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In vitro fermentative characteristics and chemical quality of Guinea grass with organic and chemical fertilization

Claudia Yanet Wilson-García[1], Paulino Sánchez-Santillán[2*], Nelson Erik López-Zerón[3], Iván Efrén Domínguez-Rodríguez[2], Marco-Antonio Ayala-Monter[2], Nicolás Torres-Salado[2], José Luis Valenzuela Lagarda[4]

[1] Universidad Autónoma Chapingo, San Luis Acatlán, Carr. San Luis Acatlán-Tlapa de Comonfort Km 5, San Luis Acatlán, Guerrero, México, C.P. 41600.
[2] Universidad Autónoma de Guerrero, Facultad de Medicina Veterinaria y Zootecnia No. 2, Carr. Acapulco-Pinotepa Nacional Km 197, Cuajinicuilapa, Guerrero, México, C.P. 41940.
[3] Centro de Bachillerato Tecnológico Agropecuario 178, Carr. San Luis Acatlán Horcasitas Km 0.5, San Luis Acatlán, Guerrero, México, C.P. 41600.
[4] Centro Regional de Educación Superior de la Costa Chica, Universidad Autónoma de Guerrero, C 6 de marzo s/n, Cruz Grande, Guerrero, México, C.P. 41800.
* Correspondence: sanchezsantillanp@gmail.com

ABSTRACT

Objective: To evaluate the chemical quality and *in vitro* fermentative characteristics of *Panicum maximum* cv. Guinea grass, in order to determine its optimum cutting point under four fertilization schemes.

Methodology: Guinea grass was fertilized with chemicals (F1), vermicompost (F2), compost (F3), and compost + leachate (F4). The grass was cut at 20, 35, 50, 50, 65, 80, and 105 days. The neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP) contents were determined, along with the dry matter (DMD), NDF (NDFD), and ADF (ADFD) degradations, as well as the *in vitro* gas production. The experimental design was a 4×6 factorial arrangement within a completely randomized design; fertilization and cutting days were used as factors.

Results: F3-65 d, F1-65 d, F4-80 d, and F1-8 d had higher NDF content; F1-65 d, higher ADF; F1-20 d, higher CP ($p \leq 0.05$); F4-50 d and F2-50 d, higher gas production; F4-20, F4-35, F4-50, F2-20, F2-35, F1-20 d, higher DMD, F2-20 and F4-20 d, higher NDFD; F4-20, F4-35, F4-65, and F2-20 d, higher ADFD ($p \leq 0.05$).

Limitations/Implications: The lack of previous research studies about the organic fertilization of Guinea grass.

Conclusions: Fertilization with vermicompost or compost + leachate improves chemical content, *in vitro* gas production, and degradation of Guinea grass.

Key words: *Panicum maximum*, *in vitro* degradation, *in vitro* gas, bromatological.

