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Harvest age of *Urochloa* hybrids regarding yield, chemical composition, and *in vitro* biogas production

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ABSTRACT

Objective: To evaluate the chemical composition, fermentation, and *in vitro* biogas production of the Cayman and Cobra cultivars, at different cutting ages.

Design/Methodology/Approach: Dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose (HEM), and cellulose (CEL) were determined at 28, 35, 42, and 49 cutting days. *In vitro* dry matter degradation (IVDMD), pH, and concentration of volatile fatty acids (VFA) were determined during fermentation. The biogas volume was estimated at 6, 12, 24, 48, and 72 h; the volume of methane (CH_4) and carbon dioxide (CO_2) in the Cobra and Cayman forages was determined at 72 h. A completely randomized design was used for the experiment.

Results: There were no differences ($P>0.05$) in DM production during the different cutting ages. CP was higher ($P<0.05$) in both cultivars, at 28 and 35 days after the cutting. The NDF, ADF, HEM, and CEL percentages were different in both cultivars. IVDMD was higher ($P<0.05$) between day 28 and day 42. Finally, CH_4 production was lower ($P<0.05$) at 28 and 35 d after the cutting.

Study Limitations/Implications: The chemical composition of pastures is influenced by climate and, therefore, further analysis must be carried out during different periods or seasons of the year.

Findings/Conclusions: The optimal cutting age of the Cobra and Cayman cultivars under drought conditions is between day 28 and day 35 of regrowth. During that period, they have the best chemical and fermentation characteristics.

Keywords: Cobra and Cayman cultivars, cutting time, fermentation, methane production.

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INTRODUCTION

The drought conditions of the tropical areas limit the productive potential of forages (Rojas-García *et al.*, 2018), causing changes in the chemical composition and low biomass production in the prairies (Pratti-Daniel *et al.*, 2019). Different improved genotypes of grasses have been selected in recent years, including hybrids of genus *Urochloa*, such

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as Cayman (BR/1752) and Cobra (BR/1794). These hybrids have recorded excellent biomass production yields and have adapted well to the tropical climate (Pizarro *et al.*, 2013; Vandremini, 2014). Arroyave *et al.* (2013) reported that these cultivars obtain an annual yield of 2 to 4 t DM ha^{-1}, without fertilization, during long drought periods. In addition, they can also grow in alkaline or acid soils (Morales-Bautista *et al.*, 2011). Age impacts the nutritional quality of forages, producing changes in the chemical composition, which affect animal production. The ruminal degradability of grasses is related to the cell wall percentages, because the degradability of the rumen decreases as the fiber content increases (Ledea *et al.*, 2018). Ku-Vera *et al.* (2014) reported that tropical forages had a low CP content and a high NDF percentage, as well as a low digestibility and a lower metabolizable energy content. When tropical grasses are harvested at an appropriate age, they have a lower cell wall content and a higher CP protein and soluble sugars content, which reduces the CH_4 and CO_2 emissions produced by ruminants (Camacho-Escobar *et al.*, 2020).

Eckard *et al.* (2010) reported that between 3% and 10% of the net energy consumed by a bovine becomes CH_4, which is mainly eliminated when the animals belch. Consequently, reducing these emissions has a double impact. On the one hand, the greenhouse gas emissions (GGE) that causes global warming to diminish and, on the other hand, the loss of energy used for animal production also diminishes. The hypothesis of this research was that, during the harvest age, the chemical composition, the fermentation parameters, and the *in vitro* biogas (CH_4 and CO_2) production of the Cobra and Cayman *Urochloa* hybrids were impacted by drought conditions. Therefore, the objective of this study was to evaluate the chemical composition, fermentation, and *in vitro* biogas production of CH_4 and CO_2 of the Cobra and Cayman cultivars, at different harvest ages, during the drought season, in the coastal region (La Costa) of Oaxaca, Mexico.

