In vitro gas production and digestibility of Echinochloa Pyramidalis (Chase) Hitchc. & Chase grown under constructed wetland treating faecal sludge as runinant feed

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Abstract— Samples of *Echinochloa pyramidalis* grown in constructed wetland treating faecal sludge were subjected to different measurements. The plants were at a vegetative stage at the end of acclimatization period, 2-, 3-, week periods of treatment and 10-week-old regrowth while samples 5-, 6-week periods of treatment were at flowering stage. The nutritive value of the experimental plant samples was evaluated by determination of *in vitro* dry matter digestibility (IVDMD), *in vitro* organic matter digestibility (IVOMD), metabolizable energy (ME), neutral detergent bound nitrogen (NDF-N), partitioning factors (PF), volatile fatty acid (VFA) and microbial biomass (MB) production. The gas production (GP) after 24hrs of incubation was assessed using an *in vitro* incubation technique with rumen fluid. The results showed that GP_{-24h} ranged from 50.6 to 54.9 ml/200 mg DM while the IVDMD varied from 38.8 to 51.9% DM and IVOMD from 67.6 to 71.5%DM. The ME observed was about 9.97 to 10.5 MJ/kg and NDF-N varied from 0.96 to 1.82%DM. PF ranged between 2.1 and 1.3 ml/mg, MB from 102.45 to 132.37 mg and VFA from 1.15 to 1.25 mmol/ml. These digestibility parameters did not change greatly with stage of maturity; *E. pyramidalis* and its fractions at five harvest periods maintained great nutritional values. Compared to most tropical forages used in ruminant feeding, the forage nutritive value of *E. pyramidalis* was equal and even better. Based on these results, *E. pyramidalis* is proved to be suitable for ruminant supplementations and this alternative is needed to be promoted in productive sanitation sector in tropical areas of sub-Saharan-Africa.

Index Terms— By-product; digestibility, *Echinochloa pyramidalis*; feed evaluation, *in vitro* gas production; nutritive value, sludge treatment

1 INTRODUCTION

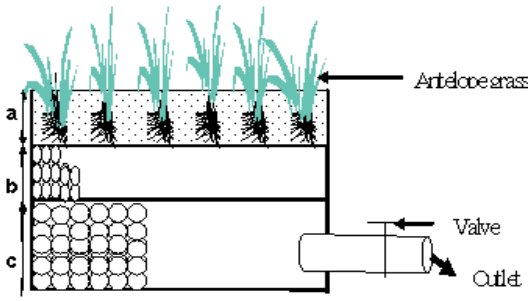
In Sub-Saharan Africa countries, the main feed resources for livestock are natural pastures consisting of grasses, legumes and browse tree species. During the dry season, the availability of the ruminant feeds is more restricted and these feeds shortage always posed a problem for ruminant production [1], [2]. Moreover, the uses of suitable industrial by-products or concentrates are restricted due to their availability and costs; then, livestock rely on natural pasture to meet their nutrient requirement. Due to rapid urbanization and increasing population growth rate, the ever increasing demand of cereal grains for human consumption coupled with the reduction of land uses for pasture purpose is decreasing the feed supply to ruminants. Thus, there is great need to explore alternate feed resources that do not compete with human feed [3], [4]. On the other hand, there is an increasing concern related to waste disposal in African cities. In the African countries, the rapid urbanization and population growth rate have led to large amount of wastewater and excreta production [5], [6]. This can create the opportunity for re-using by-products of wastewater and sanitation treatment such as plants which are presented as alternative ruminant feed resources [7], [8], [9], [6].
Constructed wetlands have been gaining in popularity as a low-cost and low maintenance technology for treating wastewater from urbanized areas [10], [11], [9], [12]. In these wetlands at the same time that pollutant removal from wastewater occurs, great quantities of biomass are produced. According to some authors such as Sartoris et al. [13], Thullen et al. [14], plants must be harvested for the most effective removal of pollutants; large amount of biomass would be available for different uses. Recycling of wastewater and sanitation treatment by-products such as plants and their utilization as alternative ruminant feed resources for livestock production is important for economical and environmental aspects [7], [8], [9], [6]. Therefore, the objective of this study was to assess the nutritional characteristics of *E. pyramidalis* by *in vitro* gas production and digestibility studies.

