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In Vitro Digestibility and Gas Production from *E. crus-pavonis* used in Wetlands from Domestic Wastewater Treatment

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Summary

In order to evaluate the possibilities of valorisation as feed of the plant biomass produced during wastewater treatment in constructed wetlands, a study of the *in vitro* digestibility and gas production of *Echinochloa crus-pavonis* was carried out in the Laboratory of Animal Production and Nutrition of the University of Dschang. The *in vitro* digestibility of *Echinochloa crus-pavonis* was evaluated at different harvesting periods. The digestibility parameters of *E. crus-pavonis* samples were determined by the *in vitro* method at different phenological stages. The gas production (GP) during 24 hours of incubation was assessed using the *in vitro* incubation technique with bovine rumen fluid. *In vitro* dry matter digestibility (IVDM) values ranged from 52.09% to 64.76% with a decrease observed with the phenological stages of *E. crus-pavonis (*from 64.76% at the leafy stage to 52.09% at the flowering stag). The microbial biomass (MB) values varied significantly between 67.99 and 88.45 mg, with no significant difference observed between the leafy (88.45 mg), bolting (82.93 mg) and early heading (80.26 mg) stages ($P>0.05$). On the other hand, changes in gas produced during 24 hours from the studied samples of *E. crus-pavonis* (34.9 and 48 ml/500mg) and volatile fatty acids (VFA) values (1.08 and 0.80 mmol/ml) decreased significantly ($P < 0.05$) with the change in phenological stage. The values of the partitioning factors (CF) of *E. crus-pavonis* in rumen fluid significantly decreased with advanced plant maturity ($P < 0.05$). The numerical values ranged from 0.52 to 1.19 ml/mg. A decrease in NDF-N was observed with the phenological stages of *E. crus-pavonis.* By combining the requirements of an optimal quantitative and qualitative production of *E. crus-pavonis*, harvesting at the bolting or early heading stage is an option of choice for exploitation as forage, under the conditions of this study. Based on the *in vitro* digestibility parameters studied, *E. crus-pavonis* is suitable as a ruminant feed.

Keywords: *Echinochloa Crus-Pavonis*; Constructed Wetlands; Ruminant Feed; Nutritional Value; *In Vitro* Digestibility

Introduction

In a developing country, the need to feed a growing population is forcing farmers to develop inappropriate lands for agriculture (Tendonkeng et *al.,* 2010) [1]. Farmers tend to increase cultivable land at the expense of rangelands; hence the iterative conflicts between farmers and livestock keepers with the main consequence that livestock keepers find it difficult to meet the needs of animals in extensive livestock systems (Asongwed and Njoya, 2002; Pamo *et al.,* 2006) [2,3]. It is therefore important to develop sedentary livestock farming that will allow intensive and sustainable use of land resources and facilitate livestock management. It is necessary to increase the supply of good quality fodder in order to facilitate the transition from nomadic to sedentary livestock farming (JGRC, 2001) [4]. Fodder crops can be an alternative that could ensure availability of fodder in tropical countries, both in favourable (rainy season) and unfavourable (dry season) periods. Some species of aquatic macrophytes used in wastewater treatment have been exploited as fodder in rabbit breeding, because of their chemical composition, which is favourable to rabbit feeding (Lebas, 2012) [5]. These species include *Eichhornia crassipes, Ipomoea aquatic* and *Pistia stratiotes* (Lebas, 2012) [5]. These plants present appropriate potentialities in livestock nutrition given their chemical composition and a source of income for producers (Abiola *et al.,* 2010; Ngoutane *et al.,* 2011; Tsetagho *et al.*, 2018) [6,7,8].

Previous studies on macrophyte species (*Echinochloa pyrmidalis*) have shown that they exhibit significant biomass production in constructed wetlands (Abiola *et al.,* 2010; Ngoutane *et al.,* 2011) [6,7]. Comparable results have been obtained with other macrophytes used in vegetated beds, like *Cyperus papyrus, Eichhornia crassipes, australis, Typha latifolia, Typha augustifolia*. In Cameroon, recent studies on the efficiencies of vegetated beds have estimated the biomass produced by *Echinochloa pyrmidalis* at 100- 150 t DM/ha and *Echinochloa crus-pavonis at* 35-45 t DM/ha (Kengne *et al.,* 2009; Lekeufack *et al.,* 2011) [9,10]. Unfortunately, all the treatment systems adopted often do not take into account the need to valorise the by-products of the purification process, particularly plant biomass, which poses significant problems from an environmental, economic, technological and even health point of view (Keraita *et al.,* 2010; Kala *et al.,* 2012) [11,12].

