 0.01 <sup>aB</sup>	3.35± 0.21 <sup>aA</sup>	52.62± 0.98 <sup>abA</sup>	45.43± 0.37 <sup>aB</sup>	0.63± 0.02 <sup>aB</sup>
	Goat	32.90± 0.09 <sup>aA</sup>	85.27± 1.73 <sup>bB</sup>	7.21± 0.01 <sup>aA</sup>	0.72± 0.001 <sup>aA</sup>	3.89± 0.20 <sup>aA</sup>	55.65± 0.13 <sup>bA</sup>	49.14± 0.08 <sup>aA</sup>	0.88± 0.23 <sup>aA</sup>
RSTPL150+Cc	Sheep	26.48± 0.89 <sup>bB</sup>	96.89± 3.22 <sup>bA</sup>	6.34± 0.12 <sup>bB</sup>	0.57± 0.02 <sup>bB</sup>	3.29± 0.98 <sup>aA</sup>	56.754± 5.00 <sup>aA</sup>	43.49± 0.79 <sup>bB</sup>	0.81± 0.30 <sup>aB</sup>
	Goat	32.12± 0.65 <sup>aA</sup>	84.49± 3.79 <sup>bB</sup>	7.10± 0.08 <sup>aA</sup>	0.70± 0.01 <sup>aA</sup>	4.33± 0.22 <sup>aA</sup>	59.93± 2.51 <sup>aA</sup>	48.50± 0.59 <sup>aA</sup>	1.15± 0.39 <sup>aA</sup>
Significance level (0.05)	Ash extract (AE)	0.000	0.000	0.000	0.000	0.636	0.000	0.000	0.380
	Rumen fluid source RFS)	0.000	0.000	0.000	0.000	0.216	0.707	0.000	0.003
	Interaction (AE ×RFS)	0.024	0.479	0.024	0.024	0.027	0.000	0.024	0.183

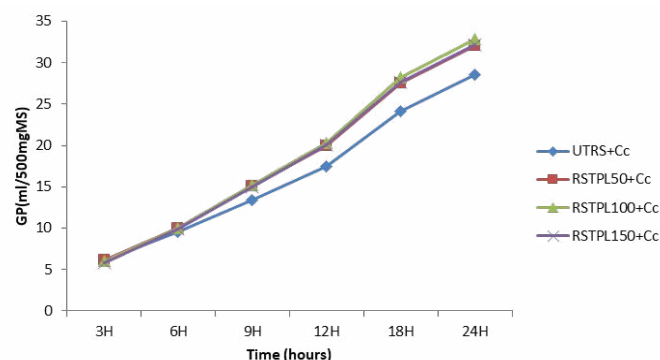
a, b, c: means bear the same superscripts in the same column are not significantly different (P>0.05); A, B : means bear the same superscripts in the same column are not significantly different (P > 0.05) UTRS + Cc: Untreated rice straw + *Calliandra calothyrsus* leaves; RSTPL50 + Cc: Rice straw treated with 50 g of plantains leaves ash extract + *Calliandra calothyrsus* leaves; RSTPL100 + Cc: Rice straw treated with 100 g of of plantains leaves ash extract + *Calliandra calothyrsus* leaves; RSTPL150 + Cc : Rice straw treated with 150 g of of plantains leaves ash extract + *Calliandra calothyrsus* leaves. GP: Gas Production; MM: Microbial mass; ME: Metabolizable Energy; VFA: Volatile Fatty Acid; PF: Partitioning Factor; IVDDM: In vitro digestibility of dry matter; IVDOM: In vitro digestibility of Organic Matter; N-NDF: Residual nitrogen.

significantly (p<0.05) decreased with ash extract treatment level compared to untreated rice straw. The amount of gas produced after 24 hours of incubation, VFA production, ME and IVDOM of the RSTPL50 + Cc and RSTPL100 + Cc diets incubated with sheep rumen fluid were comparable (p>0.05) and significantly (p<0.05) higher than that of the RSTPL150 + Cc diet. Ash extract treatment levels had no significant effect (p>0.05) on residual nitrogen (N-NDF) and amount of organic matter fermented to produce 1 ml of gas (PF).