2 MATERIALS AND METHODS

2.1 Site of Study and Experimental Design

The investigations were conducted at the experimental field of the University of Yaoundé I, Cameroon located at 760-m above sea level (3°45 N and 11°32 E) from July 2010 to April 2011. Yaoundé has a typical equatorial Guinean climate characterized by two rainy seasons (from September to mid-November and from mid-March to June) and two dry seasons (from mid-November to mid-March and from July to August). Annual rainfall was about 1600 mm and daily temperature varies between 23 and 32 °C. Rainfall and temperature values were recorded daily during the experimental period.

The studies were carried out in six lab-scale vertical-flow constructed wetland (VFCW) units vegetated initially with a density of 16 plants/m^2 of antelope grass (*Echinochloa pyramidalis* L). Each unit consisted of plastic tank of 1×1×1m^3 in size with media arrangement adapted from the design of Kottattep et al. [15]. The substrata of these VFCW units were composed of 30-cm large-sized gravel of 25 - 50 mm filled at the bottom, 20-cm of small-sized gravel of 10 - 25 mm in the middle and 15-cm of fine-sized sand of 0.30 - 0.75 mm diameter on top of the tanks (Fig.1).



a: 15-cm of fine-sized sand; **b**: 20-cm small-sized gravel; **c**: 30-cm large-sized gravel.

Fig. 1. Schematic diagram of the vertical flow constructed wetland (VFCW) used to grow *Echinochloa pyramidalis* with valve

2.2 Planting and System Operation

Sixteen young shoots or rhizomes of *E. pyramidalis* with uniform size (about 20-cm of long) were collected in the surrounding natural wetlands and transplanted the same day in the six VFCW units. After planting, the beds were flooded with raw domestic wastewater to about 5-cm above the gravel layer and the plants were left to grow for ten weeks (acclimatization) and after that they were subjected to FS application. A mixture of FS collected from traditional pit latrines, septic tanks and public toilets were delivered by the emptying trucks and collected in the storage tanks. Prior to the FS application, samples were taken at the outlet after stirring and analyzed for physico-chemical parameters such as pH, T°C, redox potential (Eh), salinity (Sal), total dissolved solids (TDS) and conductivity using a Hach HQ14d conductivity meter. Raw FS was applied twice a week to all experimental units at a variable loading rate of 50, 100 and 150 kg TS/m^2/yr during the whole period of study. FS loading rates were calculated based on the total solid (TS) content of the raw sludge. Knowing the constant annual TS contents [15]; the hydraulic load of the sludge applied to the experimental units was calculated newly before each application following this equation:

$$HL\ \ (mm) = \frac{ALR}{TSR} \times 1/52$$

Where ALR is annual loading rate (kg TS/m^2/yr) and TSR is total solid content of raw FS (kg/L) newly delivered by the mechanical emptiers.

2.3 Sampling procedures

Samples of *Echinochloa pyramidalis* used in our study were collected at different growth stage in constructed wetlands treating the faecal sludge. The harvest management systems comprised the following steps: at the beginning of the FS treatment (10-week of acclimatization period), 2-, 3-, 5-, 6-week period of treatment and 10-week of regrowth were observed in the cutting process. The cutting regimes were studied in two different seasons: September to mid-November (rainy season) and mid-November to mid-March (dry season). The entire plant samples (leaves and stems) of *E. pyramidalis* harvested in the experimental units were cut off by hand at 20-cm above the ground level, watched, plotted on the pressed paper weight and dried in a forced-draft oven to constant weight at 65°C to determine the dry matter (DM) content. The ratio of leaf to stem was not assessed. Dried samples were ground in a Moulinex Optiblend mill to pass through a 1-mm sieve, and stored for later for incubations by *in vitro* assays.

2.4 *In vitro* Gas Production Measurement

The rumen fluid was collected immediately from adult cattle after slaughter in the municipal slaughterhouse in the city of Dschang-Cameroon before 7 a.m. and they were put in a pre-warmed (39°C) CO_2-filled thermos flask and immediately transferred to the laboratory. This fluid was homogenized for 15-s in a laboratory blender with magnetic stirrer and filtered through a double layer of cheese cloth and diluted with the culture medium which contains bicarbonate buffer, macro mineral, micro mineral resazurine and reducing solution prepared according to Menke et al. [16].