MATERIALS AND METHODS

The research was carried out at the microbiology and nutrition labs and in the Área de Cultivos Forrajeros of the Campo Experimental of the Universidad del Mar, Campus Puerto Escondido, located in the Bajos de Chila, municipality of San Pedro Mixtepec, Juquila, Oaxaca (15° 55' 27.54" N and 97° 09' 04.09" W), at 12 m.a.s.l. The climate is warm sub-humid (A(c)w2 type), with an annual average precipitation of 930.8 mm-1668 mm and an annual average temperature of 26 °C (Serrano-Altamirano *et al.*, 2005).

Sampling collection

The Cayman and Cobra cultivars were sown by hand in a 480 m^2 area, using PAPALOTLA® certified seeds. The seeds were sown in small holes, one next to the other (1 cm apart), with a 6.0 kg ha^{-1} density. The distance between the furrows was 50 cm. The experimental area was divided into eight 60-m^2 plots. During the growing period, a single 50 kg ha^{-1} urea dose was applied. Auxiliary irrigation was carried out once a week. A standardization cutting was made 90 days after the sowing, when the cultivars had grown 10 cm above ground. Cutting intervals took place 4, 5, 6, and 7 weeks after the regrowth.

Chemical composition

The samples consisted of forages harvested at intervals of 4, 5, 6, and 7 weeks after regrowth. The moisture and dry matter content of the samples were determined. Dry matter content was determined following the recommendations of Herrera (2014), using a drying oven (Felisa®, México) at 65 °C for 48 h. The samples were processed in a Werker MF 10 grinder (Ika®, USA), with a 1 mm sieve. Subsequently, CP was determined using the Kjeldahl method (AOAC, 1997); meanwhile, NDF, ADF, CEL, and HEM were determined following the recommendation of Van Soest *et al.* (1991). The samples were analyzed at the Animal Nutrition lab of the Departamento de Ciencias Básicas Para la Salud, Universidad de Guadalajara, Centro Universitario del Sur, Ciudad Guzmán, Jalisco. Mexico.

Culture medium preparation

In order to determine the *in vitro* dry matter degradation (IVDMD), pH, VFA, and biogas, CH_4, and CO_2 production, a culture medium for rumen microorganisms was used. Based on Cobos and Yokoyama (1995), the culture medium was prepared with glucose, cellobiose, starch, and fresh ruminal fluid (GCA-FR). The source of the inoculum was fresh ruminal fluid from a Zebu × Brown Swiss adult, with 500 kg live weight and a rumen fistula. The fluid was extracted 2 h after the specimen was fed with a 100% forage diet. The handling of the bovine complied with the regulations for the use of animals with research purposes of the Universidad del Mar (NOM-024-ZOO-1995).

***In vitro* dry matter degradation (IVDMD)**

In order to determine *in vitro* dry matter degradation (IVDMD), 0.5 g DM from each treatment was weighted in triplicate, adding 120 mL viral serology. Forty-five mL of the sterile culture medium for total bacteria was added to each vial (Cobos and Yokoyama, 1995). The vials were inoculated with 5 mL of fresh ruminal fluid. Each vial was considered both as a bio-fermenter and an experimental unit. IVDMD was determined after 72 h, following the recommendations of Mellenberger *et al.* (1970).

***In vitro* rumen fermentation 72 h after the incubation**

An Orion® portable potentiometer (USA) was used to determine pH. A Perkin Elmer® gas chromatograph (USA) with a flame ionization detector was used to determine VFA molar concentration, based on the recommendations of Erwin *et al.* (1961). The following retention times were recorded: 1.26 m for acetate, 1.6 m for propionate, and 2.09 m for butyrate. The analysis was carried out in the Microbiología Ruminal and Genética Microbiana labs of the Colegio de Postgraduados, Mexico.

