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In vitro CHEMICAL AND FERMENTATIVE CHARACTERISTICS OF TROPICAL PASTURES AT DIFFERENT AGES OF REGROWTH

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ABSTRACT

The low nutritive value of pastures in tropical regions represents a limitation in the productivity of grazing ruminants. The objective was to determine the *in vitro* chemical and fermentative characteristics of nine tropical grasses (Aruana [*Panicum maximun* Jacq. cv. Aruana], Bermuda [*Cynodon dactylon* L.], Estrella de África [*Cynodon Cynodon nlemfuensis* Vanderyst], Insurgente [*Brachiaria brizantha* Hochst. Stapf.], Llanero [*Andropogon gayanus* Kunth], Mombaza [*Panicum maximun* Jacq. cv. Mombaza], Pará [*Brachiaria mutica* Stapf], Pangola [*Digitaria eriantha* Steud.], and Tanzania [*Panicum maximun* Jacq. cv. Tanzania]) at three regrowth ages (30, 45, and 60 days). The chemical analysis was used to determine the concentrations of dry matter (DM), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), and ash (Ce). The *in vitro* trial measured partial (24, 48, and 72 h) and cumulative biogas and methane production, fermentation kinetics estimators (*A*, *b*, and *k*), dry matter degradation (DMD) and detergent neutral fiber degradation (NDFD), pH, and ammonia nitrogen ($N\text{-}NH_3$). The experimental design was completely randomized at each regrowth age. In conclusion, based on chemical analysis and fermentative characteristics, Pangola and Estrella pastures performed better at each regrowth age.

Keywords: biogas, methane, crude protein, detergent fibers, degradations.

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INTRODUCTION

Mexico has 240 399 km^2 of dry tropics, of which 26 % is used for grazing (Enríquez-Quiroz *et al*., 2021; Torres-Torres and Rojas-Martínez, 2018). Forage production accounts for 33 % of total arable land (FIRCO, 2017). Grasses in Mexico are represented by 204 genera and 1182 species, 159 of which are cultivated or introduced (CONABIO, 2018). Grass is the primary source of food for ruminants in the tropics; therefore, its yield and nutritional quality are important to determine animal productivity in grazing systems. However, the best regrowth age is rarely considered when the highest nutritional value and maximum dry matter digestibility are to be found (Avellaneda-Avellaneda *et al*., 2020).

Grasslands in the tropics are characterized by native or introduced pastures with inadequate or no management, no paddock rotation, lack of knowledge of pasture nutrients at the time of grazing, and no control over the age of regrowth. This contributes to ruminants not receiving nutrients that tropical pastures could provide at an optimal regrowth age for grazing or cutting (Avellaneda-Avellaneda *et al*., 2020; Enríquez-Quiroz *et al*., 2021). Forages differ in structure, cell wall composition, plant species, anatomical parts, and cell wall stage. Therefore, studies are required to evaluate tropical forages nutritionally through chemical analysis, *in vitro* gas production tests, *in situ* and *in vivo* tests to characterize them, and to formulate diets or supplements that meet physiological requirements while remaining cost effective to optimize animal productivity (Almaraz-Buendía *et al*., 2019).
The hypothesis is that the *in vitro* chemical and fermentative characteristics of Aruana, Bermuda, Estrella, Insurgente, Llanero, Mombaza, Pará, Pangola, and Tanzania pastures vary at each regrowth age. Therefore, the objective was to determine the *in vitro* chemical and fermentative characteristics of nine tropical grasses at three different regrowth ages in order to find the best nutritional condition for their use in ruminant feeding by grazing or cutting.

MATERIALS AND METHODS

The research was carried out in the Animal Nutrition laboratory and zootechnical post of the Faculty of Veterinary Medicine and Zootechnics No. 2 of the Autonomous University of Guerrero. It is located in the municipality of Cuajinicuilapa, Guerrero, Mexico. The study was carried out from July to October 2019 (Table 1).

Table 1. Climatological conditions in the municipality of Cuajinicuilapa, Guerrero during the experimental period.

Month	Max. temperature (°C).	Min. temperature (°C).	Mean temperature (°C).	Rainfall (mm)	No. of irrigation
July	33.1	17.3	24.6	289	0
August	33.0	17.0	25.0	250	0
September	32.5	17.5	25.5	280	0
October	34.6	17.8	26.0	169	0

Pastures

The grass samples used were: Aruana (*Panicum maximun* Jacq. cv. Aruana), Bermuda (*Cynodon dactylon* L.), Estrella de África (*Cynodon Cynodon nlemfuensis* Vanderyst), Insurgente (*Brachiaria brizantha* Hochst. Stapf.), Llanero (*Andropogon gayanus* Kunth), Mombaza (*Panicum maximun* Jacq. cv. Mombaza), Pará (*Brachiaria mutica* Stapf), Pangola (*Digitaria eriantha* Steud.), and Tanzania (*Panicum maximun* Jacq. cv. Tanzania). The pastures were planted in 2 m^2 plots with no tillage, without fertilization. Sowing was done in rows (30 cm) using the cuttings or stakes technique at a distance of 20 cm between stakes and left for 30 d; the pastures were then cut to homogenize them and harvested at 30, 45, and 60 days after regrowth. The pastures were dried at 60 °C for 48 h in a naturally ventilated oven (Riossa® HCF-41, Mexico) to determine dry matter (DM, method 967.03) according to AOAC (2005) and ground to 1 mm size in a Thomas-Wiley Mill (Thomas Scientific®, Swedesboro, NJ, USA).

