fermentation

MDPI

Article

Rumen Fermentation Profile and Greenhouse Gas Mitigation of Three Forage Species from Agroforestry Systems in Dry and Rainy Seasons

Gary Meza-Bone [1,2], Carlos Meza-Bone [1], Juan Avellaneda-Cevallos [1,3], Ronald Cabezas-Congo [1], Ronald Villamar-Torres [1,2], María Cabanilla-Campos [1], Walter Vivas-Arturo [4], Frank Intriago-Flor [4], Fabricio Meza-Bone [5], Aurelio Zapatier-Santillan [1,2], Ricardo Bastidas-Espinoza [2], Tito Solís-Barros [1], Luz Muñoz-Arboleda [6], Walter Garcia-Cox [5] and Marcos Barros-Rodríguez [7,*]

1 Carrera Agropecuaria, Facultad de Ciencias Pecuarias y Biológicas, Universidad Técnica Estatal de Quevedo, km 7 $\frac{1}{2}$ vía Quevedo-El Empalme, Quevedo CP 120301, Los Ríos, Ecuador
2 Instituto Superior Tecnológico Ciudad de Valencia, km 3 1/2 vía a Valencia Sector El Pital 1, Quevedo CP 120301, Los Ríos, Ecuador
3 Programa de Pastos y Ganadería, Estación Experimental Tropical Pichilingue, Instituto Nacional de Investigaciones Agropecuarias-INIAP, km 7 vía Quevedo-El Empalme, Quevedo CP 120301, Los Ríos, Ecuador
4 Facultad de Ciencias Zootécnica, Universidad Técnica de Manabí, Sitio Las Animas km 2.5 vía Boyaca, Portoviejo 130709, Manabí, Ecuador
5 Carrera Forestal, Facultad de Ciencias Agrarias y Forestal, Universidad Técnica Estatal de Quevedo, km 7 $\frac{1}{2}$ vía Quevedo-El Empalme, Quevedo CP 120301, Los Ríos, Ecuador
6 Facultad de Ciencias Agropecuarias, Universidad Nacional de Colombia, Sede Palmira, Palmira AA 237, Valle del Cauca, Colombia
7 Facultad de Ciencias Agropecuarias, Universidad Técnica de Ambato, Sector el Tambo-La Universidad, Vía a Quero, Cevallos CP 1801334, Tungurahua, Ecuador
* Correspondence: ma_barrosr@yahoo.es

check for updates

Citation: Meza-Bone, G.; Meza-Bone, C.; Avellaneda-Cevallos, J.; Cabezas-Congo, R.; Villamar-Torres, R.; Cabanilla-Campos, M.; Vivas-Arturo, W.; Intriago-Flor, F.; Meza-Bone, F.; Zapatier-Santillan, A.; et al. Rumen Fermentation Profile and Greenhouse Gas Mitigation of Three Forage Species from Agroforestry Systems in Dry and Rainy Seasons. *Fermentation* **2022**, *8*, 630. https://doi.org/10.3390/fermentation8110630

Academic Editors: Peixin Fan and Qing Zhang

Received: 30 September 2022
Accepted: 8 November 2022
Published: 11 November 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: The variability of climatic conditions throughout the year and the physiological maturity of plant material can have effects on the nutritional value of plants and the productive performance of animals. The aim was to determine the effect of three tropical forage species (*Tithonia diversifolia*, *Erythrina poeppigiana* and *Cratylia argentea*) harvested at four cutting ages (30, 45, 60 and 75 days), during the dry and rainy seasons on in vitro gas production, rumen pH, volatile fatty acids (VFA) and CH_4 production. A completely randomized design in a factorial arrangement (three forage species, four cutting ages and two seasons) was used. Four Brahman bulls of 250.0 ± 20.5 kg of live weight were used, provided with a ruminal cannula for the extraction of ruminal fluid. The animals were kept separately in individual pens, where they were fed with *P. maximum* and water was provided *ad libitum*. Gas production among forage species was lower ($p = 0.0001$) in *T. diversifolia* and *E. poeppigiana* in the dry season. The cutting age had an influence on gas production, showing lower production ($p = 0.0164$) at 30 and 45 days in the dry season. The VFA production showed differences for the effect between forages in the rainy season, with higher values ($p < 0.05$) for acetic, propionic and butyric acid in the species *E. poeppigiana*, *C. argéntea* and *T. diversifolia* respectively. CH_4 production showed differences between forage species, showing the lowest ($p = 0.0001$) production of CH_4 in *T. diversifolia* for the dry and rainy seasons. It is concluded that the use of shrubby species (*T. diversifolia, E. poeppigiana and C. argéntea*) in ruminant production systems in the rainy and dry seasons, with an early regrowth age (30 and 45 days), present a promising alternative on the reduction of CH_4.