Biogas emissions measurement

Biogas production was estimated using the technique reported by Krabill *et al.* (1969) and modified by Cobos *et al.* (2018). The bio-fermenters were placed in a bath Marie (39 °C); they were connected to the biogas capture traps with a Tygon® hose (internal diameter: 3/32”). Two Terumo® 20 g × 1 inch needles were connected to each end of the hose. The bio-fermenter was connected to one end and the biogas capture trap was connected to the other. A needle was connected to the latter as a release valve. The trap was placed upside-down in a plastic test tube. Biogas volume was quantified at 6, 12, 24, 48, and 72 h of incubation.

Estimation of the *in vitro* production of CH_4 and CO_2

The CH_4 and CO_2 ratios were determined using a 500 μL sample of the biogas obtained from the traps. The sample was injected in a Perkin Elmer® gas chromatograph (USA), which had a thermal conductivity indicator and a Poropack packed column. Retention times were 0.71 m and 1.0005 m for CH_4 and CO_2, respectively. The analyses were carried out in the Microbiología Ruminal and Genética Microbiana labs of the Colegio de Postgraduados, Mexico.

Statistical analysis

The chemical and *in vitro* rumen fermentation variables were analyzed using a completely randomized design, with four treatments and three repetitions per treatment for each cultivar. All the evaluated variables were subjected to an analysis of variance, using the GLM procedure of SAS (2011) and Tukey's mean comparison test (α=0.05) (Steel and Torrie, 1988).

RESULTS AND DISCUSSION

The DM yield of the cultivars did not record statistical differences (P>0.05) in the different harvest ages. Meanwhile, there were differences (P<0.05) in the chemical composition regarding the harvest age for the Cobra and Cayman cultivars (Table 1).

Table 1. Effect of the harvest age in the yield, chemical composition, and IVDMD of the Cobra and Cayman cultivars, during the drought season in the tropic.

Cultivar	kg MS ha^{-1}	PC	FDN	FDA	HEM	CEL	DIVMS
Cobra							
28d	1858.24^{a}	10.26^{a}	52.27^{c}	26.74^{b}	25.91^{b}	10.86^{b}	61.27^{a}
35d	2273.02^{a}	9.54^{b}	56.78^{b}	35.42^{a}	22.29^{a}	14.45^{a}	61.09^{a}
42d	1877.28^{a}	7.94^{c}	61.57^{a}	32.79^{a}	27.73^{d}	11.89^{b}	56.87^{a}
49d	2596.44^{a}	7.74^{c}	54.86^{b}	29.25^{b}	24.13^{c}	11.95^{b}	49.39^{b}
MS total	6746.74						
EE	205.84	0.12	0.47	0.58	0.36	0.3	1.01
Cayman							
28d	1662.26^{a}	11.15^{a}	55.83^{b}	27.54^{b}	23.90^{a}	10.86^{b}	62.11^{a}
35d	1848.51^{a}	10.75^{a}	57.47^{b}	28.19^{b}	19.87^{b}	14.45^{a}	58.30^{ab}
42d	2201.96^{a}	9.44^{bc}	57.73^{ab}	30.34^{a}	24.43^{a}	11.89^{b}	58.80^{ab}
49d	2711.99^{a}	8.26^{c}	59.38^{a}	31.68^{a}	23.86^{a}	11.95^{b}	53.52^{b}
MS total	8424.72						
EE	179.91	0.37	0.68	0.38	0.25	0.3	1.66

abc=in the column, indicates statistical differences (P<0.05) in the harvest age.
28, 35, 42, 49=harvest age of the cultivar. EE (SE): standard error.