Chemical analysis

These analyses (3 independent samples) included crude protein (CP, method 920.105) and ash (Ce, method 942.05) using the procedures described by AOAC (2005). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the method described by van Soest *et al.* (1991). The organic matter (OM) content was quantified by subtracting ash from 100.

Culture medium and biodigesters

The culture medium was prepared as described by Herrera-Pérez *et al.* (2018). The biodigesters and their inoculation were carried out according to the methodology described by Torres-Salado *et al.* (2019), where the carbon source of the biodigester was changed to a pasture at 30, 45, or 60 d regrowth age.

In vitro biogas production and kinetics

Biogas production (5 independent samples) was measured by displacement of the plunger of a glass syringe (50 mL; BD Yale®, Brazil) as described by Hernández-Morales *et al.* (2018). Biogas production was partially reported at 24, 48, and 72 h, as well as the cumulative production at 72 h.

The cumulative biogas production values (5 independent samples) were used to estimate biogas production kinetics using the Gompertz model (Lavrenčič *et al.*, 1997). Nonlinear regression analysis was used to estimate the estimators A, b, and k using the PROC NLMIXED procedure of the SAS statistical package (2002).

In vitro methane production

Methane production (5 independent samples) was measured at 24, 48, and 72 h. A Taygon® hose (2.38 mm internal and 45 cm length) with hypodermic needles (20 G x 32 mm) at the ends was used to couple a biodigester with a trap vial filled with NaOH (2N) solution (Torres-Salado *et al.*, 2018). Methane production was partially reported at 24, 48, and 72 h, as well as the cumulative production at 72 h.

In vitro fermentative characteristics

The fermentative characteristics were determined *in vitro* after 72 h of incubation. For ammonia nitrogen ($N\text{-}NH_3$), the McCullough (1967) methodology, as modified by Herrera-Pérez *et al*. (2018), was followed. The culture medium was prepared as described by Herrera-Pérez *et al.* (2019). Dry matter degradation (DMD) and detergent neutral fiber (NDFD) were calculated using the methodology described by Hernández-Morales *et al.* (2018).

Statistical analysis

Chemical and *in vitro* assay variables were analyzed by age at regrowth with a completely randomized design using the GLM procedure of SAS (2002). The Tukey method was used for the mean comparison test ($p \leq 0.05$).

RESULTS AND DISCUSSION

The components of the nutritive value of forages are the chemical composition, digestibility, and their efficiency of utilization by the animal (Enriquez *et al.*, 2021). Therefore, in the present study, the chemical composition and fermentative characteristics were determined *in vitro*. At 30 d of regrowth, Bermuda and Pangola averaged 32.66 % DM; Bermuda, Estrella, and Pangola averaged 70.87 % NDF, respectively, representing the highest values for each nutrient ($p \leq 0.05$). Likewise, Tanzania showed a higher CP content, Aruana higher Ce, and Estrella higher OM ($p \leq 0.05$). At 45 d of regrowth, the highest nutrient contents were Bermuda in DM, Estrella in NDF and CP, and Llanero in OM. Aruana and Insurgente averaged 43.84 % of ADF, while Bermuda, Insurgente, Pará, and Tanzania averaged 8.94 % Ce, both nutrients having the most contents ($p \leq 0.05$) with no differences among pastures ($p > 0.05$). At 60 d of regrowth, Estrella exhibited the highest DM content, while Bermuda had the lowest CP content, with significant differences observed ($p \leq 0.05$). Additionally, Bermuda and Pangola demonstrated higher levels of NDF with an average of 73.54 %. These same grasses, along with Llanero, Tanzania, and Aruana, displayed elevated ADF content, averaging 43.73 %. Moreover, Aruana and Pangola exhibited OM content, averaging 92.80 % ($p \leq 0.05$) (Table 2).

In general, the evaluated pastures showed increased DM, NDF, and ADF contents and decreased CP content as regrowth days increased. This may be due to decreased biomass and nitrogen accumulation rates while carbon concentration increases, specifically through fibers (Gándara *et al*., 2017; Enriquez *et al*., 2021) due to the lignification process and xylem development, leading to cellulose and other complex carbohydrates accumulation (Maceda *et al*., 2019). Likewise, the variation in nutrient content of the pastures is assumed to be due to species and developmental stage (Reiné *et al*., 2020).