Managing plant biomass is therefore of great concern. However, studies on aquatic plants have revealed a large quantity of molecules: amino acids, long chain acids (Bodo *et al.,* 2006) [13]. The valorisation of biomass is one of the most important aspects of this approach. Nowadays, the production of plants biomass in the system can be considered as a valuable outlet. Some authors have shown that the biomass generated by macrophytes can be used as raw material for the paper industry, compost and as a feed supplement for animals during the dry seasons (Polprasert, 2007; Perbangkhem and Polprasert,2010; Lekeufack *et al.,* 2011 and Djumyom *et al.,* 2016) [10,14-16].

The work done by Tsetagho *et al.*, (2018) [8] has highlighted the chemical composition of *E. crus-pavonis* that could be used for animal nutrition. An orientation in the animal nutrition passes by a control of the nutritive value that combine the chemical composition and digestibility of fodder. It is necessary to evaluate the digestibility of *E. crus-pavonis* in order to valorise it as fodder for animal feeding.

However, no studies have been conducted on the digestibility of *E. crus-pavonis* biomass produced after wastewater treatment. The present study was conducted to assess the *in vitro* digestibility of *E. crus-pavonis* at different phenological stages.

Materials and Methods

Study site

The study was conducted in the experimental wastewater treatment plant at the University of Dschang campus. Dschang is located at the 15th degree of the East meridian, between latitudes 5°25' and 5°30' North, and between longitudes 10°0' and 10°5' East. It is located at an average altitude of 1400m above sea level. The climate is a Cameroonian equatorial climate temperate by the altitude. This climate is characterised by an average annual temperature of 20.1°C with a thermal amplitude of 2.2°C, annual rainfall varying

between 1500 and 2000 mm, total annual insolation at 1800 hours and, an average relative humidity varying between 40 and 97%. The rainy season, which corresponds to the sowing period, runs from mid-March to mid-November. February is generally the hottest month, and July and August thecoldest (Figure 1).

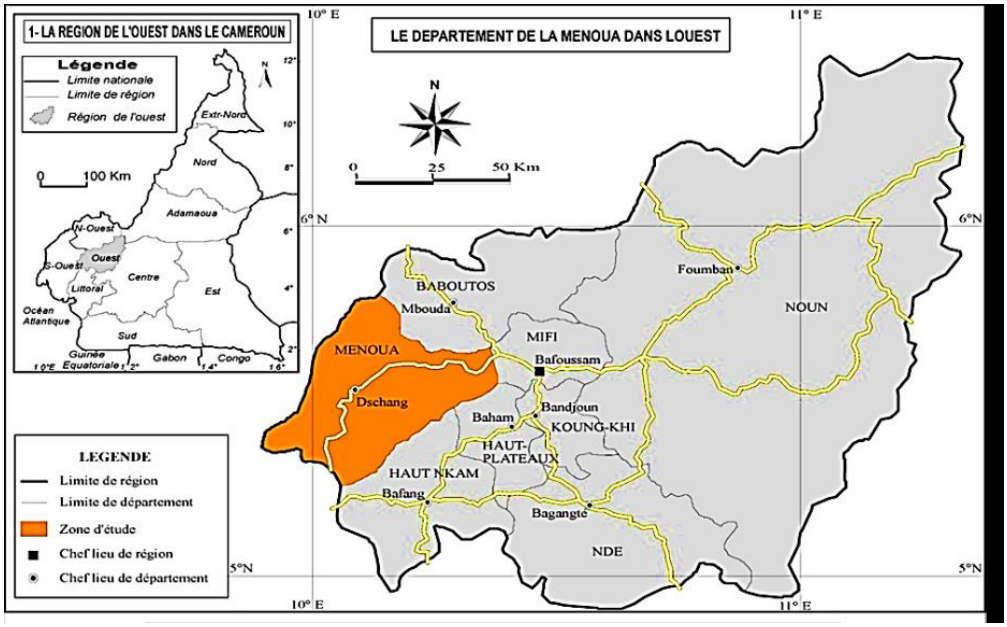

Figure 1: Geographical location of the city of Dschang

Harvesting and sowing of *E. crus-pavonis* saplings

Young axillary buds of *E. crus-pavonis* were collected from the nearby wetland located about 30 m from the study site (Figure 2). After harvesting, about 60 plants with similar morphological characteristics (2 to 3 leaves and 15 to 30 cm in height), then were selected and transplanted into prepared beds.

