NUTRITIVE VALUE AND *IN VITRO* METHANE PRODUCTION OF TWO PERENNIAL GRASSES UNDER DIFFERENT STOCKING RATES IN PERI-URBAN AREAS OF WEST-AFRICA †

[VALOR NUTRITIVO Y PRODUCCIÓN *IN VITRO* DE METANO DE DOS PASTOS PERENNES BAJO DIFERENTES CARGAS DE PASTOREO EN ZONAS PERIURBANAS DE ÁFRICA OCCIDENTAL]

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SUMMARY

Background. In sub-Saharan Africa, pasture is the most used practice in ruminant production, and grazing areas characteristics varied in different environment. To improve ruminants' performances, the quality of forages represents one of the most important factors to keep under control. **Objective.** To quantify the effects of grazing stocking rate on the nutrient composition, energy content and *in vitro* fermentation characteristics including volatile fatty acids (VFA) and methane production (CH_4) of two local perennial grasses *Dactyloctenium aegyptium* L. and *Leptochloa caerulescens* L. **Methodology.** The forage samples were collected in three locations (Zinvié, Gakpé, Adounko) characterized by different stocking rate (low, medium and high). Their chemical composition, energy content and *in vitro* fermentation characteristics, including CH_4 production, were determined. **Results.** *D. aegyptium* showed the highest crude protein, energy content, organic matter degradability and VFA production ($p<0.01$) when collected in Zinvié, whereas the highest ($p<0.01$) structural carbohydrates (NDF) content was detected in Gakpé area and the highest CH_4 production was recorded from the sample collected in Adounko ($p<0.01$). Contrariwise, *L. caerulescens* showed the highest level of crude protein and degradability ($p<0.01$) when collected in Zinvié, whereas it proved the highest NDF content and the lowest lignin values ($p<0.01$) when collected in Adounko. The highest CH_4 level was obtained from the sample collected in area of Gakpé ($p<0.01$). Higher ($p<0.01$) *in vitro* VFA production were recorded in samples collected in Adounko and Gakpé than in the Zinvié. **Implications.** The results obtained in this study highlight the significant influence of urbanization and herd concentration, as well as of other environmental conditions, on the nutritional value and potential environmental impact of the studied perennial grasses. **Conclusion.** Samples from high grazing stocking rate locations produced more *in vitro* methane. Further *in vivo* studies are needed to confirm these results.

Keywords: feed resources; greenhouse gas; nutrient degradability; ruminant production; volatile fatty acids.

RESUMEN

Antecedentes. En África subsahariana, el pastoreo es la práctica más utilizada en la producción de rumiantes, y las características de las áreas de pastoreo varían en diferentes entornos. Para mejorar el rendimiento de los rumiantes, la calidad de los forrajes representa uno de los factores más importantes a mantener bajo control. **Objetivo.** Cuantificar los efectos de la carga de pastoreo sobre la composición de nutrientes, el contenido energético y las características de fermentación *in vitro*, incluidos los ácidos grasos volátiles (AGV) y la producción de metano (CH4) de dos gramíneas perennes locales *Dactyloctenium aegyptium* L. y *Leptochloa caerulescens* L. **Metodología**. Las muestras de forraje se recolectaron en tres localidades (Zinvié, Gakpé, Adounko) caracterizadas por diferente carga animal (baja, media y alta). Se determinó su composición química, contenido energético y características de fermentación *in vitro*, incluida la producción de CH4. **Resultados**. *D. aegyptium* mostró el mayor contenido de proteína cruda, contenido energético, degradabilidad de materia orgánica y producción de AGV ($p<0.01$) cuando se recolecta en Zinvié, mientras que el contenido más alto ($p<0.01$) de carbohidratos estructurales (NDF) se detectó en el área de Gakpé y la producción más

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alta de CH4 se registró de la muestra recolectada en Adounko (p <0.01). Por el contrario, *L. caerulescens* mostró el mayor nivel de proteína cruda y degradabilidad (p <0.01) cuando fue recolectada en Zinvié, mientras que demostró el mayor contenido de FDN y los valores más bajos de lignina (p <0.01) cuando fue recolectada en Adounko. El nivel más alto de CH4 se obtuvo de la muestra recolectada en el área de Gakpé (p <0.01). Se registró una producción de AGV *in vitro* más alta (p <0.01) en las muestras recolectadas en Adounko y Gakpé que en Zinvié. **Implicaciones**. Los resultados obtenidos en este estudio destacan la influencia significativa de la urbanización y concentración del rebaño, así como de otras condiciones ambientales, sobre el valor nutricional y el potencial impacto ambiental de las gramíneas perennes estudiadas. **Conclusiónes**. Las muestras de lugares con alta tasa de pastoreo produjeron más metano *in vitro*. Se necesitan más estudios in vivo para confirmar estos resultados.
Palabras clave: recursos alimenticios; gases de efecto invernadero; degradabilidad de nutrientes; producción de rumiantes; ácidos grasos volátiles.