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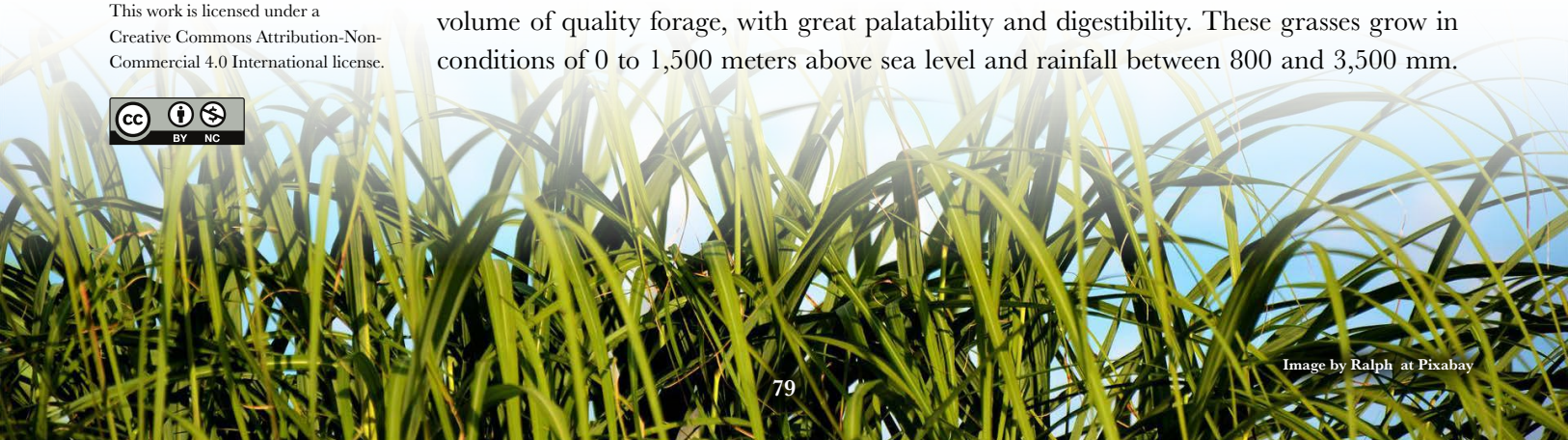
INTRODUCTION

Panicum maximum species are tolerant to trampling and drought. They produce a high volume of quality forage, with great palatability and digestibility. These grasses grow in conditions of 0 to 1,500 meters above sea level and rainfall between 800 and 3,500 mm.



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They need medium to high fertility, well-drained soils with a pH of 5 to 8 and have a tiller growth system. In addition, its biomass productivity and nutritional quality make *P. maximun* a forage alternative for ruminants (Andrade-Solórzano *et al.*, 2020).

The *in vitro* gas production method uses volume measurement to assess the rate and degree of fermentation of forages for ruminants. *In vitro* ruminal fermentation systems are adapted to forage feeding conditions; consequently, the gas produced is directly related to microbial fermentation (Amanzougarene and Fondevila, 2020). The objective of this research was to evaluate the chemical quality and biogas production of *Panicum maximum* cv. Guinea grass under four fertilization arrangements.

MATERIALS AND METHODS

Experimental Site

The work was carried out at the Facultad de Medicina Veterinaria y Zootecnia No. 2 of the Universidad Autónoma de Guerrero, located in the municipality of Cuajinicuilapa, Guerrero.

Forage and Fertilization

The ground was harrowed twice. *Panicum maximum* cv. Guinea grass was established in August 2017. Three stripes were established 1 m apart from each other. Each row included four rows separated by 20 cm. The sowing density was 6 kg ha^{-1}. The following fertilization arrangements were used: F1=inorganic, 120-60-00 of NPK; F2=application of 10 t ha^{-1} vermicompost; F3=10 t ha^{-1} compost; and F4=10 t compost + three applications of a 20% leachate dose, at 7-day intervals, using a backpack sprayer with a 40-pound pressure adjustable nozzle. The samplings were carried out 20, 35, 50, 65, 80, and 105 days after the homogenization cut.

In vitro gas production

For gas measurement, biodigesters (experimental unit) were prepared using a 120-mL serological pipette, following the method described by Torres-Salado *et al.* (2019) and using Guinea grass with different fertilization at each cutting age. Accumulated biogas production was measured at 2, 4, 6, 8, 10, 12, 24, 48, and 72 h as described by Hernández-Morales *et al.* (2018). At 72 h of incubation, the content of the biodigesters was filtered into ANKOM® bags and the dry matter (DMD), neutral detergent fiber (NDFD), acid detergent fiber (ADFD) degradations were determined, according to the methodology proposed by Hernández- Morales *et al.* (2018).

Chemical analysis

On the one hand, the crude protein (CP) and ashes (As) contents of Guinea grass —with different fertilizations at each cutting age— were determined following the methods described by the AOAC (2005). On the other hand, the neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined with the method described by Van Soest *et al.* (1991).