Keywords: gas production; methane mitigation; tropical forages; volatile fatty acids

1. Introduction

Ruminant production systems in tropical, subtropical, semi-arid and arid zones around the world are restricted by the limited availability and low quality of forage resources

throughout the year [1,2]. Forage resources characterized mainly by presenting variability in their nutritional components [3,4], probably influenced by: (a) variability of climatic conditions during the year (spring, summer, autumn and winter), (b) geographical area, (c) physiological development of the plant and (d) soil fertility [5]. The supply of poor-quality forage sources affects the productive performance of animals, by generating considerable energy losses that fluctuate between 2–15% of the energy available for production, as a consequence of the generation of greenhouse gases (GHG): CH_4, CO_2 and N_2O, mainly in response to the presence of structural carbohydrates (cellulose and hemicellulose) in the feed [6,7]. In this way, it is estimated that livestock activity is responsible for generating approximately 14.5% of global GHG emissions of anthropogenic origin. Of which, it is estimated that livestock contributes with about 47% of CH_4, 27% of CO_2 and 29% of N_2O [8–10].

The emission of GHG into the environment exacerbates the present problem of global warming. Therefore, there is currently a growing interest in reducing GHG emissions, mainly CH_4 [11], due to its warming potential 28 times more than CO_2 [12]. GHG production is an inevitable consequence of the anaerobic fermentation of feed in ruminants, observed with greater emphasis in animals that feed on low-quality forage (high in fiber), typical of semi-arid and arid areas where rain is scarce and solar radiation and environmental temperature is high [13]. An alternative to this problem is the use of tree or shrub legumes with forage potential, due to their ability to reduce GHG generation [14], in response to the presence of secondary metabolites (tannins, saponins, oils essential) that can modify the ruminal fermentation profile and reduce the production of CH_4 [15,16]. However, the quality of the forage can vary according to the time of year, even among plants of the same species, probably due to a greater deposition of structural carbohydrates in the cell wall of the plants, due to the high solar radiation and lower humidity in the dry season, compared to the rainy season [17]. Aspects that limit the productive performance of animals and increase susceptibility to diseases in response to the decrease in the nutritional value of ingested forage, evidenced mainly by the low availability of protein, highly soluble carbohydrates and the reduction in the proportion of leaf area in the plant (ratio; leaves/stems) [18].

Many plants with forage potential for ruminant feeding have been undervalued, probably due to ignorance of their benefits and the variability of their nutritional value at different times of the year (spring, summer, autumn and winter) [19]. The use of these resources could favor the yield of forage biomass per unit of land used, unlike herbaceous forages that have a shallow root system and are affected in times of little rain, with delayed growth and decreased productivity. its nutritional value [20,21]. In this context, a notable variability has been evidenced on the protein content in *Ceirchrus ciliaris* L. influenced by the time of year (17.1% in the summer and 10.5% in the winter) [22]. In addition, forage trees and shrubs are usually rich in secondary compounds, mainly condensed tannins (CT) which, when combined with traditional grasslands, have the ability to decrease CH_4 production [23]. Effects that are probably attributed to the decrease in methanogenic archaea and protozoa in the rumen in response to: (1) inhibition of H_2 exchange between species (methanogen-protozoa) due to the effect of CT on the protein adhesin and subsequent change in the permeability of the cell membrane that affects the growth and activity of methanogen microorganisms, (2) formation of tannin-protein complexes that decreases the availability of nutrients for ruminal microorganisms and compromises their development, and (3) decrease in available H_2 for CO_2 reduction to CH_4 [24]. However, the effects of tannins fluctuate depending on the type, source, molecular weight, dose and the adaptation of animals to their consumption. To such an extent that negative effects on productive performance, feed consumption, digestion and animal health have been evidenced. Effects that are probably attributed to: (i) interaction of CT with salivary glycoproteins and subsequent decrease in feed palatability, (ii) greater permanence of feed in the rumen as a consequence of decreased substrate digestibility, (iii) intoxication by the excessive

consumption of CT (>55 g CT/kg DM) and (iv) reduced intestinal enzymatic activity of trypsin, amylase and amino acid synthesis [25].