The lack of differences in DM production in the cultivars is the result of the lower moisture content of the soil, which may have been the cause of the lower nutrient availability for the grasses. Consequently, the reduction in the weight of the stems and leaves caused a lower biomass production. Pizarro *et al.* (2013) reported that, under adverse conditions, the Cayman and Cobra hybrids have a specialized genetic potential for forage production. The accumulated DM production in this study reached 8,424.72 and 6,746.74 kg ha^{-1} for Cayman and Cobra, respectively. These results match the findings of Garay-Martínez *et al.* (2018), who reported a forage production of 6,310 and 8,890 kg ha^{-1}, under drought conditions, for the Cobra and Cayman cultivars, respectively. The lowest DM yield among the cultivars under study was the consequence of the low precipitation levels recorded during the evaluation. The lack of humidity impacted the nutrient availability in the soil, changing the photosynthesis biochemical process of the plants (Cardoso *et al.*, 2015). In addition, the short intervals between harvests limit the capacity of the plants to accumulate reserves, preventing a vigorous regrowth, which results in a lower growth rate and a lower biomass accumulation (Cruz-Hernández *et al.*, 2017). Meanwhile, plant metabolism increases along with harvest age (Castro *et al.*, 2013), which causes a higher leaf production that subsequently increases the biomass of the grass.

The CP content recorded differences depending on the harvest age ($P<0.05$) of both cultivars. The Cobra hybrid recorded its highest CP content at a younger harvest age (28 days), while its percentages decreased at an older harvest age (42 and 49 d). The same trend was recorded for Cayman. The results obtained in this experiment are lower than the findings of Garay-Martínez *et al.* (2018), who reported CP percentage results, at 28 d of age, of 16.2 and 16.1 for Cayman and Cobra, respectively. For their part, Rojas-García *et al.* (2018) reported a 14.4% CP for the Cobra cultivar, at 35 d of regrowth. In this study, CP recorded a decreasing trend as the age of the plant increased. Meanwhile, Garay-Martínez *et al.* (2018) determined that the protein content of *Urochloa* hybrid grasses decreased in older plants. On more mature grasses, DM content and cell walls tend to increase and, consequently, the CP percentage tends to diminish. Therefore, determining the optimal cutting age —in which grasses have the best nutritional characteristics for the animals— is fundamental (Rojas-García *et al.*, 2018). At an early age, grasses have a higher leaf:stem ratio, which increases the plants' CP concentrations —which can be found in higher amounts in its leaves. At an older age, the cellulose, hemicellulose, and lignin content of the cell wall synthesis increases and, consequently, the nutritional quality of the grass decreases (de Dios-León *et al.*, 2022).

Differences in NDF percentages ($P<0.05$) depended on the harvest age; the lowest values were recorded at day 28 in both cultivars. The highest value recorded by Cobra grass was 61.57%, at day 42, while Cayman grass recorded 59.36%, at day 49. Overall, the NDF of both cultivars increased along with the harvest age. The NDF value for Cobra grass was higher at day 35 than the value obtained by Cayman at day 49. The ADF results have a similar trend than the results of NDF. These values do not match the findings of López-Garrido *et al.* (2022), who reported 67.75% NDF and 40.75% ADF values for Cobra grass. Regarding Cayman grass, they reported 71.55% NDF and 44.53% ADF. These percentages were obtained with a 60-day harvest age. Villalobos and Arce (2013) pointed out that a high NDF and ADF content decreases the degradation and nutrient content of the forages. On the one hand, the differences ($P<0.05$) in the hemicellulose percentage depend on the harvesting age: the highest content was recorded by Cobra at day 42 (27.73%), while Cayman recorded lower values at day 35 (19.87%). On the other hand, the highest value (24.43%) was obtained 42 days after the harvest. In this regard, Reyes-Pérez (2022) carried out a study with *Brachiaria decumbens*, during a period with scarce rain, and reported 30.18% and 30.58% values, at 30 and 45 harvest days, respectively. This study found out differences ($P<0.05$) in the cellulose percentage of the cultivars, at 42 and 49 harvest days; the Cobra grass obtained the highest value. A trend towards a cellulose content increase was recorded as the plant grew older. As the age of the plant increases, the cell wall ratio also increases. This ratio is associated with the increase in the DM, NDF, ADF, and hemicellulose content. Older plants are related to the reduction of new leaves, the increase of vascular bundles, the loss of water, a lower cell content, and a higher stem ratio (de Dios-León *et al.*, 2022).