Statistical analysis

The variables of the chemical analysis and *in vitro* gas production were examined with the SAS GLM procedure (SAS Institute Inc, 2011), in a completely randomized design with a 6×4 factorial arrangement, considering the cutting days (20, 35, 50, 65, 80, and 95 days) and the type of fertilization (F1, F2, F3, and F4) as factors. The comparison of means was carried out with the Tukey test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Fertilizing grasses with nitrogen affect the chemical quality of forages. Additionally, it causes productive and environmental damage. Consequently, organic fertilization is an agroecological alternative for the improvement of the chemical quality of forages (Álvarez *et al.*, 2016). The highest NDF contents were observed in the F3-65, F1-65, F4-80, and F1-80 d interactions, while in ADF there were achieved with F1-65 d (Table 1). These high NDF levels are observed at these ages, because, as the grass grows, it develops a support structure mainly composed of lignin and cellulose (Gándara *et al.*, 2017), components of NDF and ADF. The highest CP content was recorded in F1-20 d and the highest values occurred in the first 20 d (Table 1): as a forage ages, its CP content decreases, because the nitrogen concentration declines, as the tissues mature and less protein compounds are synthesized (Gándara *et al.*, 2017).

Regarding the cutting age, no differences between fertilizations are identified at 20 and 35 d, but a trend towards a difference starts as the grass grows older, since the use of biological fertilizers (F2, F3, and F4) decreases the detergent fiber content. In contrast, the protein content does not show a clear difference between F1 (which shows the highest values) and the rest of the biological fertilizations (F2, F3, and F4) at different cutting ages (Table 1). Sánchez-Santillan *et al.* (2021) reported lower values for aruana grass than in this study, at similar cutting ages and using the same fertilizers. They reported 17.4% CP at 20 d of cutting with chemical fertilization, 79.9% NDF at 65 d with vermicompost fertilization, and 46.8% ADF at 95 d with chemical fertilization. It should not be assumed that these differences in chemical content are only related to the type of fertilization, but that agronomic management also interferes with the chemical content of Guinea (Table 1) and aruana (Sánchez-Santillán *et al.*, 2021) grasses.

The *in vitro* gas production method is used to evaluate the fermentation rate and degree of ruminant feeds, because it is correlated with *in vivo* parameters (Amanzougarene and Fondevila, 2020). Therefore, it has been used in the present study to infer the microbial digestion of fertilizations on different cutting days and their gas production (mainly carbon dioxide and methane) as final catabolites of fermentation (Amanzougarene and Fondevila, 2020). Therefore, F4 and F2, both at 50 d of cutting, showed the highest gas production ($p \leq 0.05$) in the evaluated hours (Table 2). However, at each measurement hour, there are no differences ($p > 0.05$) between fertilizations on the different cutting days (Table 2). Overall, Table 2 shows a great variability of gas production in each measurement hour; this is assumed to be caused by the chemical nature of carbohydrates, since gas production is the result of the fermentation of acetate, propionate, and butyrate (Amanzougarene and Fondevila, 2020). Values lower than the interactions recorded in the present study

Table 1. Chemical analysis of Guinea grass at different regrowth age under different chemical and organic fertilization scheme.