INTRODUCTION

Cattle farming has existed for long time around most West-African cities and has emerged more recently in many others because of the increasing market demand for fresh milk and meat in urban centers (Roessler *et al*., 2016; Amadou *et al*., 2012). The Republic of Benin is a small and long stretched country in West Africa, characterized by different environmental and agronomic conditions. Its economy is mainly based on agriculture and natural grassland-based livestock production. Several cattle farm types have been identified in peri-urban areas of Cotonou (Koura *et al*., 2015), the biggest city in Republic of Benin. However, as observed by these authors, the occurring increasing urbanization has led to a drastic reduction of grazing areas, while the number and size of ruminant farms have been continuously increasing.

It is widely accepted that feed resources available to grazing animals in tropical countries, and particularly during dry seasons, are often of very low quality and sparsely distributed (Assouma *et al*., 2018). This is particularly true of area that also shows multiple and conflicting land uses: human settlements, infrastructures, farmlands, grazing lands, fishing, biodiversity conservation (mangroves), and tourism (beaches and wetlands). Nevertheless, the agricultural and urbanization pressures as well as the concentration of large cattle herds were the main factors affecting forage availability in peri-urban areas in Guinean areas of Benin (Koura *et al*., 2015). With regard to these factors, different levels of pressure can be found in the places around the city. It is important to highlight the impact of stocking rates on perennial grasses, especially during the dry season when they are the main available forage on routes and in wetlands.

Dactyloctenium aegyptium L. and *Leptochloa caerulescens* L. are some of the most widely known consumed grasses which persist in Guinean pasture during the dry season (Akobundu and Agyakwa, 1989). These species are different in their ecological characteristics. The first is a drought-resistant grass, mainly present in sandbanks and marginal areas (roadsides, fallows and waste lands) and it resists even in overgrazed pastures (Zaharaby *et al*., 2001). *Leptochloa caerulescens* is recognized as a weed of irrigation plots and paddy fields, due to its resistance to the weed-killer, scouring and erosion (Akobundu and Agyakwa, 1989). Although these two forages are the main annual perennial grasses species grazed during the period of feed shortage, little attention has been paid to their contribution in terms of nutritional value and environmental welfare. In particular, data in the bibliography report a relatively poor nutritive value for *Dactyloctenium aegyptium*, while few information on *Leptochloa caerulescens* exist.

Climatic and environmental conditions are commonly pointed to be the main factors influencing changes in nutritional value of forage species (Adjolohoun *et al*., 2008; Teklu *et al*., 2010; Koura *et al*., 2015; Musco *et al*., 2016). However, high stocking rate is also well known to have a great impact on plant communities and biomass, and influence on the nutritive value of perennial species (Jarillo-Rodriguez *et al*., 2011; Lin *et al*., 2011). The quality of pasture represents one of the most important factors to keep under control in order to improve ruminants' performances (Tudisco *et al*., 2014). However, considering the needs to adopt a sustainable ruminant production system (Pulina *et al*., 2017), a more complete forage evaluation also needs to include the determination of methane, which contribute to total greenhouse gas emissions. Indeed, the methane production can be affected by grazing areas management strategies, including stocking rate and grazing intensity (Savian *et al*., 2014).

This study was therefore designed with the aim to determine the *in vitro* fermentation characteristics of the two perennial grasses, including volatile fatty acids and methane production. It is hypothesized that *Dactyloctenium aegyptium* and *Leptochloa caerulescens* differ in their nutritional characteristics according to areas and stocking rate. Outputs from this research could be useful for defining strategies for efficient and sustainable management of peri-urban grazing areas in terms of animal performances and methane emissions.

MATERIALS AND METHODS

Study area

The study area covered a radius of 35 km around the city of Cotonou and includes mainly the municipalities of Abomey-Calavi and Ouidah in the same agroecological area. This area is geographically situated between 6.15° and 6.42° North latitude and between 2° and 2.15° East latitude and has a subequatorial climate with two rain seasons (from March to July and from October to November), alternated with two dry seasons of unequal duration. The annual rainfall amounts recorded by the National Direction of Meteorology (DNM) are between 739.6 mm and 2,203.3 mm with an average of 1,305.95 mm (DNM, 2017). The soil is mainly sandy and of ferralitic type (Volkoff, 1976-1978). The vegetation consists of shrubs, grassland swamps, swamp forest and mangrove forest on the coastal belt and of semi-deciduous dense forests on bar land area (Akoègninou *et al*., 2006).