All laboratory handling of rumen liquor was carried out under continuous flushing with CO_2 so as to maintain anaerobic conditions. Forage samples were weighed (500-mg) in triplicate into 100-ml calibrated glass syringes with pistons lubricated with Vaseline.

40-ml rumen fluid-buffer mixture (10-ml rumen fluid, 10-ml bicarbonate buffer, 5-ml macromineral solution, 0.0031-ml micromineral solution and 15-ml distilled water) was pumped with an automatic pipette (Fortuna Optifix) into each syringe pre-warmed at 39°C before and followed by incubation in a water bath at 39°C based on the Hohenheim gas test [16]. Parallel incubations for the measurement of gas production without substrate (blank) and gas produced from 500-mg of standard samples of known gas production parameters were included at the beginning of the incubation period. The incubation lasted 24-hours, syringes

were shaken gently at each reading and the gas volume was recorded after 3, 6, 9, 12, 18 and 24-hours of incubation. Gas production was calculated and corrected according to the following formula [17]:

$$GP\ \ (ml/200mg\ DM) = \frac{(V_{24} - V_o - GP_o) \times 200mg \times GP_h}{m \times MS}$$

Where V_{24} is the volume of gas read after 24-hours incubation; V_0 is the volume of rumen fluid-buffer mixture in the syringe at the beginning of the incubation; GP_0 is the volume of gas produced by the blank after 24-hours of the incubation and GP_h is the net volume of gas produced by the standard (ml/200 mg) after 24-hours of the incubation.

2.5 Estimation of *in Vitro* Dry Matter Digestibility (IVDMD)

At the end of incubation, the entire residue was transferred into 600-ml spotless beakers and the syringes were rinsed with two 20-ml portions of *Neutral Detergent Solution* (NDS) and digested for one hour. The contents were then washed with hot water and filtered using glass crucibles (coarse porosity n° 1, micrometer pore size porosity, Pirex) under vacuum. The crucibles were dried at 105°C overnight, weighed after cooling in a desiccator. The *in vitro* true digestibility of dry matter (IVTD) was calculated as the difference between the weight of incubated substrate and the weight of the undegraded residue after NDS treatment at the end of incubation. These residues obtained after treatment with NDS were used for the determination of NDF-N by the Kjeldahl method. After determining the weight of ash, the IVDMD was calculated by subtracting the weight of incubated substrate from the weight of non-degradable residue after the *Neutral Detergent Solution* treatment at the end of incubation using the following equation [18]:

$$IVDMD(\%) = \frac{P_e - R}{P_e}\ x\,100$$

Where P_e is weight of incubated sample and R is weight of the sample after incubation.

2.6 *In Vitro* Organic Matter Digestibility (IVOMD) and Metabolizable Energy (ME) estimations

At the end of incubation (24-h), the volume of gas produced by the standard and corrected with substrate (blank) were used for calculating the *in vitro* organic matter digestibility (IVOMD) according to the following regression equation [18]:

$$DIVMO\ (\%) = 14.88 + 0.889 \times GP + 0.45 \times CP + 0.0651 \times CA$$

Where, GP is volume of gas production at 24 h of incubation, CP is crude protein and CA is crude ash.
The metabolizable energy (ME) of samples was calculated as described by Makkar [19]:

$$EM\ (MJ/kgDM) = 2.20 + 0.136 \times GP + 0.057 \times CP$$

Where, GP is volume of gas production at 24-h of incubation and CP is crude protein

2.7 Partitioning factors (PF), microbial biomass (MB) and volatile fatty acid (VFA) determinations

Partitioning factor (PF) used as a measure of fermentation efficiency is a quantity of the truly degraded organic matter (OM) needed to produce 1-ml gas; it was calculated as the relating OM degradation to total gas production at 24-h [20], [19]:

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

Where, OMD (mg) is organic matter disappearance and GP (ml) is total gas production after 24-h of incubation.

As microbial biomass (MB) production concerned, it was calculated according to the following equation [19]:

$$MB\ (mg) = OMD - GP \times SF$$

Where, OMD (mg) is organic matter truly degraded, GP (ml) is gas volume produced after 24- hours of incubation and SF is stoichiometrical factor (2.20 for forages).