Figure 2: Young axillary buds of *E. crus-pavonis*

Once transplanted, the young buds were fed with primarily treated wastewater from the student residence of the University of Dschang with the physicochemical characteristics indicated in Table 1. The aclimatation phase lasted 1 month, after which the various analyses were carried out on the aerial part of the plant until the seeds fell.

Parameter	Dry season	Rainy season
CND (µS/cm)	3705±383	2294±354
Turbidity (FTU)	266±3	311±72
TDS (mg/L)	697±63	425±58
NO_3^-(mg/L)	8,7±2	5.1±1.2
PO^{-3}_4(mg/L)	113±18	94,3±33
$SO_4^{-2}{}_4$ (mg/L)	14,5±3	7.8±3.5
DCO (mg/L)	545±11	584±21
DBO_5 (mg/L)	229±5	244±9

Table 1: Physicochemical characteristics of sewage used

This study was carried out in a horizontal surface flow (HSF) wetland configuration vegetated with *E. crus-pavonis*. The wetlands for 4 m length, 2 m width and 0.6 m height were constructed using cement blocks (Figure 2). The inside of the structures was plastered with concrete, then cement and Lankofuge ™ for water tightness. A 1% slope was constructed on the bottom of each wetland bed to ease the movement of water from the inlet to the outlet. Gabions of 30 cm with stones of 5-8 cm in diameter were arranged at the inlet and outlet zones of the wetlands, while a drainage layer of about 10 cm was arranged at the bottom. The wetland beds are connected to a tank by PVC pipes with taps allowing the control of the flow rate (2.93 m^3/day). This flow has been calibrated in order to control the residence time of wastewater in the planted filters and to ensure a hydraulic buffer role. The outlet structures were adjustable to enable the regulation of the water level in the substrate (Figure 3).