The IVDMD in both cultivars decreased ($P<0.05$) with age; the highest value was recorded at 28, 42, and 45 harvest days. The results of this study match the findings of López-Garrido *et al.* (2022), who reported DM degradation percentages of 64.61% (Cobra) and 63.57% (Cayman), under moderate salinity conditions, at 60 harvest days. In this regard, Barrera *et al.* (2015) related the degradation decrease with the structural carbohydrates increase. Ledea-Rodríguez *et al.* (2018) pointed out that the DS degradation decrease is related to the increase of the age of the plant and the rumen environmental conditions, because DM degradation is related to the microbiota that colonizes food particles. Overall, de Dios-León *et al.* (2022) concluded that the IVDMD decrease is related to the increase of the age of the grasses, because, grasses have more leaves and a lower proportion of stems at an early harvest age (30-40 d) than at a mature harvest age (40-60 d), when high DM, NDF, and ADF percentages are recorded. As the forages mature, the stem proportion increases and the leaf proportion diminishes and, consequently, the structural carbohydrate and lignin content increases, reducing its degradation in the rumen.

No differences in pH were recorded ($P>0.05$) regarding the harvest age (Table 2). This behavior was similar for both grasses. Therefore, this situation suggests that the rumen bacteria had an appropriate development during the *in vitro* fermentation, because they require an almost neutral pH to carry out their enzymatic activity (Barboza *et al.*, 2009).

There were differences ($P<0.05$) in the concentration of acetate during the *in vitro* fermentation and the harvest age (Table 2). The Cobra grass and the Cayman grass recorded their highest concentration at day 49 and 42, respectively. López-Garrido *et al.* (2022) studied the same grass varieties, recording a lower acetate concentration at 60 harvest days, with values of 17.22 mM L^{-1} (Cobra) and 18.88 mM L^{-1} (Cayman). The propionate and butyrate concentrations of both cultivars did not record any differences ($P>0.05$) between harvest ages. A difference ($P<0.05$) in the total VFA concentration depended on the harvest age of both cultivars, recording a higher concentration at day 49 (Table 2).

Table 2. pH values and VFA concentration 72 h after the in vitro incubation of the Cobra and Cayman grasses, at different harvest ages.

Cultivar	pH	Ace	Prop	But	AGV Total	Ac:Pro
		mM L^{-1}				
Cobra						
28	6.35[a]	25.77[ab]	13.73[a]	8.15[a]	47.66[ab]	1.87[a]
35	6.29[a]	25.48[b]	12.71[a]	7.18[a]	45.37[ab]	2.00[a]
42	6.34[a]	24.08[c]	13.01[a]	7.25[a]	44.34[b]	1.85[a]
49	6.44[a]	27.03[a]	13.85[a]	7.58[a]	48.47[a]	1.95[a]
EE	0.07	0.27	0.29	0.37	0.73	0.03
Cayman						
28	6.33[a]	23.76[ab]	12.80[a]	7.33[a]	43.90[ab]	1.85[a]
35	6.37[a]	23.38[ab]	12.51[a]	7.87[a]	43.77[ab]	1.87[a]
42	6.32[a]	22.07[b]	12.32[a]	7.79[a]	42.18[b]	1.79[a]
49	6.41[a]	23.94[a]	13.25[a]	8.09[a]	45.28[a]	1.81[a]
EE	0.03	0.37	0.3	0.27	0.62	0.04

abc=in the column, indicates statistical differences ($P<0.05$) in the harvest age.
28, 35, 42, 49=harvest age of the cultivar. EE (SE): standard error.

The higher NDF percentage in the grasses provides more cellulose and hemicellulose, which can potentially be fermented by cellulolytic bacteria (such as *Ruminococcus flavefaciens*, *Ruminococcus albus*, and *Fibrobacter succinogenes*), degrading glucose and even acetate and butyrate (Ley de Coss *et al.*, 2018).