Volatile fatty acids (VFA) were estimated using the following equation [19]:

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

Where, GP is gas volume (ml) produced after 24 hours of incubation.

2.8 Statistical Analysis

Data on gas production, *in vitro* dry matter digestibility (IVDMD), *in vitro* organic matter digestibility (IVOMD), metabolizable energy (ME), neutral detergent bound nitrogen (NDF-N), *in vitro* true digestibility of dry matter (IVTD), partitioning factors (PF), microbial biomass (MB) and volatile fatty acid (VFA) were subjected to one-way analysis of variance using the general linear model of SPSS 16.0 for Windows. Each data point was the mean of three replicates (n = 3) and comparisons with P values ≤0.05 were considered significantly different. Duncan's Multiple Range Test was used to compare

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their means [21].

3 Results and Discussion

3.1 Growing Season Conditions (rainfall, temperature and soil nutrient status)

During our experimental study, the monthly rainfall and temperature distributions (Fig.2) varied from 0 to 18.9 mm and 25.2 to 30.1°C respectively.

As it can be seen on Table 1, the raw FS used during the entire experimental period exhibited wide variations which are shown by their great values of standard deviation. The sludge exhibited high BOD_5 (225 - 1800 mg/l), PO_4^{3-} (4.6 - 1762.5 mg/l), TKN (248.16 - 697.5 mg/l) and COD (1531.5 - 11140 mg/l). However, the variability of FS reported in the current study corroborated those of other Sub-Saharan countries such as Burkina-Faso, Ghana, Thailand and Argentina where, with high BOD_5 and NH_4^+- H as urine was almost disposed with faeces thus, making the FS to be biochemically instable concentrations [22], [11]. The great quantities of total nitrogen and total phosphorous found in the faecal sludge suggest their high nutrient contents.

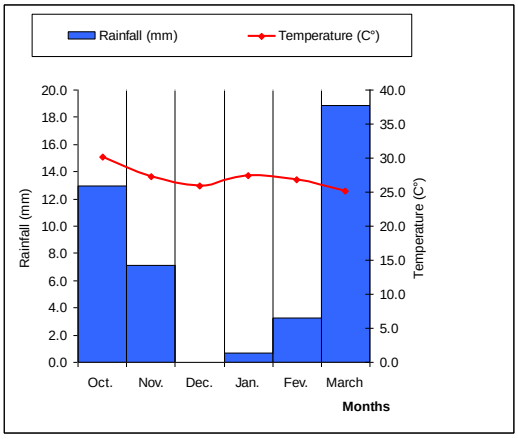

Fig.2 Monthly rainfall and temperature distributions during the entire period of study.

3.2. *In Vitro* Gas Production

Data of gas production at 24-h of incubation, *in vitro* dry matter digestibility (IVDMD), *in vitro* organic matter digestibility (IVOMD), metabolizable energy (ME), neutral detergent bound nitrogen (NDF-N), *in vitro* true digestibility of dry matter (IVTD), partitioning factors (PF), microbial biomass (MB) and volatile fatty acid (VFA) are summarized in Table 2.

During the fermentation period, data of gas production at 24-h of incubation were significantly different (P = 0.020) among samples of *E. pyramidalis*. As it has been demonstrated by Blümmel and Orskov [23] that gas production is associated with volatile fatty acid production following fermentation of substrate, the differences between gas productions could be explained by the differences in total VFA production and their molar proportion [24]. As shown in the Table 3, a strong correlation found between gas production and VFA (r = 0.998; P < 0.01), was consistent with that of Doane et al. [25] and Getachew et al. [26]. The positive correlations between gas production with ADL (r = 0.689; P < 0.01) indicated the influence of ADL concentrations on gas production. These results supported those of Nsahlai et al. [27] who reported that 70% of the variation in gas production in *Sesbania* could be explained by changes either in NDF, hemicellulose or ADL. In the other hand, ME (r = 0.618; P < 0.01) and cellulose (r = 0.722; P<0.01) showed a positive correlation individually with gas production whereas the CP contents were negatively correlated (r = -0.542; P<0.05). All samples produced approximately at least 70% of their total gas production at 24-h within first 12-h. While gas production consistently increased with advancing time of digestion (increased from the beginning at 3-h of incubation to the end at 24-h), the greatest proportion of gas production occurred during these 24-hours of incubation. However, the amount of gas produced per unit of fermented material reflects the level of fermentation of the forages. Figure1 clearly shows the trend of the *in vitro* gas production characteristics of these forage samples harvested at different periods. Although, a comparison of gas production volumes of different treatments indicated a linear increase in gas production as the forage growing period was prolonged (P<0.02), the volumes of gas production recorded among the samples were somewhat overlapping, making comparison difficult (Fig. 2). The relative gas production observed in the current study among all incubated samples was expected since these samples were highly concentrated in CP and NDF. The extent of gas volume produced from the incubation of 200 mg forage sample for 24-hours varied significantly (P<0.02 between 50.6 to 54.9 ml in the whole-forage plant of *Echinochloa pyramidalis* (L). The significant variability of the gas amount produced within the forage samples harvested at different periods could be due to the nature and the variable proportions of nutrient and the secondary compound contents (phenolic and tannin compounds). Moreover, the differences might also have been due to stage of forage growth and/or season of harvest, and/or proportions of leaves and stems sampled. The high potential of gas production seems to indicate a better nutrient availability for rumen microorganisms; it may be attributed to the high content of neutral detergent soluble fraction of carbohydrates in the forage samples [28] [29] [30].