The use of forage shrubs is presented as a promising alternative in ruminant feeding. *Tithonia diversifolia* is a shrub whose forage contains between 19.3–22.9% crude protein (CP), 18.4–25.5% dry matter (DM), 84.0–86.6% organic matter (OM) and 18.1–19.4% crude fiber (CF) [26,27] and 37.9–48.1 g/kg MS of TC [28]. *Erythrina poeppigiana* is a legume characterized by a high concentration of PC (20.2–32.0%), DM (23.2%), MO (90.7%), neutral detergent fiber (NDF: 53.4–60.9%), acid detergent fiber (ADF: 31.5–37.3%) and CT (0.4%) [29,30]. *Cratylia argentea* is also a legume with high nutritional value (CP: 19.5–24.0%, OM: 88.5%, NDF: 56.4%, ADF: 32.3% and TC: 0.2%) [30,31]. Therefore, it is hypothesized that *T. diversifolia*, *E. poeppigiana* and *C. argéntea* are forages that can be useful as feed for ruminants throughout the year and probably reduce CH_4 production. Based on this background, the aim of this research work was to determine the effect of three tropical forage species at four cutting ages, during the dry and rainy seasons, on in vitro gas production, rumen pH, volatile fatty acids and methane production.

2. Materials and Methods

2.1. Location

The research was carried out at the Experimental Farm "La María", of the State Technical University of Quevedo, located in the Province of Los Ríos, in the Canton Mocache, located at km 7 $\frac{1}{2}$ of the Quevedo-El Empalme road, in an area classified as tropical humid forest, at a height of 73 m above sea level.

2.2. Animals

Four Brahman bulls of 250.0 ± 20.5 kg of live weight were used, provided with a ruminal cannula (four inches of internal diameter, Bar Diamond, Parma, ID, USA) for the extraction of ruminal fluid. The animals were kept separately in individual pens, where they were fed with *P. maximum* and water was provided *ad libitum*

2.3. Forage Samples

The forage was harvested from an agroforestry system established in 2016, located at the following geographic coordinates (Latitude: 1°05′0.19″ South, Longitude: 79°29′59.86″ West). The entire plant (leaves and stems) of three shrub species (*T. diversifolia*, *E. poeppigiana* and *C. argentea*) was used and an equalization cut was made and subsequently experimental cuts were made 30, 45, 60 and 75 days for the dry and rainy season. The plant samples were harvested in the rainy season from January to March (with a monthly rainfall of 270–600 mm and a temperature of 22–30 °C) and for the dry season from September to November (with a monthly rainfall of 0 mm and a temperature of 20–30 °C), the samples were taken from the same plants in both seasons manually at 0.50 cm from the soil towards the apex of the plant. Each shrub species were identified by Dr. F Meza-Bone, professor at the Facultad de Ciencias Agrarias y Forestal, Universidad Técnica Estatal de Quevedo (in spanish). A voucher specimen of *T. diversifolia*, *E. poeppigiana* and *C. argentea* has been deposited in our laboratory (voucher No. UTEQ-1001, 1002 and 1003 respectively). The sampled plant material (5 kg per plant: 20 plants of each species) leaves and stems were dried at 60 °C (72 h) in a forced air oven until reaching constant weight to determine the dry matter and chemical composition, later it was ground in a hammer mill (THOMAS-Wiley, USA, Model 4) and the resulting plant material was passed through a 1 mm sieve to homogenize the particle size.