Bermuda, Estrella, Insurgente, Llanero, Mombaza, Pangola, and Pará pastures with 56 d of regrowth had an average content of 11.94, 72.27, 46.21, and 11.07 % of CP, NDF, ADF, and Ce, respectively (Almaraz-Buendía *et al*., 2019). When compared to the values obtained in this study at 60 days of regrowth, these values are lower in NDF, ADF, and Ce, and higher in CP. (Table 2). The DM values of the Tanzania pasture at each of the three regrowth ages (Table 2) are lower than those reported by Patiño-Pardo *et al*. (2018) at 35 d of regrowth (23.6 % DM). These authors published that Tanzania pasture with 25 d contained 11.7 % CP, while Polo (2021), on the same pasture with

Table 2. Chemical characteristics of nine tropical pastures at three regrowth ages.

Pasture	DM	NDF	ADF	CP	Ce	OM
			30 d of regrowth			
Aruana	21.55 cd	66.09 cd	34.55 b	8.42 bc	11.46 a	88.54 f
Bermuda	32.79 a	70.52 ab	33.73 bc	3.66 d	8.33 e	91.67 b
Estrella	28.73 b	70.00 ab	33.99 bc	9.15 bc	6.83 f	93.17 a
Insurgente	24.11 c	63.94 de	31.94 c	8.04 c	10.72 b	89.28 e
Llanero	25.02 bc	68.03 bc	34.26 bc	9.45 b	6.34 f	93.66 a
Mombaza	21.36 cd	67.36 c	37.59 a	8.8 bc	9.54 d	90.46 c
Pangola	32.52 a	72.10 a	35.61 ab	8.59 bc	9.42 d	90.58 c
Pará	18.96 de	62.83 e	33.33 bc	8.96 bc	10.18 c	89.82 d
Tanzania	17.06 e	64.54 de	34.30 b	13.92 a	10.30 bc	89.70 de
EEM	1.07	0.61	0.32	0.49	0.32	0.32
			45 d of regrowth			
Aruana	21.24 e	72.40 b	43.03 ab	2.86 e	8.33 bc	91.67 ab
Bermuda	32.37 a	68.29 c	33.23 e	6.64 bc	8.42 ab	91.58 bc
Estrella	30.59 b	76.79 a	42.10 bc	10.94 a	7.93 bc	92.07 ab
Insurgente	22.32 e	73.21 b	44.65 a	7.49 b	9.02 ab	90.98 bc
Llanero	24.48 d	67.80 cd	36.95 d	5.37 bcd	6.82 c	93.18 a
Mombaza	27.27 c	64.41 e	35.52 de	4.98 cde	7.93 bc	92.07 ab
Pangola	29.89 b	66.11 de	33.87 e	7.02 bc	8.11 bc	91.89 ab
Pará	24.51 d	64.60 e	35.38 de	4.83 cde	8.45 ab	91.55 bc
Tanzania	17.79 f	69.02 c	39.83 c	3.52 de	9.87 a	90.13 c
SEM	0.89	0.78	0.79	0.47	0.18	0.18
			60 d of regrowth			
Aruana	29.21 c	72.02 ab	45.00 a	1.00 b	7.34 de	92.66 ab
Bermuda	32.14 b	73.54 a	43.31 a	3.07 ab	16.51 a	83.49 e
Estrella	44.39 a	68.47 cd	35.03 d	3.80 ab	8.74 c	91.26 c
Insurgente	26.89 cd	66.28 d	34.46 d	2.78 ab	8.14 cd	91.86 bc
Llanero	22.37 f	71.06 abc	42.05 ab	3.44 ab	8.65 c	91.35 c
Mombaza	23.48 ef	68.68 bcd	39.90 bc	4.64 a	10.02 b	89.98 d
Pangola	33.37 b	73.53 a	43.40 a	4.37 a	7.05 e	92.95 a
Pará	24.92 de	66.10 d	37.64 cd	2.55 ab	9.85 b	90.15 d
Tanzania	22.69 ef	72.71 a	44.87 a	5.00 a	10.41 b	89.59 d
SEM	1.32	0.58	0.78	0.28	0.53	0.53

a,b,c,d,e Mean values per column with different letters are statistically different ($p \leq 0.05$). DM: percentage of dry matter; NDF: percentage of neutral detergent fiber; ADF: percentage of acid detergent fiber; CP: percentage of crude protein; Ce: percentage of ash; OM: percentage of organic matter; EEM: standard error of the mean.

30 d of regrowth, reported 7.9 % CP; therefore, the values obtained in this study are similar to Patio *et al*. (2018) and lower than Polo (2021) (Table 2).