Participatory stocking rate evaluation

As free grazing in unrestricted open natural vegetation is the main ruminant feeding practice and, due to lack of updated information on land use patterns and total livestock number in the area, it was difficult to evaluate stocking rate as number of grazing animals per grazing area unit. A participatory approach involving local farmers was therefore used to evaluate together with the farmers the status of their common grazing areas, as suggested in Wu *et al*. (2015).

For the purpose of this study, three localities were pre-selected based on their characteristics (Table 1) and distance from urban centers, and named as followed: Adounko lowlands, Gakpé fallows, Zinvié savannah. In each of these three areas, a focus group discussion was conducted to assess famers' perception of the grazing stocking rate in their area. The group discussion was constituted of twelve (12) agro-pastoral farmers randomly selected among cattle keepers, two (02) local responsible of the municipality and agricultural services, five (05) elder and experienced key farmers in each area. Discussions were conducted in local language by three experts of landscape planning, grassland management and ruminant production. Farmers were asked, with the guidance of the experts to rank how land use pressure identified in Koura *et al*. (2015), as ruminant herds' concentration, cultivated area extension, urban infrastructures (habitations, building and other presence) extension and reducing grazing areas, affected their area. The farmers were first trained, under the supervision of the two local administrative responsible, to understand the different factors and how to give the different score. Three scores can be given (1. low impact, 2. medium impact, 3. high impact). Then a final mark was given to the area, corresponding to the mean of all the four score. All the results were validated together by the five experienced farmers, and the local responsible based on their experience of the area and literature available.

Frequencies of farmer's responses were calculated and as suggested by Wu *et al*. (2015), majority of responses was presumed to be the dominant perception of land use pressure and stocking rate.

Table 1. Characteristics of the three sampling areas in the peri-urban district of Cotonou.

Areas	Zinvié	Gakpé	Adounko
Distance from urban centers (average, km)	15	30	5
Major soil type	Bar (ferralitic), hydromorphic	Bar (ferralitic)	Sandy, hydromorphic
Climatic conditions	Subequatorial climate with two rain seasons alternated with two dry seasons of unequal duration Rainfall amounts recorded 1997-2016 (DNM, 2017): 739.6 – 2,203.3 mm		
Hydrographic	Proximity of a lake	Proximity of a lake	Proximity of Atlantic Ocean and a coastal lagoon
Major vegetation	Marshy meadow and savannah	Savannah	Grassy savannah and marshy meadow
Main crop production	Food crop (cereals and legumes)	Food crop (cereals and legumes)	Commercial gardening Coconut plantations Food crop

Assembled from Volkoff (1976-78); Akoègninou *et al*. (2006); Koura *et al*. (2015), DNM (2017); INSAE (2017).

Forages selection and sampling

Focus groups organized with farmers of the three different areas, also permit to identify forage species commonly remaining during feed scarcity period (dry season) and grazed by ruminants. The discussion which focused on the forage species more ingested by animals because of they are available and preferred led to the identification of two perennial grasses species: *Dactyloctenium aegyptium* L. and *Leptochloa caerulescens* L.

Samples of both species were collected at their maturity phase, during August-September in the short dry season preceded by a long raining season. Method described in Bezabih *et al*. (2013) for forage sampling was used. Six transects were undertook across grazing areas of 16 km^2, and forage samples (100 g each) were collected from 60 quadrats of 1.0 m^2, randomly positioned along the transects in each of the three areas. The sample cuts (leaves and stems) were made at the upper part of the plants at 5.0 cm above ground surface. The 60 sub-samples of each forage species per area were subsequently pooled in order to obtain only one composite sample per forage species in each area, for the laboratory analysis.

All the forage samples were screened to assess their chemical composition and *in vitro* fermentation characteristics, including methane production.

Chemical composition

All the samples were ground to pass a 1 mm screen (Brabender Wiley mill, Brabender OHG, Duisburg, Germany) and analyzed for dry matter (DM), crude protein (CP), ether extract (EE) and ash as suggested by AOAC (2015) procedures (ID number: 2001.12, 978.04, 920.39 and 930.05 for DM, CP, EE and ash, respectively). Structural carbohydrate fractions were also analyzed: neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL), as described by Van Soest *et al*. (1991).