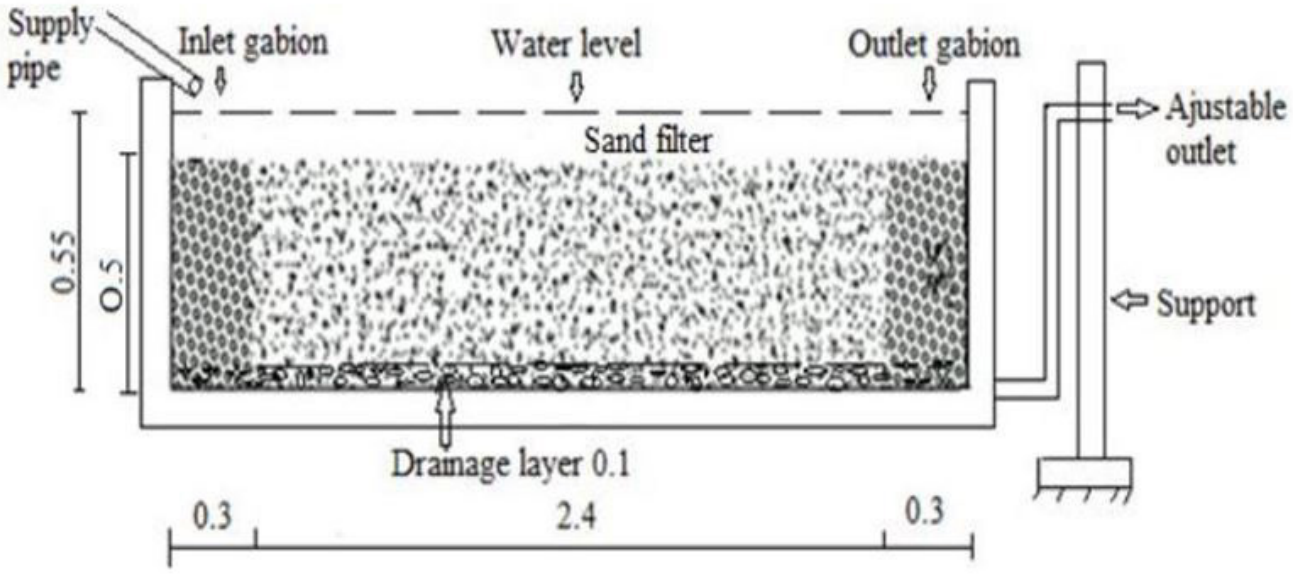

Figure 3: Longitudinal section of the experimental wetland

Sampling and analysis of *E. crus-pavonis* at different phenological stages

At the leafy, the bolting, the early heading and the flowering stages respectively, plant samples were taken at a rate of three clumps per basin in order to obtain a representative sample of the plant biomass. Transported to the laboratory of Animal Production and Nutrition of the University of Dschang, the aerial part of the plants (leaves and stems) was cut into small pieces and dried at 60°C in a

ventilated oven to constant weight. Subsequently, the samples were ground using a home-made tri-hammer mill, and stored in plastic bags for chemical composition assessment according to the methods described by AOAC (1990) [17].

In vitro digestibility assessment

Conditioning and incubation of samples and stock solution

The day before the test was to be carried out, the samples and the freshly prepared stock solution were placed in a Memmert incubator at 39°C overnight. The water bath was also switched on and the temperature was controlled by two LAUDA E300 thermostats also set at 39°C.

The morning before the ruminal fluid was added, the stock solution was placed in the water bath at 39°C where it was continuously supplied with a stream of CO_2 from a gas cylinder set at 4 bars. Sodium sulphide (417 g) and NaOH 6N (0.444 ml) were added to the stock solution, which turned from blue to colourless to red.

Collection of ruminal fluid

The ruminal fluid was collected before 7 am, just after slaughter of adult male cattle of the species *Bos taurus* of about 4years old at the municipal slaughterhouse of the city of Dschang and kept in a thermos previously kept warm with boiling water (to simulate the temperature of the rumen), and immediately transported to the laboratory where it was immediately filtered under a CO_2 flow previously described. For the preparation of 2100 ml of inoculum, 700ml of the filtered ruminal fluid was taken and introduced into the solution still under the CO_2 flow. The resulting mixture, or inoculum, was homogenised for 10 min using a magnetic rod. Then, 40 ml were taken and injected into each syringe using a FORTUNA OPTIFIX precision dispenser and the whole set (syringe + inoculum) was placed in the water bath for incubation.

The incubation period was 24 hours and the volumes of gas produced were recorded after 3 h, 6 h, 9 h, 12 h, 18 h, 24 h. The gas production after 24 hours was calculated and corrected according to the following formula (Menke and Steingass,1988):

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

Where: V_{24} = *volume of gas read at 24 hours*

V_0 = *volume of inoculum in the syringe at the beginning of the incubation*
Gp_0 = *volume of gas produced by the blank at 24 hours*
GP_h = *volume of gas produced by the standard at 24 hours*
DM = dry matter

Determination of *in vitro* dry matter digestibility (IVDMD)

At the end of the incubation, the syringes were emptied and rinsed twice in succession with two 15 ml portions of Neutral Detergent Solution (NDS) in 600 ml beakers, which were immediately brought to a low boil for 1 hour, and the contents filtered into pre-dried and weighed filter crucibles. After treatment with NDS, the sample residues were used for the determination of residual nitrogen (NDF-N) by the Kjeldahl method. The *in vitro* dry matter degradability was obtained as the difference between the weight of the incubated substrate and the weight of the undegraded residue after NDS treatment at the end of the incubation. The following formula established by Van Soest and Robertson (1985) [18] was used:

$$\text{IVDMD } (\%) = \frac{Pe - R}{Pe} \times 100$$

Where: *Pe = Weight of the incubated sample*
R = Weight of the sample after incubation

***In vitro* organic matter digestibility (IVOMD) and metabolizable energy (ME)**

To assess the IVOMD, the gases produced and corrected by the control gases after 24 hours of incubation were used according to the regression equation of Menke and Steingass (1988).