2.4. Gas, CH_4 Production and Rumen pH

The rumen content (solid and liquid fraction) was obtained from 4 bulls provided with ruminal cannula. Rumen content was collected before feeding and kept at 39 °C in a sealed plastic container during transport to the laboratory. The nitrogen-rich medium (artificial saliva) was prepared as described by Menke and Steingass [32]. Gas production was determined according to the methodology described by Theodorou et al. [33] which

consists of placing 0.400 g of dry matter from each treatment was placed in serum bottles of 100 mL nominal capacity, 60 mL of inoculum (70:30 medium; artificial saliva/inoculum; rumen content) was added under a constant CO_2 flow. The bottles were sealed and incubated at 39–40 °C. Gas pressure and volume were measured manually at 3, 6, 9, 12, 24, 48, 72 and 96 h after incubation with a DELTA OHM model DO 9704 pressure transducer (Delta OHM, Padova, Italy) and plastic syringes. CH_4 production was quantified with a GX-6000 gas monitor (RKI Instruments gas analyzer, GX-6000, Tokyo, Japan) following the methodology described by Elghandour et al. [34]. For each treatment, four bottles were used and four additional bottles as blank. At the end of 96 h, the gas and CH_4 data (mLgas or CH4/g MS incubated) were fitted to the equation $y = D\ (1 - e^{-k\ t})$ described by Krishnamoorthy et al. [35], where:

y = cumulative gas production at a given time (mL)
D = potential cumulative gas production (mL)
k = rate of gas production (h-I)
t = time of fermentation (h)

The rumen pH was measured from the same bottles with the help of a pH meter (BANTE-221 Portable pH/ORP Meter, London, UK) at 6, 12 and 24 h post incubation.

2.5. Fermentation Profile

Four additional bottles for each treatment were prepared following the same methodology indicated above for Gas and CH_4 production. 6 h after incubation, 8 mL of sample was collected from each treatment and repetition and mixed with 2 mL of 25% metaphosphoric acid and stored at −4 °C until analysis, AGVs were analyzed by means of a gas chromatograph (Clarus 400, PerkinElmer, Shelton, CT, USA) following the methodology described by Ryan [36].

2.6. Experimental Design and Statistical Analysis

A completely randomized design was used in a factorial arrangement (three forage species—four cutting ages and two seasons) with four repetitions per treatment, as follows:

$$Y_{ijk} = \mu + A_i + B_j + C_k + AB_{ij} + AC_{ik} + BC_{jk} + ABC_{ijk} + \sum_{ijk}$$

where:

Y_{ijk} = Observation of the response variable obtained from the treatment with the i-th level of A, the j-th level of B, the k-th level of C
μ = Overall mean
A_i = Effect of the i-th level of the forage species factor
B_j = Effect of the j-th factor level of the cutting ages
C_k = Effect of the k-th level of the epoch factor
AB_{ij} = Effect of the interaction of the i-th level of factor A and the j-th level of factor B
AC_{ij} = Effect of the interaction of the i-th level of factor A and the k-th level of factor C
BC_{jk} = Effect of the interaction of the j-th level of factor B and the k-th level of factor C

All the variables were analyzed according to the proposed experimental design, using the PROC GML of the SAS. All means were compared using Tukey test.

3. Results

3.1. Gas, CH_4 Production and Rumen pH

Gas production showed differences ($p < 0.05$) between forage species, cutting age, interaction between forage species and cutting age, and on the dry and rainy seasons (Table 1). The accumulated gas production between forage species was lower ($p < 0.05$) in *T. diversifolia* (108.0 mLgas/0.400 g incubated DM) and *E. poeppigiana* (114.01 mLgas/0.400 g incubated DM) in the dry season, and in the rainy season it was lower ($p < 0.05$) in *T. diversifolia* (96.99 gas/0.400 g incubated DM). On the other hand, the effect between seasons (dry and rainy) showed lower ($p < 0.05$) accumulated gas production in *T. diversifolia* (96.9 mLgas/0.400 g incubated DM) and *C. argéntea* (118.3 mLgas/0.400 g incubated DM)

in the rainy season, however, in the dry season *E. poeppigiana* showed lower ($p < 0.05$) gas production (114.01 mL gas/0.400 g incubated DM) compared to the other forages.