Ash extract treatment significantly (p <0.05) increased the amount of gas produced after 24 h of incubation (R<sup>2</sup>=0.58), VFA production (R<sup>2</sup>=0.57), IVDDM (R<sup>2</sup>=0.97), IVDOM (R<sup>2</sup>=0.60) and ME obtained with goat rumen fluid (R<sup>2</sup>=0.57) (Table 3). In contrast, MM (R<sup>2</sup>=0.69) significantly (p<0.05) decreased with treatment of rice straw with ash extract compared to untreated straw. Gas production, VFA, IVDOM and ME obtained with ash extract treated

rice straw incubated with goat rumen fluid was similar (p> 0.05) (Table 3). With the exception of the RSTPL50+Cc diet, the partitioning factor (PF) also increased with increasing level of ash extract treatment (R<sup>2</sup>=0.76), the highest value was obtained with the RSTPL150 + Cc incubated with goat rumen fluid.

Gas produced after 24 hours of incubation, *in vitro* digestibility of organic matter (IVDOM), volatile fatty acid (VFA), metabolizable energy (ME) and residual nitrogen (N-NDF) were significantly (p<0.05) higher with goat rumen fluid whatever the level of ash extract treatment considered (Table 3). Conversely, the microbial mass (MM) obtained with the sheep rumen fluid was significantly (p<0.05) greater than that obtained with goat rumen fluid whatever the diet considered. Whatever the rumen fluid considered, the *in vitro* digestibility of dry matter (IVDDM) and Partitioning factor (PF) has been improved by ash extract treatment. In fact, the IVDDM



**Figure 2:** Evolution of the gas produced of the different diets incubated with goat rumen fluid

UTRS + Cc : Untreated rice straw + *Calliandra calothyrsus* leaves ; RSTPL50 + Cc : Rice straw treated with 50 g of plantains leaves ash extract + *C. Calothyrsus* leaves ; RSTPL100 + Cc: Rice straw treated with 100 g of plantains leaves ash extract + *C. Calothyrsus* leaves RSTPL150 + Cc : Rice straw treated with 150 g of plantains leaves ash extract + *C. Calothyrsus* leaves.

and PF of the treated rice straw incubated with sheep or goat rumen fluid was similar ( $p > 0.05$ ). The residual nitrogen (N-NDF) obtained with the goat rumen fluid was significantly ( $p < 0.05$ ) higher than that recorded with the sheep rumen fluid irrespective of the diet considered. PF and *in vitro* digestibility of dry matter (IVDDM) obtained with RSTPL100 + Cc and RSTPL150 + Cc diets incubated with goat rumen fluid were similar ( $p > 0.05$ ) and higher than those obtained with sheep rumen fluid for the RSTPL50 + Cc diet. Treatment of rice straw with ash extract resulted in significantly ( $p < 0.05$ ) higher VFA production with goat rumen fluid. There was significant ash extract treatment  $\times$  rumen fluid source interaction on GP, ME, VFA, IVDDM, IVDOM and PF ( $p < 0.05$ ). Treatment of rice straw with ash extract significantly ( $p < 0.05$ ) influenced all *in vitro* digestibility parameters irrespective of the rumen fluid source consider with the exception of microbial mass and residual nitrogen which were similar ( $p > 0.05$ ).

## DISCUSSION

The gas produced after 24 hours of incubation was significantly ( $p < 0.05$ ) improved by the treatment of rice straw with 50 or 100 g of ash extract. The values obtained were close to those reported by Pi et al. (2005) with untreated and granulated rice straw (28.7 ml), pretreated (1% NaOH + 4% Ca(OH)<sub>2</sub>) and non-granulated straw (29.8 ml) combined with a concentrate in Boer goats. However, they were lower than those reported by the same authors following the incubation of pretreated and granulated rice straw associated with a concentrate (33.7 ml). The increase in gas production would be related to the decrease in MM, as noted by (Menke et al., 1979), who observed that forages with