***In vitro* gas production**

The fermentation characteristics were studied using the *in vitro* gas production technique by incubating all the forage samples at 39 °C under anaerobic condition with buffered rumen fluid (Calabrò *et al*., 2015). The substrates to test were weighted (1.0004 ± 0.0003 g) in four replicates 120 ml serum flasks, where 74 ml of anaerobic medium were added. The rumen fluid was collected in a pre-warmed thermos at a slaughterhouse authorized according to EU legislation (2004) from six adult cows (*Bos taurus*) fed a standard diet (NDF 45.5 % and CP 12 % DM). The collected material was rapidly transported to the laboratory, where it was pooled, flushed with CO_2, filtered through a cheesecloth and added in each flask (5 ml). Four flasks containing no substrate were incubated as blanks to correct organic matter degraded (OMD), gas production (GP24) and volatile fatty acids production (VFA).

During the incubation time, the gas production of the fermenting cultures (GP_{24}, ml/g) was recorded in two of the four bottles using a manual pressure transducer (Cole and Palmer Instrument Co, Illinois, USA). The fermentation was stopped at 24 h and the fermentation liquor was analyzed for pH with a pH-meter (model 3030 Alessandrini Instrument glass electrode, Jenway, Dunmow, UK) and sampled for VFA analysis. At the end of the experiment, the extent of sample disappearance, expressed as organic matter degradability (OMD, %), was determined by difference of the incubated OM and the undegraded filtered (sintered glass crucibles; Schott Duran, Mainz, Germany, porosity # 2) and burned at 550 °C for 5 h.

For VFA determination, fermenting liquors were centrifuged at 12,000 g for 10 min at 4 °C (Universal 32R centrifuge, Hettich Furn Tech Division DIY, Germany). One milliliter of supernatant was then mixed with 1 ml of oxalic acid (0.06 mol). VFAs were measured by gas chromatography (Thermo Quest 8000top Italia SpA, Rodano, Milan, Italy; fused silica capillary column 30 m, 0.25 mm ID, 0.25 µm film thickness), using external standard solution composed of acetic, propionic, butyric, iso-butyric, valeric and iso-valeric acids (Musco *et al*., 2017). Branched-chain fatty acids proportion (BCFA) was calculated as follows: (iso-butyric + iso-valeric) / tVFA.

Methane determination

The methane (CH_4) production was determined in two bottles per sample from *in vitro* gas production test (Boussaada *et al*., 2018), sampling the head-space gas (3 ml) from each serum flask in duplicate with a gastight syringe. The analysis was carried out using a gas chromatograph (GC Trace 1310, Thermo Scientific, Waltham, MA USA) equipped with a loop TC detector and a packed column (HaySepQ SUPELCO, 3/16-inch, 80/100 mesh). The methane production was expressed as a percentage of the total gas (pCH_4, %), related to the incubated OM (iCH_4, ml/g) and related to the degraded OM (dCH_4, ml/g).

Data processing and statistical analysis

Statistical analysis of data collected for the stocking rate evaluation was implemented in IBM SPSS 20 software, to compare farmers' responses between the three areas. For nominal variables, cross-tabulations with chi-square statistics were used, while means and

standard deviations were calculated for stocking rate mean score and compare across areas using the non-parametric Kruskal-Wallis test.

The nutritive value of forages was estimated as metabolizable energy for lactation (ME, MJ/kg DM) using the equation proposed by Menke and Steingass (1988):

$$ME = 2.2 + 0.1357 \times GP + 0.0057 \times CP + 0.0002859 \times CP^2$$

where CP is the content (g/kg DM) of crude protein and GP is the gas obtained *in vitro* (ml/200 mg incubated DM) after 24 h of incubation.

Chemical composition parameters (DM, CP, EE, ash, NDF, ADF, ADL and ME), *in vitro* fermentation characteristics (OMD, GP24, pH and VFA), and methane production (pCH_4, iCH_4 and dCH_4) were subjected to the analysis of variance (PROC GLM, SAS, 2000) according to the model:

$$\gamma_{ijk} = \mu + S_i + A_j + SA_{ij} + \varepsilon_{ijk}$$

where, y is the single data, μ is the mean, S is the sample effect (i = 1-2), A is the area effect (j = 1-3), SA is the interaction of both effects (sample x area) and ε is the error term. Differences between means were compared using the Tukey test and were considered statistically significant at $p<0.05$ and $p<0.01$.

RESULTS

Perception of land use pressure by the farmers' revealed the heterogeneity of the land use pattern in peri-urban areas (Table 2). The Adounko lowlands, close to the urban centers, was perceived as of highest ($p<0.05$) stocking rate. High influence of coconut and commercial vegetables cultivation all over the year (100% of respondent), and of cattle herd concentration in the area (66.7% of respondents) on reducing grazing lands (75%) were pointed as main reasons. On the contrary, the lowest stocking rate was mentioned in Zinvié grassy savannah and marshy meadow. Indeed, the farmers' perceived that the medium influence of urban and agricultural extension had not affected grazing land availability in this agricultural area with a medium herd concentration. Gakpé, with characteristics close to Zinvié, showed medium stocking rate due to high agricultural practice which affected grazing lands availability.