TABLE 1
Characteristics of the raw faecal sludge (FS)

Parameters	n	Mean	Maximum	Minimum	Median	Standard deviation
T°C	10	26.33	30.3	16.4	27.7	4.77
pH	10	7.53	8.85	6.99	7.31	0.86
EH (mv)	10	-10.79	11.7	-47.83	-8	20.58
TDS (mg/l)	10	1238.21	2720	423	776	937.18
CND (µS/cm)	10	2496.71	5230	849	1815	1764.96
Sal (%)	10	1.23	2.83	0.4	0.8	0.95
COD (mg/l)	10	7380.21	11140	1531.5	7345	3488.48
BOD_5 (mg/l)	10	1383.43	1800	225	1725	602.31
TKN (mg/l)	10	526.64	697.5	248.16	538.78	177.32
NH_4^+ (mg/l)	10	339.558	914.65	28.75	77.725	440.74
NO_3 (mg/l)	10	509.472	1730	0.00	249	667.113
NO_2 (mg/l)	10	0.006	0.034	0.00	0.00	0.014
PO_4^{3-} (mg/l)	10	423.858	1762.5	4.6	144.375	677.747

n: number of samples. EH: Redox potential; Sal: Salinity; TDS: Total dissolved solids; CND: Conductivity; TKN: Total Kjeldahl nitrogen, COD: Chemical oxygen demand, BOD_5: Biochemical oxygen demand for five days, PO_4^{3-}: Ortho-phosphate, NH_4^+: ammonium, NO_3^-: nitrate and NO_2^-: nitrite.

TABLE 2
Effect of Harvest time on the Gas Production at 24-h of incubation, *In Vitro* Dry Matter Digestibility (IVDMD), *In Vitro* Organic Matter Digestibility (IVOMD), Metabolizable Energy (ME), Neutral Detergent bound Nitrogen (NDF-N), Partitioning Factors (PF), Microbial Biomass (MB) and Volatile Fatty Acid (VFA) of *E. pyramidalis*.

Item	10-week[a]	12-week[b]	13-week[c]	15-week[d]	16-week[e]	10-week[f]	P-value
DIVMS	40.83±0.85[a]	48.07±1.10[f]	43.94±1.74[d]	51.93±2.38[f]	38.78±0.43[a]	40.11±0.46[a]	0.000
NDF-N	1.82±0.17[ab]	1.72±0.49[a]	1.54±0.13[a]	0.96±0.05[a]	2.25±1.05[b]	1.92±0.09[a]	0.099
PF	1.31±0.17[ab]	1.31±0.65[a]	1.48±0.25[a]	2.06±0.23[b]	1.16±0.76[a]	1.30±0.27[a]	0.254
IVOMD	69.76±0.73[abc]	67.63±0.87[a]	68.25±1.37[ab]	69.59±1.19[abc]	70.31±1.22[bc]	71.55±0.83[c]	0.008
ME	10.25±0.21[ab]	9.97±0.19[a]	10.33±0.26[ab]	10.26±0.18[ab]	10.27±0.24[ab]	10.52±0.13[b]	0.108
VFA	1.24±0.03[bc]	1.17±0.03[a]	1.25±0.05[c]	1.18±0.03[abc]	1.15±0.04[a]	1.21±0.02[abc]	0.022
GP_{24h}	54.57±1.28[bc]	51.73±1.29[ab]	54.94±1.96[c]	52.12±1.28[abc]	50.64±1.49[a]	53.20±1.12[abc]	0.020
MB	122.61±1.95[b]	132.37±2.23[d]	102.45±2.19[a]	129.00±0.30[c]	122.01±0.92[b]	127.62±1.14[c]	0.000