Table 1. In vitro gas production parameters (mL gas/0.400 g incubated DM) and rumen pH of shrub and tree species at different cutting ages, in the dry and rainy seasons.

		Dry Seasons					Rainy Seasons				
		Gas Production		Rumen pH			Gas Production		Rumen pH		
		D	*k*	6 h	12 h	24 h	D	*k*	6 h	12 h	24 h
Forage											
T. diversifolia		108.0 ^{bA}	0.045 ^{bA}	7.62 ^{aA}	7.62 ^{aA}	7.70 ^{aA}	96.9 ^{bB}	0.047 ^{bA}	7.62 ^{aA}	7.63 ^{aA}	7.67 ^{aA}
E. poeppigiana		114.0 ^{bB}	0.040 ^{bA}	7.60 ^{aA}	7.66 ^{aA}	7.65 ^{aA}	116.3 ^{aA}	0.037 ^{cB}	7.60 ^{aA}	7.65 ^{aA}	7.62 ^{aA}
C. argentea		121.7 ^{aA}	0.053 ^{aA}	7.62 ^{aA}	7.67 ^{aA}	7.67 ^{aA}	118.3 ^{aB}	0.054 ^{aA}	7.63 ^{aA}	7.69 ^{aA}	7.65 ^{aA}
Ages (day)											
30		108.2 ^{bA}	0.037 ^{cA}	7.60 ^{aA}	7.67 ^{aA}	7.67 ^{aA}	103.5 ^{cB}	0.037 ^{cA}	7.61 ^{aA}	7.68 ^{aA}	7.64 ^{aA}
45		114.3 ^{abA}	0.037 ^{cB}	7.61 ^{aA}	7.66 ^{aA}	7.65 ^{aA}	108.9 ^{bB}	0.044 ^{bcA}	7.61 ^{aA}	7.68 ^{aA}	7.63 ^{aA}
60		117.3 ^{aA}	0.049 ^{bA}	7.62 ^{aA}	7.63 ^{aA}	7.65 ^{aA}	112.6 ^{abB}	0.047 ^{bA}	7.63 ^{aA}	7.65 ^{aA}	7.62 ^{aA}
75		118.4 ^{aA}	0.062 ^{aA}	7.61 ^{aA}	7.62 ^{aA}	7.71 ^{aA}	117.1 ^{aA}	0.056 ^{aB}	7.62 ^{aA}	7.62 ^{aA}	7.69 ^{aA}
Inteaction											
T. diversifolia	30	100.8 ^{bA}	0.034 ^{bA}	7.65 ^{aA}	7.70 ^{aA}	7.73 ^{aA}	88.8 ^{dA}	0.035 ^{dA}	7.65 ^{aA}	7.70 ^{aA}	7.71 ^{aA}
	45	105.4 ^{abA}	0.033 ^{bB}	7.61 ^{aA}	7.67 ^{aA}	7.70 ^{aA}	95.0 ^{dB}	0.039 ^{cdA}	7.62 ^{aA}	7.67 ^{aA}	7.67 ^{aA}
	60	112.5 ^{abA}	0.040 ^{bB}	7.61 ^{aA}	7.57 ^{aA}	7.67 ^{aA}	96.1 ^{cdB}	0.045 ^{bcdA}	7.62 ^{aA}	7.61 ^{aA}	7.64 ^{aA}
	75	113.1 ^{abA}	0.073 ^{aA}	7.61 ^{aA}	7.53 ^{aA}	7.69 ^{aA}	107.8 ^{bB}	0.068 ^{aB}	7.60 ^{aA}	7.53 ^{aA}	7.67 ^{aA}
E. poeppigiana	30	105.2 ^{abA}	0.036 ^{bA}	7.57 ^{aA}	7.66 ^{aA}	7.62 ^{aA}	107.4 ^{bcA}	0.033 ^{dB}	7.58 ^{aA}	7.67 ^{aA}	7.58 ^{aA}
	45	115.0 ^{abA}	0.036 ^{bB}	7.61 ^{aA}	7.69 ^{aA}	7.64 ^{aA}	113.0 ^{abA}	0.038 ^{cdA}	7.61 ^{aA}	7.65 ^{aA}	7.61 ^{aA}
	60	116.8 ^{abB}	0.064 ^{aA}	7.59 ^{aA}	7.67 ^{aA}	7.62 ^{aA}	122.1 ^{aA}	0.038 ^{cdB}	7.59 ^{aA}	7.66 ^{aA}	7.61 ^{aA}
	75	118.9 ^{abB}	0.067 ^{aA}	7.63 ^{aA}	7.62 ^{aA}	7.71 ^{aA}	122.6 ^{aA}	0.039 ^{cdB}	7.62 ^{aA}	7.62 ^{aA}	7.69 ^{aA}
C. argentea	30	118.5 ^{abA}	0.041 ^{bA}	7.59 ^{aA}	7.65 ^{aA}	7.68 ^{aA}	114.3 ^{abB}	0.042 ^{cdA}	7.60 ^{aA}	7.66 ^{aA}	7.64 ^{aA}
	45	122.4 ^{aA}	0.040 ^{bB}	7.61 ^{aA}	7.64 ^{aA}	7.62 ^{aA}	118.6 ^{abB}	0.054 ^{abcA}	7.61 ^{aA}	7.71 ^{aA}	7.61 ^{aA}
	60	122.6 ^{aA}	0.064 ^{aA}	7.68 ^{aA}	7.67 ^{aA}	7.66 ^{aA}	119.5 ^{aB}	0.059 ^{abB}	7.68 ^{aA}	7.69 ^{aA}	7.62 ^{aA}
	75	123.3 ^{aA}	0.067 ^{aA}	7.61 ^{aA}	7.71 ^{aA}	7.73 ^{aA}	121.0 ^{aB}	0.068 ^{abA}	7.63 ^{aA}	7.71 ^{aA}	7.72 ^{aA}
SE		4.03	0.003	0.02	0.06	0.04	1.35	0.003	0.03	0.05	0.04
Forage		0.0001	0.0001	0.2711	0.4566	0.1411	0.0001	0.0001	0.2475	0.2236	0.3252
Ages		0.0164	0.0001	0.7753	0.6894	0.1754	0.0001	0.0001	0.8662	0.4363	0.2363
Forage × Ages		0.8737	0.0001	0.1073	0.4777	0.5102	0.0552	0.0016	0.3361	0.5281	0.6432