The gas production technique is based on the proportional relationship between microbial digestion and the production of volatile fatty acids, resulting in the production of CO_2 and CH_4 as final catabolites of fermentation (Amanzougarene and Fondevila, 2020). Pangola produced the most biogas after 30 d of regrowth, Estrella and Pangola after 45 d, and Insurgente after 60 d ($p \leq 0.05$) (Table 3). The importance of

Table 3. Partial and cumulative *in vitro* biogas production of nine tropical pastures at three regrowth ages.

Pastures	Partial production (mL g^{-1} DM)			Accumulated (mL g^{-1} DM)
	24 h	48 h	72 h	
		30 d of regrowth		
Aruana	82.54 cd	18.51 b	42.42 cd	143.47 de
Bermuda	123.02 b	57.66 a	25.36 d	206.04 b
Estrella	98.72 c	50.12 a	26.17 d	175.01 c
Insurgente	70.08 de	30.03 b	56.97 abc	157.08 cde
Llanero	53.93 ef	67.79a	49.20 bc	170.91 c
Mombaza	51.67 ef	23.96 b	61.41 ab	137.04 de
Pangola	178.52 a	68.12 a	30.53 d	277.17 a
Pará	41.40 f	21.83 b	67.74 a	130.96 e
Tanzania	67.74 de	63.98 a	27.10 d	158.81 cd
SEM	8.06	4.03	3.21	8.49
		45 d of regrowth		
Aruana	155.00 c	62.60 b	28.32 bc	245.92 c
Bermuda	124.46 d	67.48 b	25.49 bc	217.43 de
Estrella	181.26 ab	77.33 a	26.61 bc	285.20 a
Insurgente	190.86 a	52.47 cd	28.14 bc	271.46 b
Llanero	110.58 e	58.93 bc	37.83 a	207.33 e
Mombaza	144.92 c	46.34 de	30.90 abc	222.16 d
Pangola	176.44 b	63.06 b	33.79 ab	273.30 ab
Pará	180.07 ab	42.07 e	24.35 c	246.48 c
Tanzania	145.15 c	59.43 bc	26.32 bc	230.91 d
SEM	5.17	2.05	0.94	5.13
		60 d of regrowth		
Aruana	109.94 bc	72.55 ab	28.42 b	210.92 c
Bermuda	58.54 ef	87.38a	38.17 ab	184.09 d
Estrella	43.09 f	55.39 b	44.63 ab	143.12 e
Insurgente	121.92 ab	85.26a	37.40 ab	244.59 a
Llanero	77.52 d	69.36 ab	35.79 ab	182.66 d
Mombaza	76.84 d	72.27 ab	38.04 ab	187.15 d
Pangola	60.67 de	69.64 ab	49.43 a	179.73 d
Pará	129.34a	72.59 ab	31.76 ab	233.69 b
Tanzania	99.20 c	72.70 ab	37.10 ab	209.00 c
SEM	5.67	2.02	1.59	5.74

a,b,c,d,e Mean values per column with different letters are statistically different ($p \leq 0.05$). EEM: standard error of the mean.

biogas production from the mentioned grasses is due to the direct relationship with the fermentation of carbohydrates to acetate, propionate, and butyrate. Biogas production from protein or fat is not significant, due to the conditions of the technique itself (Amanzougarene and Fondevila, 2020). Furthermore, the amount of biogas released is a consequence of the fermentation time (Texta-Nogueda *et al*., 2019), chemical nature, and relationship with other components such as lignin or protein (Amanzougarene and Fondevila, 2020).

At 30 d of regrowth, Pangola pasture had the highest partial biogas production at 24 hours, followed by Estrella, Insurgente, and Pará pastures at 45 d and Insurgente at 60 d, indicating that these pastures showed the highest amount of soluble carbohydrates (Texta-Nogueda *et al*., 2019). In the partial biogas production at 48 h, the pastures showed great variation in values at each regrowth age because structural carbohydrates that are not attached to lignin are fermented, and the availability of these carbohydrates depends on their chemical composition (Gándara *et al*., 2017). At 30 d of regrowth, Insurgente, Mombaza, and Pará pastures produced the most biogas at 72 h, followed by Llanero, Mombaza, and Pangola at 45 d ($p \leq 0.05$). However, at 60 d of regrowth, the difference in biogas production was between Pangola and Aruana ($p \leq 0.05$), with no differences between the other pastures. This can be assumed to be due to the amount of carbohydrates attached to lignin, which limits the amount available for microbial fermentation (Texta-Nogueda *et al*., 2019).