Means in rows without common letters are significantly different (P < 0.05). DIVMS: in vitro dry matter digestibility (%), NDF-N: neutral detergent bound nitrogen (%), PF: partitioning factors (ml/mg), DIVMO: in vitro organic matter digestibility (%), EM: metabolizable energy (MJ/kgDM), VFA: volatile fatty acid (mmol / ml), GP 24 h: gas production at 24-h of incubation (ml/200mg DM), MB: microbial biomass (mg).

3.3. *In Vitro* Dry Matter Digestibility (IVDMD)

The IVDMD of the whole-forage plant of *E. pyramidalis* harvested at different periods greatly varied from 38.8 to 51.9% (P = 0.000) among the different samples (Table 2). Their variability may be attributed to their DM (r = 0.562; P < 0.05), PF (r = 0.597; P < 0.01) and hemi cellulose (r = 0.482; P < 0.05) concentrations as there are positively correlated. Digestibility of forage dry matter reflected the sum total of the digestibility of the component tissues as affected by morphology, anatomy and chemical composition [31]. The results of our study were in line with those of Sultan et al. [32] who reported a positive correlation of hemi cellulose with dry matter digestibility. However, some of these IVDMD values ranged above 45% which, according to Youngquist et al. [33], is the level needed for maintenance of cattle in the tropics.

The low digestibility of *E. pyramidalis* harvested at 16-week old might be due to their stage of maturity with the morphological development that caused the forage to be stemmy with higher fiber content than other samples. This result is in agreement with findings of El-Shatnawi and Mohawesh [34] and Ganskopp and Bohnert [35] who demonstrated that stems having relatively high fiber content may not have a great DMD. It has been stated by Van Soest [36] that fiber is usually detrimentally associated with digestibility. In general their low DMD may be explained by their anatomic characteristics as many C4 grass, *E. pyramidalis* has thinner leaves, more bundle sheaths and smaller interveinal distances; and it become stemmy with advancing maturity [37]. Other reasons for the decline of DMD include a reduction leaf to stem ratio in the analyzed samples with dilution due to higher DM accumulation.

Surprisingly, *in vitro* OMD (Table 2) of whole-plant of *E. pyramidalis* subjected to FS treatment significantly increased (P = 0.008) from 68.2% to 70.3% from the earliest to the oldest analyzed stage. The differences in IVOMD between the samples reflected the differences in their chemical compositions such as the levels of CP, ash, OM and hemi cellulose. This might have resulted to a positive correlation found between IVOMD with CP (r = 0.617; P < 0.01), ash (r = 0.618; P < 0.01), OM (r = 0.618; P < 0.01) and a negative correlation with hemi cellulose (r = -0.541; P < 0.05) and IVDMD (r = -0.526; P < 0.05).

In fact, the increased of IVOMD may be explained by factors such as the increase of CP in plant as the decline of digestibility as plant matures largely depends on the forage quality [31]. The increase of CP in plant may have permitted the release of greater amounts of rumen metabolites, which probably enhanced rumen microbial function and proliferation that might have improved OM digestibility [38], [39]. Hence, the increased of OM digestibility of these high fibrous forage could be due to maintenance of adequate levels of rumen ammonia. However, it has been stated that cell-wall concentration has a large influence on forage digestibility because of the increase of fibre fractions in plant tissues [40], the increase of lignification during plant development [41] and the different ratio between plant tissue components as plant advanced in maturity [42]. Our data did not obtained the trend of the decline in forage OM digestibility with advancing maturity generally ob-

served on other forage grasses however, our findings corroborated those of 66 native mountain forages mixture presented by Andrighetto et al. [43] who showed higher *in vitro* OMD values of 65.0 %DM. Although, it has been defined by Meissner et al. [44] that high quality forage might contained more than 70% digestibility, generally, about 50% digestibility is sufficient for animal maintenance [45], [46]. This suggested that the whole-plant forage of *E. pyramidalis* studied here were all above the minimum recommendation for OM digestibility.