$^{a–d}$ The letters in lowercase superscript in the column make significant reference ($p < 0.05$) to the forage species and cutting ages.; ^{AB} The letters in uppercase superscript in the rows make a significant reference ($p < 0.05$) between dry and rainy seasons.; D = potential cumulative gas production (mL), *k* = rate of gas production (h-I).; SE = standard error.

Regarding the cutting age effect in the dry season, it showed lower ($p = 0.0164$) gas production at 30 and 45 days (108.20 and 114.31 mLgas/0.400 g incubated DM, respectively), however, in the dry season lower ($p = 0.0001$) gas production at 30 days (103.53 mLgas/0.400 g incubated DM) was evidenced. The effect between seasons (dry and rainy) in the cutting ages showed differences, obtaining 4.3% lower ($p < 0.05$) gas production at 30, 45 and 60 days in the rainy season compared to the dry season. Regarding the interaction between forage species and cutting ages, no differences were observed for the two seasons (dry and rainy: $p = 0.8737$ and $p = 0.0552$, respectively) (Table 1).

CH_4 production showed an effect between forage species, showing the lowest CH_4 production in *T. diversifolia* for both the dry and rainy seasons, 73.5 and 51.7 mL CH_4/0.400 g incubated DM, respectively. On the other hand, the effect between seasons on the CH_4 production was lower in *E. poeppigiana* and *C. argéntea* for the rainy season (64.0 and 73.5 mL CH_4/0.400 g incubated DM, respectively). Regarding the production of CH_4 influenced by the cutting age effect, it was less than 30 days for both the dry and rainy seasons ($p = 0.0001$ and $p = 0.0001$, respectively). However, the effect between seasons (dry and rainy) showed lower ($p < 0.05$) CH_4 production at 45, 60 and 75 days of the rainy season. The interaction between forage species and cutting ages showed an effect ($p = 0.0001$) on CH_4 production in the dry season (Table 2).