Cumulative CH_4 production showed the highest values at 30 d for Estrella and Pangola, 45 d for Estrella, and 60 d for Insurgente, Mombaza, and Pará ($p \leq 0.05$) (Table 4). Furthermore, pasture behavior in partial CH_4 productions is similar to biogas production (Table 3). Therefore, the CH_4 values reported in this study are assumed to be the carbohydrate fermentation products, as they are primarily produced when carbohydrates are fermented directly or indirectly to acetate and butyrate (Amanzougarene and Fondevila, 2020). Almaraz-Buendía *et al*. (2019) published that Bermuda, Estrella, Insurgente, Llanero, Mombaza, Pangola, and Pará pastures produced 47.28, 86.35, 68.53, 83.71, 105.78, 83.18, and 54.12 mL g^{-1} DM of biogas, as well as 27. 28, 44.90, 44.62, 43.23, 42.63, 40.62, and 27.97 mL g^{-1} DM of CH_4. These values are lower than those reported in the present study for the same pastures at any of the regrowth ages evaluated (Tables 3 and 4). The difference in biogas and methane production can be assumed to be due to the methodology used for the determination and the age of the pastures used.

The characteristics of the culture medium at the end of the fermentation test of the grasses at the 3 regrowth ages indicated pH values ranging from 6.69 to 6.19 and N-NH_3 values ranging from 0.28 to 2.65 The carbohydrate fermentation products decreased the pH values after the pH of the initial culture medium was adjusted to 6.8 (Amanzougarene and Fondevila, 2020). The N-NH_3 content of the medium depends on the degradability of nitrogen fractions (Ahvenjärvi *et al*., 2018), so the values in the present study (Table 5) are based on the CP content of the evaluated pastures (Table 3), since no nitrogen source was added during the culture medium preparation.

Degradation expresses the proportion of feed retained by the animal in relation to the total ingested. Llanero had the lowest DMD and NDFD at 30 d, and Mombaza had the lowest at 45 d ($p \leq 0.05$) (Table 5). However, at 60 d, the DMD for Insurgente and Pangola pastures differed from Estrella and Aruana, while for NDFD, Pangola differed from Estrella and Aruana ($p \leq 0.05$) (Table 5). These differences are assumed to be due to differences in cell wall structure, organization, and composition (Zhang *et al*., 2021). DM and NDF degradation of pastures at any regrowth age has no effect

Table 4. Partial and cumulative in vitro methane production of nine tropical pastures at three regrowth ages.

Pastures	Partial production (mL g^{-1} DM)			Accumulated (mL g^{-1} DM)
	24 h	48 h	72 h	
		30 d of regrowth		
Aruana	45.49a	13.88 d	3.08 c	62.46 b
Bermuda	29.22 bc	16.92 bcd	7.69 b	53.83 de
Estrella	50.85a	15.70 cd	3.36 c	69.92a
Insurgente	35.41 b	22.33a	3.47 c	61.21 bc
Llanero	26.25 c	18.23 abc	10.94a	55.41 cd
Mombaza	35.22 b	15.74 cd	2.25 c	53.21 de
Pangola	44.65a	18.81 abc	7.05 b	70.50a
Pará	26.36 c	18.83 abc	8.28 b	53.47 de
Tanzania	21.83 c	21.08 ab	4.52 c	47.43 e
SEM	1.92	0.55	0.57	1.51
		45 d of regrowth		
Aruana	32.78 b	17.13 c	8.57a	58.48 c
Bermuda	34.50 b	15.74 c	6.75 ab	56.99 c
Estrella	47.41a	22.45a	8.31a	78.17a
Insurgente	47.14a	15.97 c	6.84 ab	69.94 b
Llanero	20.37 c	19.64 b	8.73a	48.73 e
Mombaza	33.86 b	17.67 bc	6.99 ab	58.52 c
Pangola	32.29 b	17.27 c	2.63 c	52.19 d
Pará	33.95 b	17.71 bc	5.53 b	57.20 c
Tanzania	31.58 b	16.55 c	4.89 bc	53.03 d
SEM	1.54	0.4	0.4	1.72
		60 d of regrowth		
Aruana	29.17 b	15.71 b	6.36 bc	51.25 c
Bermuda	25.43 c	21.35a	4.35 cd	51.13 c
Estrella	20.01 d	14.62 b	2.69 d	37.33 e
Insurgente	34.43a	20.21a	8.98a	63.63a
Llanero	28.34 bc	17.15 b	9.69a	55.18 b
Mombaza	29.65 b	20.53a	10.26a	60.45a
Pangola	12.74 e	20.98a	8.24 ab	41.96 d
Pará	31.74 ab	20.39a	9.83a	61.96a
Tanzania	28.78 bc	16.67 b	10.61a	56.06 b
SEM	1.24	0.5	0.54	1.66

a,b,c,d,e Mean values per column with different letters are statistically different ($p \leq 0.05$). EEM: standard error of the mean.

on their energy content or potential DM consumption when offered to the animal (Torres-Salado *et al*., 2019).

The digestibility of grasses decreases after 72 d of regrowth since cellulose and hemicellulose have variable digestibility depending on their degree of lignification (Villareal, 1994). The older the plant, the lower the digestibility because lignin

Table 5. Fermentative characteristics and estimators of *in vitro* biogas fermentation kinetics of nine tropical pastures at three regrowth ages.