high gas production have low microbial mass. Treatment of rice straw with ash extract significantly ( $p < 0.05$ ) improved its ME content, IVDDM, IVDOM and the production of VFA. These results are similar to Guérin's (1999) report increase in ME leads to an increase in the digestibility of organic matter. This could be explained by the improvement of the digestibility of the cell walls fraction of straw by the alkaline treatment resulting in the release of the energy contained in soluble carbohydrates to microorganisms (Pi et al., 2005), the VFA production and microbial cells. Indeed, more the organic matter is degraded, more energy is produced. Treatment of rice straw with ash extract significantly ( $p < 0.05$ ) decreased MM. This decrease could be explained by the contribution of the alkaline treatment modifying the rumen pH resulting in the reduction of the fermentative activity of certain microorganisms. This observation is contrary to that of Pi et al. (2005) who showed that the alkaline treatment of rice straw causes an increase in microbial mass. Treatment of rice straw with different levels of ash extract had no significant effect ( $p > 0.05$ ) on PF values (3.69, 3.35 and 3.29 mg / ml). However, these values were still close to those of conventional feeds (2.74 and 4.65 mg / ml) reported by Blümmel and Bullerdieck (1997). The decline in PF may be related to the significant decrease ( $p < 0.05$ ) in MM (Tendonkeng et al., 2016). The IVDDM of rice straw treated with 50 or 100 g ash extract was significantly ( $p < 0.05$ ) improved compared to untreated straw. The results obtained were higher than that obtained by Nolte et al. (1987) (44%) and Tendonkeng et al. (2018) (41.07%) when wheat straw or rice straw was treated respectively with 30 and 10 % ash extract mixed with *Calliandra calothyrsus* leaves, incubated with goat rumen fluid and less than *in vivo* digestibility of DM (63.5%) obtained by Abdulazeez et al. (2016) when maize cobs were treated with 50 % urea + 50 % ash extract in sheep feeding .The high value of *in vitro* of DM digestibility of straw treated with ash extract can be attributed to the use of minerals provided by this treatment, which are essential for rumen microorganisms (Navarro and Rodriguez, 1990, Van Ryssen and Ndlovu, 2018). In fact, rumen bacteria use minerals for maintenance and growth of their cellulolytic activity (Jouany et al., 1995). The source of rumen fluid significantly ( $p < 0.05$ ) influenced the *in vitro* digestibility of rice straw treated with ash extract associated with leaves of *Calliandra calothyrsus*. The quantity of gas produced, the IVDOM and the metabolizable energy of the diets were significantly ( $p < 0.05$ ) higher with the goat rumen fluid whatever the level of treatment with the ash extract. The same observation was made by Tendonkeng et al. (2016) who showed in a comparative study of two inoculum (goat and sheep) for the study of *in vitro* digestibility of rice straw treated with 5% urea, that gas produced after 24 hours of incubation was significantly ( $p < 0.05$ ) higher with goat rumen fluid. This result corroborate with that of Leng (1992) and Silanikove (2000) who observed that the goat's



rumen fluid has a great ability to tolerate the negative effects associated with increasing concentrations of secondary compounds such as silica and lignin. Microbial mass obtained with sheep rumen fluid was significantly ( $p < 0.05$ ) greater than that obtained with goat rumen fluid whatever the diet considered. This observation confirms that reported by Tendonkeng et al. (2016); this increase explains that the microorganisms of the sheep rumen liquid would have better valorized the nutrients (carbohydrate and protein) resulting from the degradation to ensure their growth. Whatever the rumen fluid considered, the IVDDM has been improved by ash extract treatment. This result is similar to the findings of Ramirez et al. (1992); Kanyinji et al. (2014) who reported that the high digestibility value of DM, obtained with ash extract treated fodder, can be attributed to its high supply in minerals and their use by rumen microorganisms.

## CONCLUSION

This study shows that the *in vitro* digestibility parameters of the different diets varied regardless of the source of the ruminal fluid. In fact, the production of gases, VFA, IVDDM, IVDOM and ME obtained with the rice straw treated with the extract of 50 or 100 g of ash incubated with the goat or sheep rumen fluid were similar but significantly higher than those recorded with the straw treated with the extract of 150 g of ash. Ash extract treatment levels had no significant effect on residual nitrogen (N-NDF) and amount of organic matter fermented to produce 1 ml of gas (PF) with sheep rumen fluid. Gas production, VFA, ME, and IVDOM were comparatively higher with goat rumen fluid than sheep. In general, the best results have been obtained with goat rumen fluid. Conversely, the microbial mass obtained with the sheep rumen fluid was greater than that obtained with goat rumen fluid whatever the diet considered.