Table 2. CH_4 production parameters ($mLCH_4$/0.400 g incubated DM) of shrub and tree species at different cutting ages, in the dry and rainy seasons.

		Dry Seasons		Rainy Seasons	
		CH_4 Production		CH_4 Production	
		D	*k*	D	*k*
Forage					
T. diversifolia		73.5 ^{cA}	1.23 ^{aA}	51.7 ^{cA}	1.21 ^{aA}
E. poeppigiana		95.7 ^{bA}	1.06 ^{bA}	64.0 ^{bB}	1.20 ^{aA}
C. argéntea		132.1 ^{aA}	1.04 ^{bA}	73.5 ^{aB}	0.91 ^{bB}
Ages (days)					
30		42.1 ^{dA}	1.24 ^{aA}	35.7 ^{dA}	1.34 ^{aA}
45		74.2 ^{cA}	1.18 ^{aA}	54.1 ^{cB}	1.11 ^{bA}
60		136.2 ^{bA}	1.14 ^{aA}	75.2 ^{bB}	0.90 ^{cA}
75		149.3 ^{aA}	0.88 ^{bA}	87.2 ^{aB}	1.08 ^{bA}
Inteaction					
T. diversifolia	30	21.3 ^{fA}	1.39 ^{aA}	19.9 ^{dA}	1.39 ^{bA}
	45	50.9 ^{eA}	0.92 ^{aA}	38.0 ^{cdA}	1.39 ^{abA}
	60	116.0 ^{bcA}	0.92 ^{deA}	73.7 ^{abB}	0.88 ^{eA}
	75	105.7 ^{cA}	1.22 ^{abcA}	75.0 ^{abB}	1.25 ^{bcA}
E. poeppigiana	30	52.8 ^{eA}	1.26 ^{abB}	33.8 ^{cdB}	1.64 ^{aA}
	45	92.0 ^{cdA}	1.10 ^{bcdA}	54.1 ^{bcB}	1.04 ^{cdeA}
	60	182.6 ^{aA}	0.75 ^{eB}	74.7 ^{abB}	0.95 ^{deA}
	75	201.1 ^{aA}	1.12 ^{bcdA}	93.3 ^{aB}	1.16 ^{bcdA}
C. argéntea	30	52.1 ^{eA}	1.05 ^{bcdA}	53.3 ^{bcA}	1.05 ^{cdeA}
	45	79.5 ^{dA}	1.04 ^{bcdA}	70.1 ^{abA}	0.89 ^{eB}
	60	110.2 ^{cA}	0.97 ^{cdeA}	77.2 ^{abA}	0.87 ^{eA}
	75	141.2 ^{bA}	1.08 ^{bcdA}	93.3 ^{aB}	0.83 ^{eB}
SE		5.22	0.05	5.01	0.05
Forage		0.0001	0.0001	0.0001	0.0001
Ages		0.0001	0.0001	0.0001	0.0001
Forage × Ages		0.0001	0.0013	0.0527	0.0001

$^{a–f}$ The letters in lowercase superscript in the column make significant reference ($p < 0.05$) to the forage species and cutting ages.; ^{AB} The letters in uppercase superscript in the rows make a significant reference ($p < 0.05$) between dry and rainy seasons.; D = potential cumulative CH_4 production (mL), *k* = rate of CH_4 production (h-I).; SE = standard error.

The rumen pH at 6, 12 and 24 h did not show differences ($p > 0.05$) in the variables evaluated (Table 1).

3.2. Fermentation Profile

Volatile Fatty Acids and total VFAs in the dry season did not show differences in the effect of forage, age and interaction between forage and age ($p = 0.5218$, $p = 0.9306$ and $p = 0.9547$, respectively). However, in the rainy season, the production of acetic acid, propionic acid and butyric acid among forage species was higher in *E. poeppigiana* (76.39 mol %), *C. argéntea* (16.31 mol %) and *T. diversifolia* (8.16 mol %) respectively. On the other hand, the effect between seasons (dry and rainy) showed differences, obtaining higher mol % of propionic acid and butyric acid in the dry season for *T. diversifolia*, *E. poeppigiana* and *C. argéntea* respectively ($p < 0.05$). Regarding the effect of cutting ages in both the dry and rainy seasons, no differences were observed for all the variables evaluated ($p > 0.05$). However, the effect between seasons (dry and rainy) the dry season showed higher ($p < 0.05$) mol % of butyric acid in all cutting ages, while in the rainy season the acetic acid was higher ($p < 0.05$) for all cutting ages. The interaction between forage species and cutting age did not show differences ($p > 0.05$) on VFA production in the dry season. However, a higher production of acetic acid was observed in the rainy season ($p = 0.0007$) (Table 3).