$$\text{IVOMD } (\%) = 14,88 + 0,889\text{Gp} + 0,45\text{CP} + 0,0651\text{C}$$

Where: *Gp = Gas produced after 24 hours of incubation*
CP = Crude Protein
C = Ash

At the same time, the metabolizable energy (ME) content was calculated according to the equation of Makkar (2002):

$$\text{ME } ({}^{Mj}/_{Kg}\,.\text{MS}) = 2,20 + 0,136\text{Gp} + 0,057\text{CP}$$

Where: *Gp = gas produced after 24 hours of incubation*
CP = crude protein

Partitioning Factors (PF), microbial biomass (MB) and volatile fatty acids (VFA)

The PF, which is defined as the amount of organic matter producing 1 ml of gas, was obtained by calculation from the following formula by Makkar (2002):

$$PF({}^{mg}/_{ml}) = \frac{OMD}{Gp}$$

Where: *OMD (mg) = organic matter disappearance*
Gp (ml) = gas produced after 24 hours of incubation

The MM was calculated by the following formula (Makkar, 2002):

$$MB(mg) = OMD - (Gp \times FS)$$

Where: *OMD (mg) = Degraded Organic Matter*
Gp (ml) = Gas produced after 24 hours of incubation
FS = Stoichiometric factor (2.20 for forages)

Volatile fatty acids (VFA) were obtained by calculation from the formula of Makkar (2002)

$$\text{VFA } \left({}^{mmol}/_{ml}\right) = 0,0239\text{Gp} - 0,0601$$

Where: *Gp = gas produced after 24 hours of incubation*

Statistical analysis

The collected data on *in vitro* digestibility of the samples obtained at the different phenological stages were entered into an Excel 2016 spreadsheet. The *in vitro* digestibility data of the samples obtained at the different phenological stages were subjected to an analysis of variance (ANOVA) using XLSTAT 2017 software. An important assumption in the ANOVA is that the variances in the different groups are homogeneous. When differences existed between the different treatments, the means were separated by the Waller Duncan test at the 5% significance level.

Results

Gas production kinetics of *Echinochloa crus-pavonis at* different phenological stages

The present study shows that the values of gas produced are respectively between 34.9 and 48.0 ml/500mg (Figure 4). Statistical analyses showed a significant ($p < 0.05$) variation with the phenological stages. The highest values were obtained at the leaf stage (48.0 ml/500mg) and the bolting stage (44.3 ml/500mg). The value of gas produced at flowering stage was the lowest in this study. The kinetics of *Echinochloa crus-pavonis* gas production had a similar profile regardless of the phenological stage. Between the third and sixth hour, there is no significant difference in gas production regardless of the harvest stage of the plant.

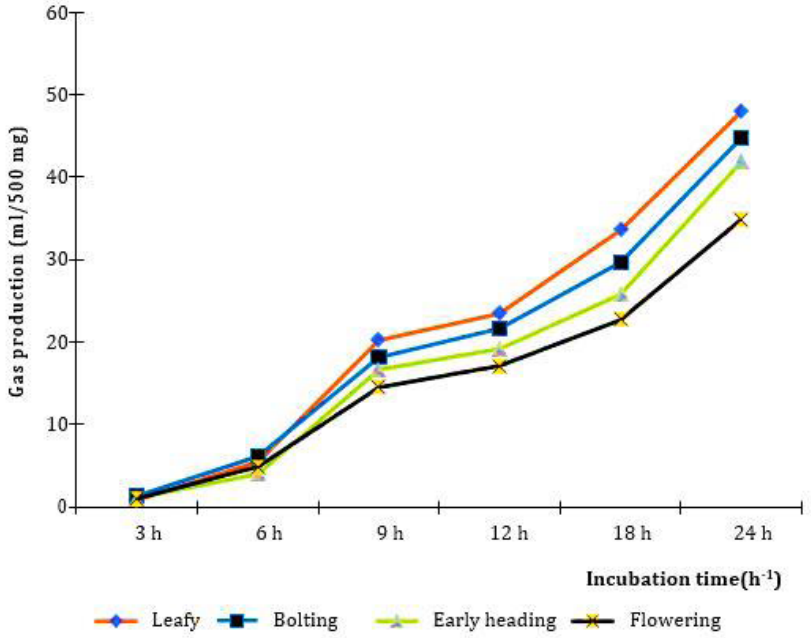

Figure 4: Gas production kinetics (ml/500 mg) of *Echinochloa crus-pavonis* at different phenological stages