Fertilization	Age (days)	NDF (%)	ADF (%)	Hemi (%)	CP (%)	Ash (%)	OM (%)
F1	20	64.6 h	34.6 l	30.0 defgh	20.3 a	12.8 ab	87.2 lm
F2	20	65.3 h	36.6 k	28.7 ghij	10.9 d	13.6 a	86.4 m
F3	20	71.6 g	39.6 j	32.0 ab	9.9 e	12.0 bcd	88.0 jkl
F4	20	64.3 h	35.0 kl	29.3 fghij	13.6 b	11.8 cde	88.2 ijk
F1	35	74.4 def	42.2 ghi	32.2 ab	12.5 c	11.0 ef	89.1 hi
F2	35	72.9 efg	41.9 hi	31.1 bcde	8.0 fg	11.6 cde	88.4 ijk
F3	35	75.1 cde	43.9 efg	31.1 bcde	6.8 hi	10.9 ef	89.1 hi
F4	35	73.4 efg	40.4 ij	32.9 a	7.0 gh	7.3 l	92.7 b
F1	50	78.6 b	46.8 cd	31.8 abc	8.6 f	8.7 ijk	91.3 cde
F2	50	71.3 g	40.5 ij	30.8 bcdef	5.7 jkl	12.2 bc	87.8 kl
F3	50	77.6 bc	46.8 cd	30.8 bcdef	5.2 jklm	9.7 gh	90.3 fg
F4	50	75.0 cde	42.8 fgh	32.2 ab	7.1 gh	6.2 m	93.8 a
F1	65	81.5 a	52.9 a	28.6 ghij	6.2 hij	8.1 jkl	91.9 bcd
F2	65	75.2 cde	44.1 efg	31.2 bcde	6.0 ijk	9.0 hij	91.0 def
F3	65	79.0 ab	47.6 c	31.5 abcd	5.8 ijk	9.0 hij	91.0 def
F4	65	70.8 g	42.7 fgh	28.0 ij	5.9 ijk	10.0 fg	90.0 gh
F1	80	79.2 ab	50.4 b	28.8 ghij	5.3 jklm	7.8 kl	92.2 bc
F2	80	74.6 de	43.4 efgh	31.2 abcde	5.9 ijk	9.0 hij	91.0 def
F3	60	77.9 b	48.2 c	29.7 efghi	4.8 lmn	9.4 ghi	90.6 efg
F4	80	71.8 fg	44.1 efg	27.6 j	6.0 ijk	11.1 e	88.9 i
F1	95	76.5 bcd	48.2 c	28.3 hij	4.3 n	9.4 ghi	90.6 efg
F2	95	74.8 de	45.0 de	29.8 defgh	5.2 klmn	9.1 ghi	90.9 efg
F3	95	79.0 ab	50.6 b	28.4 hij	4.4 mn	8.9 hij	91.1 def
F4	95	74.6 de	44.4 ef	30.3 cdefg	5.8 ijk	11.1 de	88.9 ij
SEM		0.54	0.55	0.18	0.43	0.21	0.21

Different literals within each column indicate significant difference ($p \leq 0.05$).
NDF=neutral detergent fiber; ADF=acid detergent fiber; Hemi=hemicellulose; CP=crude protein; OM=organic matter; SEM=standard error of the mean.

(Table 2) were reported by Sánchez-Santillan *et al.* (2021), who fertilized aruana grass with vermicompost, compost, compost + leachate, and chemical fertilizers, at 20, 35, 50, 65, and 90 d of cut.

The determination of *in vivo* forage digestibility is expensive and laborious, it harms animal welfare, and is unsuitable for routine analysis; therefore, the *in vitro* method correlates well with *in vivo* digestibility (Gosselink *et al.*, 2004). The highest DMD ($p \leq 0.05$) was recorded with F4 at 20, 35, and 50 d; F2 at 20 and 35 d; and F1 at 20 d, without differences between them ($p > 0.05$; Table 3). NDFD is used to predict the energy content of the evaluated substrate and animal performance (Hoffman *et al.*, 2006), as well as to improve the prediction of animal weight gain measurement (Hoffman *et al.*, 2007). Therefore, Guinea grass could be considered to be in a vegetative state, with F2 and F4

Table 2. Cumulative biogas production (mL g^{-1} DM) of Guinea grass at different regrowth age under different chemical and organic fertilization schemes.