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## CONFLICT OF INTEREST

There is not conflict of interest

## AUTHORS CONTRIBUTION

Chounna, Tendonkeng and Defang participated in the design and planning of the study; Chounna and Mbodou

collected the data and wrote the first draft of the manuscript; Lemoufouet and Miegoue participated in the planning of the study; Chounna and Mekuiko performed the statistical analyses; Tendonkeng, Defang, Lemoufouet, Miegoue and Pamo revised the manuscript.

## REFERENCES

- Abdulazeez A, Tsopito CM, Madibela OR, Kamau JM (2016). Effect of urea/wood ash-treated maize cobs as substitute for maize grain in sheep diet on intake, digestibility, nitrogen utilization, rumen  $\text{NH}_3$ -N and pH. *J. Anim. Sci. Adv.* 6(2): 1580-1585. <https://doi.org/10.5455/jasa.20160222115144>
- AOAC (2000). Association of Official Analytical Chemist. Official method of analysis, 15<sup>th</sup> edition. AOAC. Washington D.C.
- Asma Hamed HM, Mohamed Elimam E (2008). Degradation of sorghum stover treated with Rabaa ash alkali in the rumen of Nubian goats. *G. J. Agric. Sci.* 6(2): 138-147.
- Blümmel M, Bullerdieck (1997). The need to compliment *in vitro* gas production measurements with residue determinations from *in Sacco* degradability to improve the prediction of voluntary intake of hays. *Anim. Sci. Feed. Technol.* 64: 71-75. <https://doi.org/10.1017/S1357729800015563>
- Chen XL, Wang JK, Wu YM, Liu JX (2008). Effects of chemical treatments of rice straw on rumen fermentation characteristics, fibrolytic enzyme activities and populations of liquid- and solid-associated ruminal microbes *in vitro*. *Anim. Feed. Sci. Technol.* 141: 1-14. <https://doi.org/10.1016/j.anifeedsci.2007.04.006>
- FAO (2004). Données de la FAOSTAT. Rapport N°1 sur l'Afrique [www.fao.org/docrep/006/J1954F/J1954f04.htm](http://www.fao.org/docrep/006/J1954F/J1954f04.htm).
- FAO, FIDA, WFP (2012). State of food insecurity in the world 2012. Economic growth is necessary but not enough to accelerate hunger reduction and undernourishment reduction. Rome. 73 P.
- Getachew G, Makkar HPS, Becker K (2000). Tannins in tropical browses: Effects on *in vitro* microbial fermentation and microbial protein synthesis in media containing different amounts of nitrogen. *J. Agric. Food. Chem.* 48: 3581-3588. <https://doi.org/10.1021/jf990740v>
- Guérin H (1999). Feeding value of cultivated fodders. In : Roberge G. et Toutain B. (eds). Cultures fourragères tropicales. CIRAD Pp. 93-145.
- Jami E, Shterzer N, Yosef E, Nikbachat M, Miron J, Mizrahi I (2014). Effects of including NaOH-treated corn straw as a substitute for wheat hay in the ration of lactating cows on performance, digestibility and rumen microbial profile. *J. Dairy Sci.* 97: 1623-1633. <https://doi.org/10.3168/jds.2013-7192>
- Jouany JP, Broudiscou L, Prins RA, Komisarczuk-Bony S (1995). Metabolism and nutrition of the rumen microbial population. In: Jarrige R., Ruckebush Y., Demarquilly C., Farce M.H., Journet M. (eds). Nutrition des ruminants domestiques. INRA. Pp 349-381.
- Kanyinji F, Chongo J, Simbaya J (2014). Intake and digestibility of Rhodes Grass (*Chloris Gayana*) hay treated with graded levels of concentration of banana leaf ash solution in local goats. *J. Anim. Prod. Adv.* 4(6): 448-454.
- Laswai G, Mtamakaya J, Kimambo A, Aboud A and Mtakwa P (2007). Dry matter intake, *in vivo* nutrient digestibility and concentration of minerals in the blood and urine of