Pasture	pH	$N\text{-}NH_3$	DMD	NDFD	*A*	*k*	*b*
			30 d of regrowth				
Aruana	6.49 abc	0.68 abc	76.6a	73.73a	222.85 ab	2.23 de	0.04 b
Bermuda	6.38 c	1.07 abc	74.72a	71.08a	201.3 ab	2.73 cde	0.09a
Estrella	6.41 bc	1.22 ab	74.34a	70.20a	232.81a	2.13 e	0.09a
Insurgente	6.61a	0.44 bc	74.3a	71.25a	138.34 de	4.09a	0.01 d
Llanero	6.63a	0.28 c	64.14 b	58.49 b	188.41 bc	3.00 bc	0.04 b
Mombaza	6.37 c	0.85 abc	75.22a	70.01a	121.17 de	2.14 e	0.01 d
Pangola	6.48 abc	0.98 abc	75.49a	74.28a	241.27a	2.15 e	0.09a
Pará	6.57 ab	1.12 abc	76.05a	70.13a	110.14 e	2.92 cd	0.01 d
Tanzania	6.50 abc	1.40a	76.53a	75.33a	154.58 cd	3.68 ab	0.05 b
SEM	0.02	0.09	0.75	1.01	9.43	0.14	0.01
			45 d of regrowth				
Aruana	6.37 ab	0.54 c	70.55 ab	66.42 b	215.57 c	2.56 bcd	0.08 bc
Bermuda	6.34 b	1.47 ab	72.65 ab	67.14 ab	197.51 e	2.74 bc	0.07 c
Estrella	6.44 ab	1.65a	74.73a	73.97 ab	242.85a	2.51 cde	0.09 bc
Insurgente	6.29 b	1.87a	79.12a	79.93a	231.78 b	2.34 de	0.11 ab
Llanero	6.33 b	0.62 c	73.60a	70.29 ab	184.5 f	3.13a	0.07 c
Mombaza	6.56a	0.96 bc	63.05 b	50.22 c	188.62 f	2.25 e	0.08 bc
Pangola	6.39 ab	1.77a	72.45 ab	65.81 b	222.01 c	2.36 de	0.10 bc
Pará	6.39 ab	0.95 bc	72.57 ab	64.47 b	205.93 d	2.47 cde	0.12a
Tanzania	6.45 ab	1.33 ab	77.51a	75.31 ab	201.93 de	2.83 b	0.08 bc
SEM	0.02	0.1	1.00	1.73	3.65	0.05	0.0035
			60 d of regrowth				
Aruana	6.38 bcd	1.34 bc	59.83 b	51.41 b	197.50 cd	2.38 c	0.06a
Bermuda	6.56 ab	1.36 bc	70.80 ab	66.19 ab	157.98 e	6.58a	0.07a
Estrella	6.43 bc	1.92 b	58.27 b	49.74 b	113.32 f	2.59 bc	0.004 b
Insurgente	6.69a	2.65a	77.80a	75.38 ab	223.13 ab	2.59 bc	0.06a
Llanero	6.19 d	1.02 c	72.98 ab	69.85 ab	198.22 bcd	3.55 b	0.07a
Mombaza	6.45 bc	1.57 bc	70.07 ab	64.90 ab	215.85 abc	2.87 bc	0.05a
Pangola	6.35 cd	0.93 c	79.57a	81.39a	229.47a	2.65 bc	0.04a
Pará	6.43 bc	1.55 bc	73.00 ab	66.30 ab	211.60 abc	2.63 bc	0.05a
Tanzania	6.53 abc	1.44 bc	67.6 ab	61.15 ab	185.08 d	3.04 bc	0.05a
SEM	0.03	0.10	1.66	2.37	6.93	0.25	0.0041

a,b,c,d,e,f Mean values per column with different letter are statistically different ($p \leq 0.05$). pH: hydrogen potential; $N\text{-}NH_3$: mg dL^{-1} of ammonia nitrogen; DMD: dry matter degradation percent; NDFD: degradation percent of detergent neutral fiber; *A*: mL g^{-1} DM of total biogas production; *b*: mL h^{-1} of biogas production rate; *k*: lag time hours; EEM: standard error of the mean.

synthesis begins. Bermuda, Estrella, Insurgente, Llanero, Mombaza, Pangola, and Pará pastures with 56 d of regrowth showed 34.46, 39.92, 42.72, 34.17, 46.96, 37.42, and 41.24 % of DMD, as well as 37.28, 40.34, 47.66, 26.73, 52.82, 35.58, and 40.80 % of NDFD, respectively (Almaraz-Buendía *et al*., 2019). These values are lower than the same pastures with 45 or 60 d of regrowth (Table 5).