Fermentation profiles at different phenological stages were significantly different ($p<0.05$) from the 6th hour. At the end of the 24 hours, the leafy stage was most productive in gas with 48, ml/500mg, followed by the bolting stage with 44.81 ml/500mg. The lowest performance was recorded at the flowering stage with 34.93 ml/500mg. Although there was a significant difference in the volumes of gas produced at different phenological stages, a linear increase in gas production was observed as the growth period of *Echinochloa crus-pavonis was* extended. The volumes of gas production recorded among the samples were distinct, making it easy to compare between phenological stages.

In vitro digestibility of *E. crus-pavonis* according to different phenological stages with bovine ruminal fluid

Data on gas production during 24 h incubation, *in vitro* dry matter digestibility (IVDMD), *in vitro* organic matter digestibility

(IVOMD), metabolizable energy (ME), residual nitrogen (NDF-N), partitioning factors (PF), microbial biomass (MB) and volatile fatty acids (VFA) are summarized in Table 2.

	GP after 24h (ml/500mg)	IVDMD	IVOMD (%)	ME (MJ/kg, MS)	PF (mg/ml)	MB (mg)	VFA (mmol/ml)	NDF-N
Leafy	48,0±2,62a	50,97±0,69a	64,76±0,31a	9,56±0,08a	1,19±0,26a	88,45±6,25a	1,08±0,0,6a	2,40±0,18a
Bolting	44,3±0,72ab	48,38±1,23b	62,71±1,02a	9,23±0,12ab	0,81±0,06b	80,26±1,69a	0,99±0,03ab	1,81±0,49ab
Early heading	41,9±0,99b	45,87±0,37c	59,14±1,60b	8,72±0,21b	0,64±0,02c	82,93±2.10a	0,93±0,06b	2,02±0,92ab
Flowering	34,9±6,87c	42,83±2,01d	52,09±5,04c	7,64±0,80c	0,52±0,05c	67,99±10,62b	0,80±0,18c	1,50±0,43b

a, b, c: means with the same letter on the same column are statistically equal (p>0.05) at the 5% threshold

Table 2: *In vitro* digestibility parameters of *E. crus-pavonis* at different phenological stages with bovine ruminal fluid

The *in vitro* dry matter digestibility (IVDMD) obtained in this study decreased with the age of *E. crus-pavo*nis (Table 2). The IVDMD value obtained at the flowering stage (42.83%) was significantly low compared to those at the leafy (50.97%), bolting (48.38%) and early heading (45.87%) stages (P<0.05).

The *in vitro* organic matter digestibility (IVOMD) values obtained in this study decreased with the phenological stages of *E. crus-pavonis* (Table 2). The IVOMD values obtained at the leaf stage (64.76%) and the bolting stage (62.70%) were statistically similar (P>0.05), but these values were statistically higher than those obtained at the early heading (59.14%) and flowering (52.09%) stages (P < 0.05).

The metabolizable energy values recorded in Table 2 range from 7.64 to 9.56MJ/kg. Their ME values obtained at the leaf stage (9.56MJ/kg, DM), bolting stage (9.23 MJ/kg, DM) are statistically comparable (P>0.05) and significantly higher than that of other phenological stages. The lowest value was observed at flowering stage (7.664 MJ/kg, DM).

Microbial biomass (MB) values varied significantly between 67.99 and 88.45 mg. The MM values obtained at the leafy (88.45 mg), bolting (82.93 mg) and early heading (80.26 mg) stages were statistically comparable (P>0.05). The lowest value was observed at the flowering stage (67.99 mg).

As well as the variation in gas produced from the studied samples of E. crus-pavonis, the values of volatile fatty acids (VFA) varied significantly (P < 0.05) between 1.08 and 0.80 mmol/ml. The lowest VFA value was observed at the flowering stage (0.80 mmol/ml). The VFA values obtained at the leaf stage (1.08 mmol/ml) and the bolting stage (0.99 mmol/ml) were statistically comparable (P>0.05). The same observations were noted between the bolting stage (0.99 mmol /ml) and the early heading stage (0.80 mmol /ml).