- steers fed rice straw treated with wood ash extract. *Anim. Feed Sci. Technol.* (137): 25–34. <https://doi.org/10.1016/j.anifeedsci.2006.09.015>
- Leng (1992) L'application de la biotechnologie à l'alimentation animale dans les pays en développement (FAO). No 90. 97p.
  - Makkar HPS (2002). Application of the *in vitro* method in the evaluation of feed resources, and enhancement of nutritional value of tannin-rich tree/browse leaves and agro-industrial by-products. In: *Development and field evaluation of Animal Feed supplementation packages*. Proceeding of the final review meeting of an IAEA Technical Co-operation Regional AFRA Project organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and held in Cairo, Egypt, 25-29 November 2000. Pp 23-40.
  - Matumuini NEF, Tendonkeng F, Mboko AV, Zougou TG, Boukila B, Pamo TE (2013). Intake and *in vivo* digestibility of molasses-treated *Tithonia diversifolia* leaves associated with maize stover in Djallonké sheep (*Ovis aries*). *Livest. Res. Rural Dev. Volume 25, (8) Article #142*. Retrieved august 30, 2018, from <http://www.lrrd.org/lrrd25/8/matu2542>
  - Mc Sweeney C S, Palmer B, Bunch R and Krause D O (1999). *In vitro* quality assessment of tannin-containing tropical shrub legumes: protein and fibre digestion. *Anim. Feed Sci. Technol.* 82: 227-241. [https://doi.org/10.1016/S0377-8401\(99\)00103-0](https://doi.org/10.1016/S0377-8401(99)00103-0)
  - Menke KH, Raab L, Salewski A, Steingass H, Fritz D, Schneider W (1979). The estimation of digestibility and metabolizable energy content of ruminant feedstuffs from gas production when they are incubated with rumen liquor. *J. Agric. Sci.* 93: 217-222. <https://doi.org/10.1017/S0021859600086305>
  - Nader GA, Cun GS, Robinson PH (2012). Impacts of silica levels, and location in the detergent fiber matrix, on *in vitro* gas production of rice straw. *Anim. Feed Sci. Technol.* 174: 140–147. <https://doi.org/10.1016/j.anifeedsci.2012.03.009>
  - Navarro F, Rodríguez EF (1990). Study of several bromatological aspects of Mirasol (*Tithonia diversifolia* Hemsl y Gray) as a potential alternative for animal feeding. Tesis Universidad del Tolima. Ibagué, Tolima
  - Nolte ME, Cline JH, Dehority BA, Loerch SC, Parker CF (1987). Treatment of wheat straw with alkaline solutions prepared from wood ashes to improve fiber utilization by ruminants. *J. Anim. Sci.* 64:669-677. <https://doi.org/10.2527/jas1987.643669x>
  - Pamo E Tendonkeng, Kana JR, Tendonkeng F, Betfiang ME (2004). Effect of Polyethylene glycol (PEG, MW6000) on *in vitro* digestibility of *Calliandra calothyrsus* alone or mixed with *Brachiaria ruziziensis*, *Trypsacum laxum* or *Pennisetum purpureum* in dry and rainy seasons in West Cameroon. *Livest. Res. Rural Dev.* Vol. 16, Art. # 49. Retrieved June 5, 2004, from <http://www.cipav.org.co/lrrd/lrrd16/7/tedo16049.htm>
  - Pamo E Tendonkeng, Boukila B, Fonteh F, Tendonkeng F, Kana JR (2005). Chemical composition and effect of supplementation with the leaves of *Calliandra calothyrsus* and *Leucaena leucocephala* on the milk production and growth of kids of west African dwarf goats. *Livest. Res. Rural Dev.* 17(3):[http://www.lrrd.org/lrrd17/3\\_tedo17030.htm](http://www.lrrd.org/lrrd17/3_tedo17030.htm)