Fertilization	Age (day)	2 h	4 h	6 h	8 h	10 h	12 h	24 h	48 h	72 h
F1	20	21 hij	40 ef	40 i	46 h	51 jk	56 gh	87 h	107 gh	153 efgh
F2	20	46 a	57 a	63 abcde	73 abc	85 ab	99 a	140 ab	177 ab	219 a
F3	20	36 bcdef	49 cd	52 h	58 fg	65 ghi	74 def	97 fgh	119 efgh	147 gh
F4	20	33 cdefg	50 bcd	58 defgh	67 bcde	75 cde	84 cde	132 abcd	170 abc	206 abc
F1	35	12 k	29 g	29 j	32 i	40 l	41 i	88 h	114 fgh	150 fgh
F2	35	40 abc	52 abcd	61 bcdef	69 abcd	81 abc	92 abc	140 ab	185 a	216 a
F3	35	37 abcdef	49 cd	52 h	58 fg	67 efghi	77 de	113 defg	129 defg	152 efgh
F4	35	31 defg	52 abcd	66 abc	75 ab	88 a	99 a	141 ab	179 ab	223 a
F1	50	18 jk	37 f	37 i	40 h	47 k	51 hi	91 gh	111 fgh	148 gh
F2	50	45 a	57 ab	67 ab	76 a	87 a	101 a	146 a	184 a	208 abc
F3	50	38 abcde	51 abcd	52 h	58 fg	64 hi	77 de	128 abcde	150 bcde	166 defg
F4	50	42 ab	57 a	68 a	76 a	88 a	97 ab	138 abc	159 abcd	212 ab
F1	65	19 ijk	35 fg	35 ij	40 h	46 k	49 hi	53 i	67 i	107 i
F2	65	39 abcd	52 abcd	58 efgh	63 def	68 defghi	74 def	106 efgh	127 defg	155 efgh
F3	65	37 abcde	50 abcd	54 fgh	61 efg	68 defghi	77 de	116 cdef	137 defg	157 efgh
F4	65	41 abc	50 bcd	60 cdefg	65 cdef	72 cdefgh	80 cde	122 bcde	155 abcd	195 abcd
F1	80	28 fghi	50 bcd	51 h	54 g	66 fghi	72 ef	84 h	94 hi	104 i
F2	80	35 bcdefg	55 abc	65 abcd	72 abc	74 cdefg	86 bcd	122 bcde	142 cdef	171 defg
F3	80	35 bcdefg	51 abcd	60 cdefg	67 bcde	77 bcd	92 abc	121 bcde	150 bcde	182 bcde
F4	80	26 ghij	46 de	56 efgh	67 bcde	75 cdef	78 de	114 def	147 bcde	181 bcdef
F1	95	32 cdefg	51 abcd	52 h	58 fg	60 ij	64 fg	94 fgh	92 hi	128 hi
F2	95	26 ghij	49 cd	57 efgh	66 cdef	74 cdefg	83 cde	124 abcde	139 cdefg	171 defg
F3	95	30 efgh	48 cd	53 gh	60 efg	72 cdefgh	82 cde	111 defg	154 abcd	180 cdef
F4	95	18 jk	40 ef	52 h	60 efg	68 defghi	72 ef	113 defg	134 defg	172 defg
SEM		1.0	0.7	1.0	1.2	1.3	1.7	2.4	3.3	3.5

Different literals within each column indicate significant difference ($p \leq 0.05$).
SEM=standard error of the mean.

at 20 d of cutting (Hoffman *et al.*, 2007), since they presented degradations greater than 70% (Table 3). Maturity, growth conditions, and forage management at cutting and after cutting are assumed to be responsible for the variability of the NDFD values (Reuss, 2001). Sánchez-Santillan *et al.* (2021) reported higher DMD and NDFD values in aruana grass, fertilized with compost + leachate at 20, 35, and 50 days of cutting, than in the present study (Table 3).