The partitioning factors (CF) obtained in this study decreased with the age of E. crus-pavonis (Table 1). The partitioning factors (PF), value obtained at flowering stage (0.52 mg/ml) was significantly low compared to those at leafy stage (1.19 mg/ml), bolting stage (0.81 mg/ml), early heading (0.64 mg/ml) (P<0.05).

The residual nitrogen values (NDF-N) obtained in this study ranged from 2.40 to 1.50. A decrease in NDF-N was observed with the phenological stages of *E. crus-pavonis*. The NDF- N obtained at the leaf (2.40), bolting (1.81) and early heading (2.02) stages were statistically comparable (P>0.05). The lowest value was observed at flowering stage (1.50). (P<0,05).

Discussion

Gas production, *in vitro* dry matter digestibility (IVDMD), *in vitro* organic matter digestibility (IVOMD), metabolizable energy (ME), residual nitrogen (NDF-N), partitioning factors (PF), microbial biomass (MB) and volatile fatty acids (VFA) varied with the phenological stages of *E. crus-pavonis*. Indeed, *in vitro* digestibility is a process that simulates the fermentation of feed in the rumen from which certain gases such as CO_2 and CH_4 are produced. Gas production at each phenological stage during digestion of *E. crus-pavonis* evolves progressively just after incubation to reach a peak at around 24 h for most samples. Looking at the digestibility profiles proposed by Chermiti (1997) [19], the profile of *E. crus-pavonis* would be characteristic of fibrous forages because there is a more or less high latency time due to a long stay in the rumen. Indeed, for gas production to occur, a fermentable substrate and microorganisms capable of degrading it are required. The low gas production at the flowering stage could be due, on the one hand, to the existence of anti-nutritional substances and, on the other hand, to the rigidity of the plant's cell wall, which would inhibit bacterial growth (Bouazza, 2014) [20]. According to Boufennara (2012) [21], the variation in gas production is associated with the composition of the substrates and the content of phenolic compounds and condensed tannins, which vary according to plant species and family. In addition, Aregheore (2000) [22] shows that the low gas production in some species may be related to the very high protein content. The variation in relative gas production at each stage for the incubated samples was to be expected as these samples were highly concentrated in crude protein (CP) and neutral detergent fiber NDF. This high potential for gas production seems to indicate good nutrient availability for the rumen microorganisms. This could be attributed to the high content of neutral detergent soluble fraction of carbohydrates in the forage samples (Salem *et al*., 2006; Mbugua *et al*., 2008) [23,24]. Furthermore, the variation in volatile fatty acids (VFAs) during the different growth stages can be attributed to the presence of soluble carbohydrates that were transformed into VFAs after fermentation by the micro-organisms with energy production in the form of ATP.

Overall, the IVDMD *E. crus-pavonis* values at different phenological stages varied greatly between samples. The observed variability in the *in vitro* dry matter digestibility could be attributed to the concentration of dry matter (DM) and hemicellulose. Numerous studies by Sultan *et al* (2008) [25], Ngoutane *et al* (2012) [26] have shown that these parameters correlate positively with *in vitro* dry matter digestibility. The high values (above 45%) of IVDMD at the leafy, bolting and early heading stages could be explained by their fibre content. Indeed, it has been shown by several authors (Van Soest, 1982; El-Shatnawi and Mohawesh., 2000; Ganskopp and Bohnert., 2001) [18,27,28] that stems with a relatively high fibre content have a mainly negative influence on digestibility. Moreover, these values correspond to a level necessary for the nutrition of livestock in the tropics (Youngquist *et al.* ,1990) [29], Compared to other forage species, the dry matter digestibility of *E. crus-pavonis* is low. This may be explained by anatomical features, as many C_4 grasses like *E. crus- pavonis* have thinner leaves that lignify with maturity (Heckathorn *et al.*, 1999) [30]. In addition, the significant accumulation of dry matter due to the reduction of the leaf/stem ratio could be considered.