Table 3 shows the chemical composition of *Dactyloctenium aegyptium* L. and *Leptochloa caerulescens* L. in the three different areas situated in the Guinean zone of Benin. Except for ADF and EE, all the parameters showed significant differences between the two forages and within the three areas. In particular, in *D. aegyptium* the crude protein content ranked the areas as follows ($p<0.01$): Zinvié > Gakpé > Adounko. Moreover, Zinvié area also showed the highest energy content ($p<0.01$). The highest ($p<0.01$) structural carbohydrates content (NDF) was found in Gakpé area, even if for this area the lignin content of forage was the lowest. Regarding *L. caerulescens*, the lowest ($p<0.05$) crude protein content was found in the forage collected in Adounko area, which also showed the highest ($p<0.05$) NDF level but the lowest ($p<0.01$) ash content. The energy content was little variable among areas.

Tables 4 shows the *in vitro* fermentation characteristics and methane production recorded after 24 h of incubation for both studied forages in the three areas. Except for GP24 differences ($p<0.01$) were found for all the parameters obtained, comparing substrates and areas, even if more differences appear for *L. caerulescens* compared to *D. aegyptium*. Regarding the first, the lowest ($p<0.01$) OM degradability was found in Zinvié area, the highest ($p<0.01$) CH_4 production in Gakpé, when reported as percentage of total gas produced, and the lowest ($p<0.01$) CH_4 production in Zinvié area, when related to degraded OM. On the contrary, the samples of *D. aegyptium* collected in Zinvié and Adounko areas showed the highest ($p<0.01$) values compared to Zinvié area for all the parameters. Methane production ranked the three areas as follows: Adounko with high stocking rate > Zinvié with low stocking rate > Gakpé with moderate stocking rate.

Regarding the end-products after 24 hours of incubation (Table 5), the total VFA were clearly affected by the main factors considered (substrate, area, interaction), whereas the other parameters (e.g. individual acids, BCFA, pH) showed a different trend. In particular, *D. aegyptium* showed the highest ($p<0.01$) production of total VFA in Zinvié area compared to the other areas, mainly represented by acetate and propionate; on the contrary, samples of *L. caerulescens* collected in Adounko and Gakpé areas showed the highest ($p<0.01$) values for these parameters. The two species also showed differences among areas for branched chain fatty acids; in particular, for *Dactyloctenium* iso-butyrate and iso-valerate values were the lowest in Gakpé, whereas for *Leptochloa* these parameters showed the highest values in this area. In addition, butyrate production resulted significantly higher in Adounko area for *D. aegyptium* and in Gakpé area for *L. caerulescens.*

Table 6 presents the significance of correlation between some chemical parameters and in vitro fermentation data such as OM degradability and tVFA.

Table 2. Farmers' perceptions of land use pressure resulting of focus group discussions.

Pressure perceived		Zinvié	Gakpé	Adounko	Chi²	P value
Urban infrastructures extension					33.83	0.001
	1	25.0	83.3	16.7		
	2	75.0	8.3	8.3		
	3	8.3	8.3	75.0		
Cultivated land extension					29.61	0.001
	1	16.7	25.0	0.0		
	2	75.0	0.0	0.0		
	3	8.3	75.5	100		
Reducing grazing areas					29.43	0.001
	1	83.3	8.3	0		
	2	16.7	58.3	25.0		
	3	0.0	33.3	75.0		
Cattle herds concentration					12.15	0.016
	1	33.3	16.7	8.3		
	2	58.3	66.7	25.0		
	3	8.3	16.7	66.7		
Resulting perceived stocking rate		1.65^{c}	2.00^{b}	2.73^{a}		

Pressure estimation score: 1 = low; 2 = medium; 3 = high (focus group results).
^{a,b,c}Significant differences between means on the same line at $p<0.05$; Kruskal–Wallis test.

Table 3. Chemical composition and nutritive value of the two forages in the three areas.

Areas	DM	CP	NDF	ADF	ADL	EE	Ash	ME
	%			*% DM*				*MJ/kg DM*
				Dactyloctenium aegyptium L.				
Zinvié	89.41^{c}	12.52^{a}	63.60^{b}	45.36	8.69^{a}	1.36	11.62^{a}	10.45^{A}
Gakpé	90.00^{b}	8.66^{b}	71.29^{a}	45.06	5.86^{b}	1.54	10.57^{b}	7.97^{B}
Adounko	91.69^{a}	7.94^{c}	62.00^{b}	44.70	7.33^{ab}	1.73	11.36^{a}	7.54^{B}
				Leptochloa caerulescens L.				
Zinvié	88.55^{c}	7.62^{a}	70.50^{b}	47.01	6.17^{a}	2.25	11.08^{a}	7.00
Gakpé	92.65^{a}	7.24^{a}	70.30^{b}	41.51	6.44^{a}	1.18	9.55^{b}	7.11
Adounko	91.30^{b}	5.51^{b}	74.85^{a}	46.76	4.66^{b}	1.27	8.91^{c}	6.43
Substrate	***	***	***	NS	**	NS	***	**
Area	***	***	***	NS	*	NS	***	**
Interaction	***	***	***	NS	*	NS	***	**
MSE	0.18	0.025	0.82	2.27	0.45	0.16	0.22	0.315

DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; EE: ether extract; ME: metabolizable energy. MSE: mean square error. Along the column, for each fodder, different letters indicate statistically significant differences at 5%. NS, *, **, ***: not significant, $p<0.05$, $p<0.01$, $p<0.001$.

DISCUSSION

Stocking rate is the simplest parameter for evaluating the grazing areas management strategies in livestock farms. However, its evaluation as number of grazing animals per grazing area unit remains difficult in grassland-based systems where unrestricted open natural vegetation is grazed. One alternative is farmers' self-evaluation of their production factors' quality or suitability for their production objectives. Farmers' perception is widely used for assessing rangeland degradation (Solomon *et al.*, 2007; Wu *et*

al., 2015). In particular, Messing and Fagerström (2011) acknowledged appropriateness of farmer's evaluation of physical land quality for their production objectives. The farmers' evaluation in this study corroborated results reported by Koura *et al*. (2015) regarding increasing trends of infrastructures and houses, and of ruminant herd number and size in Adounko and Zinvié areas. Thus, the participatory approach was useful to evaluate pressure on grazing lands for stocking rate assessment.

The chemical composition and *in vitro* fermentation characteristics at 24 hours, including VFA and CH_4 production, of the two forage species (*Dactyloctenium aegyptium* L. and *Leptochloa caerulescens* L.) varied quite in the three sampling areas of the peri-urban zone of Cotonou, suggesting that sampling sites different for soil characteristics, pasture management and environment conditions affect nutrient accumulation in these forages (Adjolohoun *et al*., 2013).

Table 4. *In vitro* fermentation characteristics at 24 h of the two forages in the three areas.

Areas	OMD	GP24	pCH_4	iCH_4	dCH_4
	%	*ml/g*	*%*	*ml/g*	*ml/g*
			Dactyloctenium aegyptium L.		
Zinvié	42.42^{A}	100.80	10.73^{AB}	11.51	27.30^{Bb}
Gakpé	32.19^{Ba}	103.95	10.10^{B}	10.73	33.11^{Ba}
Adounko	28.91^{Bb}	101.10	11.43^{A}	11.90	44.74^{A}
			Leptochloa caerulescens L.		
Zinvié	16.54^{B}	88.05	7.63^{C}	5.63^{B}	34.17^{b}
Gakpé	24.82^{Ab}	98.05	11.16^{A}	10.21^{A}	39.99^{a}
Adounko	27.39^{Aa}	103.05	9.90^{B}	10.43^{A}	37.58^{ab}
Substrate	***	NS	***	**	NS
Area	NS	NS	**	**	**
Interaction	***	NS	***	**	**
MSE	2.60	56.9	0.112	0.64	5.34

OMD: organic matter degradability; GP24: gas produced after 24 hours of incubation; pCH_4: methane production expressed as a percentage of the total gas; iCH_4: methane production related to the incubated OM; dCH_4: production of methane related to the degraded OM. MSE: mean square error. Along the column, for each fodder, different letters indicate statistically significant differences at 5% and 1%. NS, **, ***: not significant, $p<0.01$, $p<0.001$.

Table 5. Volatile fatty acids and pH recorded after 24 h of *in vitro* incubation

Areas	pH	Acetate	Propionate	Iso-Butyrate	Butyrate	Iso-Valerate	Valerate	BCFA	tVFA
					mM/g iOM				
					Dactyloctenium aegyptium L.				
Zinvié	6.45^{Bc}	71.95^{A}	18.43^{A}	0.63^{a}	5.58^{B}	0.99	1.22^{A}	0.023	98.79^{A}
Gakpé	6.59^{Ab}	54.88^{B}	15.61^{B}	0.51^{b}	4.95^{B}	0.75	0.63^{B}	0.018	77.34^{B}
Adounko	6.70^{Aa}	58.57^{B}	17.49^{AB}	0.63^{a}	6.89^{A}	1.05	1.04^{A}	0.024	85.67^{B}
					Leptochloa caerulescens L.				
Zinvié	6.74	45.43^{Bb}	11.90^{B}	0.50^{B}	4.02^{C}	0.80^{B}	0.49^{B}	0.021^{B}	63.14^{B}
Gakpé	6.76	53.70^{Ba}	13.63^{B}	0.72^{A}	7.62^{A}	1.28^{A}	1.06^{A}	0.030^{A}	77.99^{A}
Adounko	6.72	57.97^{A}	16.23^{A}	0.57^{B}	5.90^{B}	0.94^{AB}	0.65^{B}	0.020^{B}	82.25^{A}
Substrate	***	***	***	***	NS	NS	NS	**	**
Area	*	*	NS	**	NS	***	NS	NS	**
Interaction	***	**	***	**	***	***	***	***	**
MSE	0.0034	19.6	1.63	0.034	0.26	0.025	0.027	0.00017	34.9