Identifying factors that limit cell wall degradation is a complex process (Ramírez *et al.*, 2002). Consequently, the ADFD refers to the portion of the cell wall composed of cellulose and lignin, which are associated with the ability to digest forage. Therefore, the highest values were F4 at 20, 35, and 65 d and F2 at 20 d ($p \leq 0.05$; Table 3). Lower ADFD values were reported in Bermudagrass (*Cynodon dactylon*), Mulato II grass (*Brachiaria*

hybrid), Palisade grass (*Brachiaria brizantha*), Star grass (*Cynodon nlemfuensis*), Quackgrass (*Elytrichia repens*), Gamba grass (*Andropogon gayanus*), Guinea grass (*Panicum maximun*), Para grass (*Brachiaria mutica*), and Pangola grass (*Digitaria decumbens*), with 56 days of regrowth and without any fertilization (Almaraz-Buendía *et al.*, 2019); for its part, Guinea grass was fertilized with vermicompost and compost + leachate at 35 and 50 d (Table 3).

From another perspective, the Guinea grass with 20 d had the greatest degradations as a result of its chemical content, which stands out for its lower amount of cellulose and lignin and greater amount of cellular content. This is assumed to be associated with the maturity degree of the cut; as the plant matures, it undergoes physiological changes and develops the xylem tissue for water transport, accumulates cellulose, and begins the lignification process, resulting in a cell wall that is difficult for ruminal bacteria to adhere to and to digest (Hoffman *et al.*, 2007).

Table 3. Degradations of dry matter and detergent fibers of Guinea grass at different regrowth age under different chemical and organic fertilization schemes.

Fertilization	Age (days)	DMD (%)	NDFD (%)	ADFD (%)
F1	20	63.39 abc	61.62 ab	59.75 abcd
F2	20	73.25 a	70.18 a	69.45 a
F3	20	38.23 fghi	28.72 cde	25.11 jk
F4	20	71.73 ab	70.61 a	68.81 a
F1	35	50.51 cdefg	47.32 abcde	44.05 efghi
F2	35	62.46 abc	35.6 bcde	58.56 abcde
F3	35	34.79 ghi	26.88 de	25.19 jk
F4	35	64.55 abc	62.87 ab	64.41 ab
F1	50	43.22 defghi	38.52 abcde	37.7 ghij
F2	50	56.95 bcde	50.64 abcde	47.44 defgh
F3	50	41.26 efghi	32.66 bcde	28.57 jk
F4	50	58.71 abcd	55.98 abcd	56.51 abcde
F1	65	32.6 hi	26.7 de	27.34 jk
F2	65	42.14 efghi	33.98 bcde	29.51 ijk
F3	65	42.65 efghi	36.25 bcde	33.14 hijk
F4	65	56.01 bcde	61.27 abc	62.77 abc
F1	80	27.87 i	18.29 e	17.89 l
F2	80	43.09 defghi	34.76 bcde	28.66 jk
F3	80	53.76 cdef	49.67 abcde	48.78 cdefg
F4	80	53.61 cdef	48.11 abcde	53.35 bcdef
F1	95	31.47 hi	20.97 e	21.23 k
F2	95	46.29 defgh	39.79 abcde	38.79 fghij
F3	95	51.73 cdef	46.98 abcde	44.78 efgh
F4	95	52.75 cdef	47.72 abcde	49.68 bcdefg
SEM		1.53	2.01	1.91

Different literals within each column indicate significant difference ($p \leq 0.05$). DMD=dry matter degradation; NDFD=neutral detergent fiber degradation; ADFD=acid detergent fiber degradation; SEM = standard error of the mean.

CONCLUSIONS

Fertilization with biological products, such as vermicompost or compost + leachate, improve the chemical content, gas production, and *in vitro* degradation of Guinea grass with regard to chemical fertilization or compost. It has been confirmed that the maturity of grass decreases its protein content, increases the components of the cell wall, and decreases its degradation in *in vitro* tests.

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