3.4 Metabolizable Energy (ME)

ME of whole-plant forage at different harvesting periods (Table 2) were almost unvariable (P > 0.05) with the mean value ranging from 9.97 to 10.52 MJ/kg DM making them acceptable feeds for beef, cattle, sheep and some classes of dairy cattle. In general the ME values below 7 MJ/kg DM is considered to be unacceptable for cattle and goats [43]. These ME values were higher than those (6.9-7.6 MJ/kg DM) reported by Al-Masri [43] for some range plants such as *Enodium cicutarium*, *Schismus arabiscus*, *Alhagi camelorum and Salsola vermiculata*. These ME reflected their OM digestibility and their ether extract contents. The ME were positively correlated (Table 2) with EE (r = 0.494; P<0.05), IVOMD (r = 0.687; P<0.01), VFA (r = 0.628; P < 0.01) and GP (r = 0.618; P <0.01). However, the metabolizable energy values were found within the range of reported values for a large number of tropical forages (Sen et al., 1978; Krishnamurthy et al., 1995). There were also within various European feeds ranges of ME values (4.5-15 MJ kg^{-1} DM) as reported by Menke and Steingass [17].

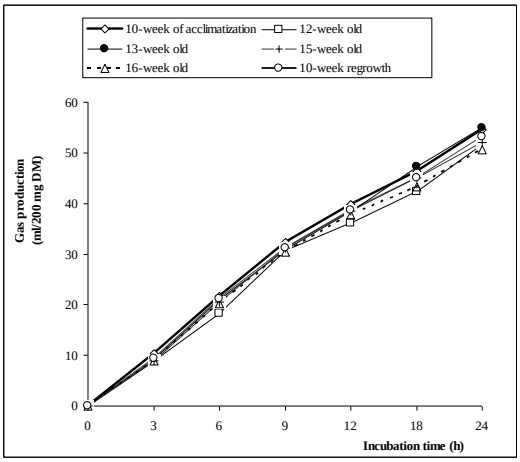

Fig. 3 Cumulative gas production profiles of *Echinochloa pyramidalis* (leaves and stems) from *in vitro* fermentation (ml/200 mg DM) within the different incubation times.

3.5 Partitioning factors (PF) and Volatile fatty acid (VFA)

PF values of whole-plant forage of *E. pyramidalis* in rumen fluid were not significantly variable (P > 0.05) with advanced maturity with the numeric the values ranged between 2.1 and 1.3 ml/mg. There were positively correlated (Table 3) with IVDMD (r = 0.562; P < 0.05). These values suggested the extent and rate of forage fermentation and energy utilization. Despite the fact that forage samples in this study had low FC compared to some feedstuffs, our results showed that the incubation of *E. pyramidalis* samples could produce sufficient energy and ammonia and thus, enhanced growth and microbial activities. In addition, they might serve as an index to assess the differences in efficiency of microbial biomass synthesis of animal feed. Moreover, the concept of partitioning of fermentation products defined as partitioning factor (PF) was introduced recently to express the conversion of energy from truly degraded substrate required to yield 1ml of gas [20].

TABLE 3
Correlation coefficient (r) of relationship between Gas Production at 24-h of incubation, *In Vitro* Dry Matter Digestibility (IVDMD), *In Vitro* Organic Matter Digestibility (IVOMD), Metabolizable Energy (ME), Neutral Detergent bound Nitrogen (NDF-N), Partitioning Factors (PF), Microbial Biomass (MB), Volatile Fatty Acid (VFA).