tVFA: total volatile fatty acids; BCFA: branched chain fatty acids. MSE: mean square error. Along the column, for each fodder, different letters indicate statistically significant differences at 5% and 1%. NS, *, **, ***: not significant, $p<0.05$, $p<0.01$, $p<0.001$.

Table 6. Significance of correlation between some chemical parameters and *in vitro* fermentation data.

	OMD %	tVFA
CP	0.75 **	0.62 *
NDF	-0.54 NS	-0.50 NS
ADF	-0.12 NS	-0.07 NS
ADL	-0.61 *	0.59 *
EE	-0.37 NS	-0.44 NS
ME	0.83 **	0.72 **

CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; EE: ether extract; ME: metabolizable energy; OMD: organic matter degradability; tVFA: total volatile fatty acids.
NS, *, **: not significant, $p<0.05$, $p<0.01$.

As reported by previous studies (Koura *et al*., 2015), the Adounko area, because it is subjected to high land use pressure, is characterized by degradation of pasture and lost in biodiversity of forage species, with also low yields and vegetative development of plants due to the sandy-soil. In Gakpé and Zinvié areas where bigger lands size is available, agricultural pressure is still a limitative factor. The results obtained on nutritional characteristics and methane production for *Dactyloctenium aegyptium* are partially agree with the data reported in literature (Bezabih *et al*., 2013). In contrast, only few comparisons are possible for *Leptochloa caerulescens* which had received less scientific attention.

Chemical composition

It is known that, during the dry season, forage roughages which are poor-quality feed can be valorized by ruminants when other resources are not available (Alexandre and Mandonnet, 2005). The crude protein values of the forages investigated were quite low (8.24% DM), when compared to results reported by Musco *et al*. (2016) for cultivated forage in Guinean areas (16.10% DM). On the other hand, these findings partially agree with the data of Babatounde *et al*. (2011) in forage consumed by sheep in the humid tropic of West Africa (7.4-15.9% DM). Moreover, lower value of crude protein (9.71% DM) and higher of structural carbohydrates (65.63% DM) content was found in *Dactyloctenium aegyptium* in all the studied areas, Adounko, Gakpé and Zinvié compared with data reported in literature for forages grown in semi-arid areas (Tefera *et al*., 2009; Bezabih *et al*., 2013). This suggests that a high depreciation in quality can be found in tropical forage due to the dry season; the tested forages' samples were collected in the dry season. According to McDonald *et al*. (2010), the low protein contents was also inherent to characteristic of C_4 plant metabolism, which is associated with survival under low soil fertility conditions. The high values of lignin, found especially in forages collected in Adounko and Zinvié areas, may have decreased the OM digestibility. Energy content follows the trend volatile fatty acids produced *in vitro*, indicating that most of energy produced comes from the degradation in the rumen (Gemeda and Hassen, 2015). *Leptochloa caerulescens* showed lower a nutritive value, in terms of crude protein (6.79% DM) and structural carbohydrates (71.88% DM) compared to *D. aegyptium*. The high level of structural carbohydrates could negatively affect the dry matter intake (Van Soest *et al*., 1991). Comparing the three areas, significant differences emerged between Zinvié and Gakpé vs. Adounko for crude protein and NDF, even if no significant differences emerged in nutritive value. The gas production recorded after 24 hours of incubation have influenced this trend, in fact both GP24 and CP values are considered in Menke formula.

Comparing the studied areas, for both forages, crude protein level and energy value were quite high in Zinvié and Gakpé, characterized by a lower land pressure compared to Adounko, whereas for structural carbohydrates the trend was not linear in the two forages. Difference in plant types as well as their adaptation to the areas, due to soil characteristics and fertility levels, could explain these values (Adjolohoun *et al*., 2008). Indeed, hydromorphic soils (Adounko and Zinvié) are known to have higher fertility level than ferralitic (Gakpé) and sandy (Akoundo) soils (Igue *et al*., 2013).