The adaptation process of microorganisms to the conditions of the *in vitro* technique depends on several factors that can affect the prediction of microorganism kinetics. The growth conditions are evidenced by *k*, which allows estimating the adjustment period where bacteria adapt to the medium to initiate exponential growth (Swinnen *et al*., 2004). Insurgente and Tanzania had the highest k values at 30 d, Llanero at 45 d, and Bermuda at 60 d ($p \leq 0.05$) (Table 5). *b* indicates the fermentation rate and can be assumed indirectly as the exponential growth rate of the inoculum (Rojas-García *et al*., 2020), because the rate of increase of bacteria in a time is proportional to the product of their fermentation, in this case, biogas. At 30 days, Bermuda, Estrella, and Pangola had the highest *b* values; at 45 days, Pará had the highest *b*; and at 60 days, Estrella had the lowest *b* ($p \leq 0.05$) (Table 5).

Almaraz-Buendía *et al*. (2019) reported values of 45.67, 82.60, 70.32, 85.08, 105.17, 82.44, and 66.70 mL g^{-1} DM for estimator A, as well as 0.037, 0.037, 0.054, 0.032, 0.034, 0.028, and 0.012 ml h^{-1} for estimator *b*. In addition, 22.81, 13.89, 38.04, 11.52, 17.30, 7.64, and 28. 62 h were registered for the *k* estimator of gas production kinetics for Bermuda, Estrella, Insurgente, Llanero, Mombaza, Pangola, and Pará pastures, respectively. Lower values for estimators *A* and *b* and higher for the *k* estimator were found for the same pastures evaluated in the present study for any of the regrowth days evaluated (Table 5).

CONCLUSIONS

In each regrowth age evaluated in the present study, Pangola and Estrella grasses showed higher content in some chemical characteristics and variables measured by the *in vitro* gas production technique, implying that they presented better chemical and fermentative results *in vitro*.

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REFERENCES

Ahvenjärvi S, Vaga M, Vanhatalo A, Huhtanen P. 2018. Ruminal metabolism of grass silage soluble nitrogen fractions. Journal of Dairy Science 101 (1): 279–294. https://doi.org/10.3168/jds.2016-12316

Amanzougarene Z, Fondevila M. 2020. Fitting of the *in vitro* gas production technique to the study of high concentrate diets. Animals 10 (10): 1935. https://doi.org/10.3390/ani10101935

AOAC (Association of Official Analytic Chemists). 2005. Official methods of analysis (18th edition). Association of Official Analytic Chemists: Washington, DC, USA. 1094 p.

Almaraz-Buendía I, García AM, Sánchez-Santillán P, Torres-Salado N, Herrera-Pérez J, Bottini-Luzardo MB, Rojas-García R. 2019. Análisis bromatológico y producción de gas *in vitro* de forrajes utilizados en el trópico seco mexicano. Archivos de Zootecnia 68 (262): 260–266. https://doi.org/10.21071/az.v68i262.4145

Avellaneda-Avellaneda Y, Mancipe-Muñoz EA, Vargas-Martínez J de J. 2020. Efecto de la edad de rebrote sobre el desarrollo morfológico y la composición química del pasto kikuyo (*Cenchrus clandestinus*) en el trópico alto colombiano. CES Medicina Veterinaria y Zootecnia 15 (2): 23–37. https://doi.org/10.21615/cesmvz.15.2.2

CONABIO (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad). 2018. La biodiversidad en Veracruz: Estudio de estado. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. Veracruz, México. https://www.biodiversidad.gob.mx/region/EEB/estudios/ee_veracruz (Retrieved: August 2021).

Enríquez-Quiroz JF, Esqueda-Esquivel VA, Martínez-Méndez D. 2021. Rehabilitación de praderas degradadas en el trópico de México. Revista Mexicana de Ciencias Pecuarias 12 (S-3): 243–260. https://doi.org/10.22319/rmcp.v12s3.5876

FIRCO (Fideicomiso de Riesgo Compartido). 2017. Fortalecimiento productivo para la producción de forrajes. Fideicomiso de Riesgo Compartido. Ciudad de México, México. http://www.gob.mx/firco/articulos/fortalecimiento-productivo-para-la-produccion-de-forrajes?idiom=es (Retrieved: July 2022).

Gándara L, Borrajo CI, Fernández JA, Pereira MM. 2017. Efecto de la fertilización nitrogenada y la edad del rebrote sobre el valor nutritivo de *Brachiaria brizantha* cv. «Marandú». Revista de la Facultad de Ciencias Agrarias 49 (1): 69–77.