	PF	IVDMD	NDF-N	IVOMD	ME	VFA	GP_{24h}	MB
PF	1							
IVDMD	.562*	1						
XNDF-N	0.028	-.593**	1					
IVOMD	-0.305	-.526*	0.101	1				
ME	-0.312	-0.362	-0.068	.687**	1			
VFA	-0.242	-0.151	-0.177	0.106	.628**	1		
GP_{24h}	-0.228	-0.118	-0.207	0.099	.618**	.998**	1	
MB	0.073	0.277	-0.018	0.163	-0.235	-.484*	-.485*	1

**Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level.*
DIVMS: in vitro dry matter digestibility (%), XNDF-N: neutral detergent bound nitrogen (%), PF: partitioning factors (ml/mg), DIVMO: in vitro organic matter digestibility (%), EM: metabolizable energy (MJ/kg

Similarly to the variable amount of gas produced from *E. pyramidalis* studied samples, the VFA values varied significantly (P = 0.02) between 1.15 and 1.25 mmol/ml. The amount of volatile fatty acids produced may be a reflection of gas production from incubated forage as there were strongly correlated (r = 0.998; P < 0.01). The positive correlation (Table 3) between gas production and VFA production (r = 0.998) was slightly greater than that reported in a study of Getachew et al. [26] with 12 feedstuffs but consistent with this study and others [49]. However, the variation of VFA may be attributed to the presence of soluble carbohydrates in *E. pyramidalis* compositions whose fermentation by micro-organisms mainly produced volatile fatty acids (VFA), gas and energy (ATP) for the growth of the micro-organisms. Regarding the strong positive

correlation between VFA and cellulose (r = 0.717; P < 0.01), these findings supported the fact that the VFA could be fermented from carbohydrates [50]. The variability of VFA may also be due to the variability of ADL (r = 0.688; P < 0.01), ME (r = 0.618; P < 0.01) as there were positively correlated. Moreover, there were a negative correlation between VFA and CP (r = -0.530; P < 0.05); VFA and MB (r = -0.484; P<0.05), suggesting that the concentration of each volatile fatty acid may be varied and affected by the composition of rumen microbial populations and the quality of forage.

3.6 Microbial biomass (MB) and Neutral detergent bound nitrogen (NDF-N)

The values of MB significantly varied between 102.45 and 132.37 mg (P = 0.000). These values were probably influenced by the levels of fat (EE) content on forage as there were inversely correlated (r = -0.669; P < 0.01) as well as ADL (r = -0.640; P < 0.01). Moreover, the negative correlated found between the MB and VFA (r = -0.484; P < 0.05) and gas production at 24-h (r = -0.485; P < 0.05) may be a consequence of their variability. This may arise because, under the anaerobic conditions of the rumen, the feed nutrients provide both the substrate for microbial cell synthesis and also the potential energy as ATP generated through conversion of feed biomass to VFA, meaning that the yield of microbes relative to VFA produced is variable, that is, ATP is used with variable efficiency [51].
NDF-N ranged between 0.96 and 2.25% did not varied significantly (P < 0.10). The negative correlations were found between NDF-N with DM (r = -0.631; P < 0.05) and IVDMD (r = -0.593; P <0.01). Differences in cell-wall lignification and the ratio of leaf to stems in edible samples of *E. pyramidalis* used for analysis could be partly responsible. Thus, NDF-N values reflected the N contents and high values of cell wall fractions in forage.

4. CONCLUSION

The reuse potential of *E. pyramidalis* after wetland treatment of FS as an alternative to conventional forage sources for animal nutrition was assessed by quantifying its nutritional characteristics. Moreover, the study monitored changes in nutritional characteristics as age advanced. The gas production, IVDMD, IVOMD, VFA, MB, NDF-N and ME were selected parameters and did not change greatly by stage of maturity. *E. pyramidalis* and its fractions at five harvest periods had great nutritional values. On the basis of these understudied parameters, *E. pyramidalis* were judged to be better and more suitable as ruminant feeds in Sub-Saharan countries where the availability of the ruminant feeds is more restricted. Compared to most tropical forages used in ruminant feeding, the forage nutritive value of *E. pyramidalis* was equal to or better. The use of *E. pyramidalis* is of economical benefit as it may lead to a reduction in the cost of ruminant rations and consequently in the cost livestock production. However, more work is needed to broaden our knowledge on the feed value of *E. pyramidalis*.

5. Acknowledgements

The authors are grateful to the Swiss Federal Institute of Aquatic Science and Technology (Eawag), Department of Water and Sanitation in Developing Countries (Sandec), to the Commission for Research Partnerships with Developing Countries (KFPE), Swiss National Centre of Competence in Research (NCCR) North-South and to the International Foundation for Science for their financial and logistic support (IFS, Sweden grant No. W/4262–1).

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