***In vitro* fermentation characteristics**

A significant influence of the sampling area on the *in vitro* fermentation characteristics of the tested forage was found in the current study. As, it can be expected, OM degradability increases when the structural carbohydrate content decreases in *Dactyloctenium aegyptium*. On the contrary, in *Leptochloa caerulescens*, the results appeared to be different and can be justified by type of environment in which the plant grows, a wet area of coastal lagoon in Adounko, and lakes environment in Gakpé and Zinvié. In general, the total volatile fatty acids produced, as well as results for the singular acids, were quite close to the values generally obtained in tropical grasses species in Guinean areas (Musco *et al*., 2016). In addition, our findings revealed that the total VFA produced followed the trend of energy content in all the samples,

confirming the connections between both these two parameters, as also found in tropical grasses species by the same author. On the other hand, OM degradability is significantly ($p<0.05$) correlated with some chemical characteristics (CP: $r = 0.75$, ADL: $r = -0.61$), confirming negative influence of cell wall fractions on this parameter (Calabrò *et al*., 2007; Musco *et al*., 2016).

Methane production

Regarding methane emission, the information is still limited on tropical forage species, however this aspect should be of a scientific interest mainly in peri-urban areas, where the number of cattle farms is increasing to the disadvantage of grazing areas. Apart from the investigation of Bezabih *et al*. (2013), very low is known on greenhouse gases emission of the forage species tested in this study. In particular, these authors had investigated methane emission in some forages, including *Dactyloctenium*, in semi-arid area of Ethiopia. Our results, partially in agreement with their findings in terms of chemical composition, show quite low values for OM degradability and CH_4 emission, probably because in this study the fermentation was stopped at 24 hours. Our data on CH_4 were quite close to results of Grazioli *et al*. (2016) in Mediterranean hay, where also high structural carbohydrates content was found. *Dactyloctenium*, the forage species from sandbanks, when collected in Zinvié savannah, showed a good nutritional potentiality and was the least negatively impacting on the environment. The samples of *Leptochloa* from Zinvié and Gakpé had the best nutritional potentiality but showed the highest CH_4 emission when it was collected in Gakpé and Adounko areas. As a whole, Zinvié seems the most interesting areas for using both forages, while Adounko low-lands close to the urban centers could be considered as the least suitable.

The high CH_4 emissions in Adounko area for *Dactyloctenium* and in Gakpé area for *Leptochloa*, can be related to the poor nutritional quality of the forage species found in these areas. Indeed, compared to Zinvié, the sandy soil in Adounko with the high pressure of herds on the pasture and the lateritic soil in Gakpé with the high agricultural pressure are probable causes of soil fertility reduction, and worsen the growth conditions, respectively for *Dactyloctenium*, a drought resistant species, and *Leptochloa*, a wetland species. These findings suggest that unfavorable agronomic conditions increase methane emissions from plants.

Irrespective of species, samples collected in Zinvié savannah had the highest nutritional values; this was also reflected in their low methane emission. This result indicates that in favorable agronomic conditions, characterized by less pressure on grasslands, with less growth stress in the forage, the nutritional value can be high also with a low environmental impact. High structural carbohydrates content in *Leptochloa* can also explain the quite low CH_4 emissions found in Adounko brackish water and in Gakpé lateritic soil. These results in methane production for both forages from different areas, were therefore quite different, indicating as found by Hook *et al*. (2010), that CH_4 emissions vary based on many factors, including the animal (i.e. species, physiological stage, feed intake, etc.), the feed (i.e. composition, quality, processing, etc.) and the geographical location.

CONCLUSIONS

This research revealed variations in terms of chemical composition, *in vitro* fermentation characteristics, volatile fatty acids and methane production for the two forage species collected in areas with different grazing management characteristics. The obtained results highlight the significant influence of urbanization and herd concentration, as well as of other environmental conditions, on the nutritional value and potential environmental impact of the two agricultural weeds during the dry season. Considering these aspects, the areas can be ranked as follows: Zinvié > Gakpé > Adounko. Arguing that extensive cattle production on communal pasture in low-land is not suitable as strategies for sustainable land use in peri-urban livestock production. The use of agricultural by-products could be a solution to enhance the nutritional value of the diets ingested by the grazing ruminants. Further *in vivo* studies are necessary to confirm these results, especially in terms of *in vivo* evaluation of methane production of these forages and soil characterization of the different areas.

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Compliance with ethical standards. All procedures involved animals were approved by the Ethical Animal Care and Use Committee of the University of Napoli Federico II (Prot. 2019/0013729 of 08/02/2019).

Data availability. Data are available with Nadia Musco, nadia.musco@unina.it upon reasonable request.

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