Hernández-Morales J, Sánchez-Santillán P, Torres-Salado N, Herrera-Pérez J, Rojas-García AR, Reyes-Vázquez I, Mendoza-Núñez MA. 2018. Composición química y degradaciones *in vitro* de vainas y hojas de leguminosas arbóreas del trópico seco de México. Revista Mexicana de Ciencias Pecuarias 9 (1): 105–120. https://doi.org/10.22319/rmcp.v9i1.4332

Herrera-Pérez J, Vélez-Regino LG, Sánchez-Santillán P, Torres-Salado N, Rojas-García AR, Maldonado-Peralta MA. 2018. *In vitro* fermentation of fibrous substrates by water buffalo ruminal cellulolytic bacteria consortia. Revista MVZ Córdoba 23 (3): 6860–6870. https://doi.org/10.21897/rmvz.1374

Lavrenčič A, Stefanon B, Susmel P. 1997. An evaluation of the Gompertz model in degradability studies of forage chemical components. Animal Science 64 (3): 423–431. https://doi.org/10.1017/S1357729800016027

Maceda A, Soto-Hernández M, Peña-Valdivia CB, Trejo C, Terrazas T. 2019. Differences in the structural chemical composition of the primary xylem of Cactaceae: A topochemical perspective. Frontiers in Plant Science 10: 1497. https://doi.org/10.3389/fpls.2019.01497

McCullough H. 1967. The determination of ammonia in whole blood by a direct colorimetric method. Clinica Chimica Acta 17 (2): 297–304. https://doi.org/10.1016/0009-8981(67)90133-7

Patiño-Pardo RM, Gómez-Salcedo R, Navarro-Mejía OA. 2018. Calidad nutricional de Mombaza y Tanzania (*Megathyrsus maximum* Jacq.) manejada a diferentes frecuencias y alturas de corte en Sucre Colombia. Revista CES Medicina Veterinaria y Zootecnia 13 (1): 17–30. https://doi.org/10.21615/cesmvz.13.1.2

Polo LEA. 2021. Rendimiento y componentes de valor nutritivo de tres cultivares *Panicum maximum*. Scientia Revista de Investigación 31 (1): 44–50.

Reiné R, Ascaso J, Barrantes O. 2020. Nutritional quality of plant species in Pyrenean hay meadows of high diversity. Agronomy 10 (6): 883. https://doi.org/10.3390/agronomy10060883

Rojas-García AR, Orocio-Martínez RK, Sánchez-Santillán P, Ayala-Monter MA, Maldonado-Peralta MA, Valenzuela-Lagarda JL. 2020. Características bromatológicas y fermentativas *in vitro* de complementos con *Enterolobium cyclocarpum* (Jacq.) Griseb y cáscara de *Cucurbita argyrosperma* Huber. Agroproductividad 13 (7): 67–74. https://doi.org/10.32854/agrop.vi.1677

SAS Institute Inc. 2002. Statistical Analysis System User´s Guide. SAS Institute, Inc. Cary, NC, USA. 956 p.

Swinnen IAM, Bernaerts K, Dens EJJ, Geeraerd AH, van Impe JF. 2004. Predictive modelling oh the microbial lag phase: a review. International Journal on Food Microbiology 94: 137–159. https://doi.org/10.1016/j.ijfoodmicro.2004.01.006

Texta-Nogueda J, Sánchez-Santillán P, Hernández-Sánchez D, Torres-Salado N, Crosby-Galvan M, Herrera-Pérez J, Rojas-García RA. 2019. Use of disaccharides and activated carbon to preserve cellulolytic ruminal bacterial consortiums lyophilized. Revista MVZ Córdoba 24 (3): 7305–7313. https://doi.org/10.21897/rmvz.1412

Torres-Salado N, Sánchez-Santillán P, Rojas-García AR, Herrera-Pérez J, Hernández-Morales J. 2018. Producción de gases efecto invernadero *in vitro* de leguminosas arbóreas del trópico seco mexicano. Archivos de Zootecnia 67 (257): 55–59. https://doi.org/10.21071/az.v67i257.3491

Torres-Salado N, Sánchez-Santillán P, Rojas-García AR, Almaraz-Buendía I, Herrera-Pérez J, Reyes-Vázquez I, Mayren-Mendoza FJ. 2019. *In vitro* gas production and fermentative characteristics of ruminal cellulolytic bacterial consortia of water buffalo (*Bubalus bubalis*) and Suiz-bu cow. Agrociencia 53 (2): 145–159.

Torres-Torres F, Rojas-Martínez A. 2018. Suelo agrícola en México: Retrospección y prospectiva para la seguridad alimentaria. Revista Internacional de Estadística y Geografía 9 (3): 137–155.

van Soest PJ, Roberton JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal Dairy Science 74 (10): 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Villareal M. 1994. Valor nutritivo de gramíneas y leguminosas forrajeras en San Carlos, Costa Rica. Pasturas Tropicales 16 (1): 27–31.

Zhang B, Gao Y, Zhang L, Zhou Y. 2021. The plant cell wall: Biosynthesis, construction, and functions. Journal of Integrative Plant Biology 63 (1): 251–272. https://doi.org/10.1111/jipb.13055