The *in vitro* digestibility of *E. crus-pavonis* organic matter (IVOMD) decreased significantly with different phenological stages. These results are in contrast to those obtained by Ngoutane *et al,* (2012) [26] who had a significant increase in IVOMD of *E. pyramidalis at* different harvesting periods during wastewater treatment. The fluctuation of IVOMD values observed at each stage would be inherent either to their crude protein, ash, OM and hemicellulose contents (Ngoutane *et al.,* 2012) [26] or to the increase of NDF, ADF in the plant. In addition, cell wall concentration has a great influence on forage digestibility due to increased fibre fractions in plant tissues and increased lignification during plant development (Borreani *et al.,* 1998; Mbugua *et al.,* 2008) [24,31]. It could also be thought that the crude protein in the plant would have allowed the release of large amounts of metabolites into the rumen, thereby promoting microbial function and proliferation in the rumen which could have improved the digestibility of organic matter. The combination of these factors decreases the digestibility of organic matter in most forage grasses such as *E. crus-pavonis*. However, these results do not corroborate those of Andrighetto *et al.* (1992) [32] obtained on 66 native mountain forage mixtures. Although Meissner *et al.* (2000) [33] defined that a high-quality forage could have a digestibility of more than 70%, a digestibility of about 50% is generally sufficient for animal nutrition. This suggests that the *E. crus-pavonis* forages studied here could be recommended for the percentage of organic matter digestibility.

The metabolizable energy (ME) of the whole plant harvested at different periods varied from 7.64 and 9.56MJ/kg, DM. These values are roughly equal to those obtained by Ngoutane *et al*, (2012) [26] with *E. pyramidalis*. These EM values are higher than those (6.9-7.6 MJ/kg DM) reported by Al-Masri (2006) [34] for some plants such as *Enodium cicutarium, Schismus arabiscus, Alhagi camelorum* and *Salsola vermiculata*. In general, ME values below 7 MJ/kg DM are considered unacceptable for cattle and goats, making *E. crus-pavonis* an acceptable forage for these mammals.

The partitioning factors (PF) values of *E. crus-pavonis* in rumen fluid decreased significantly with advancing maturity ($P > 0.05$). These values are lower than those obtained by Ngoutane *et al,* (2012) [26] with *E. pyramidalis* who showed that PF values of *E. pyramidalis did* not significantly vary with advanced maturity of the plant, with numerical values ranging from 2.1 to 1.3 ml/mg. Despite a low PF compared to some feeds, these results showed that incubation of *E. crus- pavonis* samples could produce sufficient energy and ammonia and thus enhance microbial growth and activities. They could be used as an index to assess differences in the efficiency of microbial biomass synthesis of feeds (Blümmel *et al.*, 1997) [35].

In general, the MM values vary between 67.99 and 88.45 mg at all growth stages. These values are lower than those obtained by Ngoutane *et al,* (2012) [26] with *E. pyramidalis* where the values are between 102.45 and 132.37 mg. Chemicals including fodder fat such as tannins and mimosine inhibit the enzymatic activity of microorganisms thus reducing their growth and multiplication (Chesworth 1996; Makkar 2003) [36-38]. The NDF-N values are globally low and decrease with the growth of the plant. These values are roughly equal to those obtained by Ngoutane *et al* [26]. The decrease in NDF-N observed with thephenological stages in *E. crus-pavonis.* This is believed to be due to the difference in cell wall lignification and the leaf/stem ratio [39,40].

Conclusion

The potential for use of *E. crus-pavonis* biomass from wastewater treatment in tropical environments as an alternative feed for animals was evaluated in this study. The considered parameters such as gas production (GP_{24h}), *in vitro* dry matter digestibility (IVDMD), *in vitro organic* matter digestibility (IVOMD), metabolizable energy (ME), microbial biomass (MB), volatile fatty acids (VFA), partitioning factors (PF) and residual nitrogen (NDF-N) changed significantly with plant maturity. By combining the requirements of optimal quantitative and qualitative production of a forage crop, harvesting at the bolting or early heading stage is a preferred farming option under the conditions of this study. Based on these parameters studied, *E. crus-pavonis* is suitable as a ruminant feed in sub-Saharan countries where the availability of ruminant feed is more limited. The use of *E. crus-pavonis* has an economic advantage as it can lead to a reduction in the cost of ruminant rations and hence livestock production.

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