There were no differences ($P>0.05$) in biomass production, resulting from the harvest ages under study (Table 3). However, there were differences related to harvest age during the estimation of the CH_4 and CO_2 of the grasses. The highest CH_4 production was recorded at day 35 for both cultivars. Meanwhile, Cobra recorded the highest CO_2 production at day 49, while Cayman reported its highest production at day 42.

Biogas production increases or decreases during the *in vitro* fermentation depending on the nutritional value of the forage (Camacho-Escobar *et al.*, 2020). Feeds with a higher structural carbohydrate content and a lower CP content tend to produce a higher biogas volume, while feeds with a higher soluble carbohydrate content and higher CP percentages produce a lower biogas volume.

The results of this study confirm the findings of previous studies. A lower CH_4 production —along with a higher CP content and a lower NDF, ADF, and cellulose percentage— was recorded at day 28 in both cultivars. These elements are components of the cell wall of vegetables and of the acetate:propionate ratio recorded between both evaluated cultivars. Methane production is the result of the fermentation of the structural carbohydrates. Rumen with a higher quantity of acetate results in the creation of two molecules: CO_2 and 8 H^+. Butyrate production also develops two molecules: CO_2 and 4 H^+. As a result of their metabolism, *Archaeas* use CO_2 and hydrogen to produce CH_4 and ATP (Bedoya-Mazo *et al.*, 2016).

Table 3. Biogas, CH_4, and CO_2 (mL g^{-1} DM) in vitro production of the Cobra and Cayman grasses, at different harvest ages.

Cultivar	Producción de biogás total (mL g^{-1} MS)					CH_4	CO_2
	6 h	12 h	24 h	48 h	72 h	72 h	
Cobra							
28d	23.46^{a}	25.20^{a}	56.13^{a}	34.00^{a}	10.66^{a}	37.06^{c}	104.41^{c}
35d	29.00^{a}	25.40^{a}	49.33^{a}	27.73^{a}	11.73^{a}	43.55^{b}	117.38^{b}
42d	31.86^{a}	26.66^{a}	54.86^{a}	35.80^{a}	8.73^{a}	47.53^{a}	120.25^{b}
49d	27.13^{a}	30.33^{a}	33.20^{a}	29.33^{a}	6.20^{a}	45.01^{a}	135.28^{a}
EE	2.98	6.16	5.82	3.27	2.25	0.84	2.18
Cayman							
28d	35.46^{a}	25.26^{a}	58.20^{a}	37.33^{a}	9.66^{a}	41.29^{c}	121.06^{b}
35d	37.33^{a}	29.20^{a}	44.20^{a}	24.80^{a}	12.20^{a}	43.66^{b}	134.05^{a}
42d	36.53^{a}	31.13^{a}	51.53^{a}	23.66^{a}	13.86^{a}	46.54^{a}	135.38^{a}
49d	18.66^{a}	14.60^{a}	27.20^{a}	30.33^{a}	10.40^{a}	42.48^{bc}	120.31^{b}
EE	8.52	4.55	9.11	8.71	2.77	0.51	0.87

abc=in the column, indicates statistical differences ($P<0.05$) in the harvest age.
28, 35, 42, 49=harvest age of the cultivar. EE (SE): standard error.

CONCLUSIONS

The drought season had an impact on the DM production of the Cobra and Cayman cultivars. The best age to harvest these cultivars ranges from 28 to 35 d, when they have a higher CP content and a lower NDF, ADF, hemicellulose, and cellulose content. This phenomenon resulted in a higher *in vitro* degradation of the DM of both cultivars.

During the *in vitro* fermentation, both cultivars recorded their lowest methane production between 28 and 35 harvest days; consequently, under drought conditions, the best age to harvest the Cobra and Cayman grasses, in the coastal region of Oaxaca, is between 28 and 35 d after the regrowth.

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