  - Pamo E Tendonkeng, Kana JR, Tendonkeng F, Betfiang ME (2006). Effect of Polyethylene glycol (PEG, MW6000) on *in vitro* digestibility of *Calliandra calothyrsus* alone or mixed with *Brachiaria ruziziensis*, *Trypsacum laxum* or *Pennisetum purpureum* in dry and rainy seasons in West Cameroon. *Livest. Res. Rural Dev.* 16, Art. # 49. Retrieved June 5, 2004, from <http://www.cipav.org.co/lrrd/lrrd16/7/tedo16049.htm>
  - Pauwels JM, Van Ranst E, Verloo M, Mvondo ZA (1992). *Pedology laboratory manual guide*, Agricultures publication, 28, AGCD, Brussels.
  - Pi ZK, Wu YM, Liu JX (2005). Effect of pretreatment and pelletization on nutritive value of rice straw-based ration total mixed ration, and growth performance and meat quality meat quality of growing Boer goat fed TMR. *Small Rum. Res.* 56: 81-88. <https://doi.org/10.1016/j.smallrumres.2004.02.010>
  - Rahman M, Akbar A, Islam S, Khaleduzzaman M and Bostami R (2009). Nutrient digestibility and growth rate of bull calves fed rice straw treated with wood ash extract. *Bang. J. Anim. Sci.* 38(1&2): 42 – 52. <https://doi.org/10.3329/bjas.v38i1-2.9911>
  - Ramirez RG, Cruz C, Gonzalez CC (1992). Effect of treating corn stover with wood ashes and hydroxide on nutrient digestibility by sheep and goats. *Small Rum. Res.* 7: 225–232. [https://doi.org/10.1016/0921-4488\(92\)90227-U](https://doi.org/10.1016/0921-4488(92)90227-U)
  - Van Ryssen JBJ, Ndlovu H (2018). Wood ash in livestock nutrition: 1. Factors affecting the mineral composition of wood ash. *Appl. Anim. Husb. Rural Develop.* 11. 53-61.
  - Silanikove N (2000). The physiological basis of adaptation in goats to harsh environments. *Small Rum. Res.* 35: 181-193.
  - Steele RG, Torrie JH (1980). *Principles and procedures of statistics*. Mc Graws hill book C, New York, 633 pp.
  - Tendonkeng F, Pamo TE, Boukila B, Defang FH, Nijki EW, Miégoúé E, Fogang ZB, Lemoufouet J, Djiomika TJ (2013). Socio-economic and technical characteristic of small ruminant rearing in south region of Cameroon: Case of Mvilla Division. *Livest. Res. Rural Dev.* Volume 25, article # 64. Retrieved April 5, 2013 from <http://www.lrrd.org/lrrd25/4/fern25064.htm>
  - Tendonkeng F, Matumuini Ndzani Essie F, Mboko AV, Lemoufouet J, Miégoúé E, Fogang Zogang B, Mekuiko Watsop H, Chounna A, Boukila B, Pamo TE (2016). Effect of the level of incorporation of the molasses and the source of ruminal fluid on *in vitro* digestibility of rice straw treated with urea. *Livest. Res. Rural Dev.* 2 (28). Retrieved, 03 October 2019 from <http://www.lrrd.org/lrrd28/2/tend28019.htm>
  - Tendonkeng F, Lemoufouet J, Miégoúé E, Fogang Zogang B, Matumuini Ndzani Essie F, Chounna A, Ndonkou FD, Pamo TE (2018). Effect of *Calliandra calothyrsus* associated with PEG 6000 on *in vitro* digestibility of rice straw treated with ash extract in goat. *Int. J. Biol. Chem. Sci.* 12(2):975-989. <https://doi.org/10.4314/ijbcs.v12i2.27>
  - Tiisekwa B, Huygherbaert A, De Mey L (1999). The use of wood ash and “Magadi soda” (sodium sesquicarbonate) as material for sorghum tannin detoxification. *Proc. Ann. Res. Conf. FoA. Tanzania* 4, 298–305.
  - Van Soest PJ, Robertson JB, Lewis BA (1991). Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583-3597.
  - Yaning Zhang AE, Ghaly, Bingxi Li (2012). Physical properties of rice residues as affected by variety and climatic and cultivation conditions in three continents. *Am. J. Appl. Sci.* 9(11): 1757-1768. DOI ISSN15469239 Science Publication. <https://doi.org/10.3844/ajassp.2012.1757.1768>