Table 3. In vitro proportion of volatile fatty acids in the rumen (mol %) of shrub and tree species at different cutting ages, in the dry and rainy seasons.

		Dry Seasons				Rainy Seasons			
		Rumen VFA (mol %)				Rumen VFA (mol %)			
		Acetic	Propionic	Butyric	Total FVA (mmol/L)	Acetic	Propionic	Butyric	Total FVA (mmol/L)
Forage									
T. diversifolia		73.19 ^{aB}	16.54 ^{aA}	10.27 ^{aA}	35.56 ^{aA}	76.03 ^{abA}	15.81 ^{bB}	8.16 ^{aB}	30.18 ^{aB}
E. poeppigiana		73.36 ^{aB}	16.69 ^{aA}	9.94 ^{aA}	34.28 ^{aA}	76.39 ^{aA}	15.92 ^{bA}	7.70 ^{bB}	29.34 ^{aA}
C. argéntea		73.82 ^{aB}	16.75 ^{aA}	9.43 ^{aA}	34.04 ^{aA}	75.72 ^{bA}	16.31 ^{aA}	7.97 ^{abB}	32.28 ^{aA}
Ages (days)									
30		73.53 ^{aB}	16.49 ^{aA}	9.99 ^{aA}	34.70 ^{aA}	76.31 ^{aA}	15.83 ^{aA}	7.86 ^{aB}	29.94 ^{aA}
45		73.29 ^{aB}	16.66 ^{aA}	10.06 ^{aA}	33.84 ^{aA}	75.88 ^{aA}	16.11 ^{aA}	8.01 ^{aB}	28.02 ^{aB}
60		73.34 ^{aB}	16.65 ^{aA}	10.01 ^{aA}	34.65 ^{aA}	76.02 ^{aA}	16.00 ^{aA}	7.98 ^{aB}	32.56 ^{aA}
75		73.67 ^{aB}	16.86 ^{aA}	9.47 ^{aA}	35.32 ^{aA}	75.96 ^{aA}	16.10 ^{aA}	7.93 ^{aB}	31.88 ^{aA}
Inteaction									
T. diversifolia	30	73.08 ^{aB}	16.17 ^{aA}	10.74 ^{aA}	35.57 ^{aA}	76.50 ^{abcA}	15.53 ^{aA}	7.97 ^{aA}	29.07 ^{aA}
	45	73.51 ^{aB}	16.34 ^{aA}	10.16 ^{aA}	36.51 ^{aA}	75.65 ^{bcdA}	15.90 ^{aA}	8.45 ^{aA}	29.36 ^{aA}
	60	73.18 ^{aB}	16.58 ^{aA}	10.25 ^{aA}	36.19 ^{aA}	75.89 ^{abcdA}	15.98 ^{aA}	8.13 ^{aB}	28.59 ^{aA}
	75	72.98 ^{aB}	7.09 ^{aA}	9.93 ^{aA}	33.99 ^{aA}	76.07 ^{abcdA}	15.84 ^{aB}	8.10 ^{aA}	33.72 ^{aA}
E. poeppigiana	30	73.61 ^{aB}	16.50 ^{aA}	9.89 ^{aA}	35.17 ^{aA}	76.10 ^{abcdA}	16.01 ^{aA}	7.90 ^{aA}	27.57 ^{aA}
	45	72.65 ^{aB}	17.20 ^{aA}	10.16 ^{aA}	32.12 ^{aA}	75.99 ^{abcdA}	16.17 ^{aA}	7.85 ^{aA}	28.31 ^{aA}
	60	73.39 ^{aB}	16.66 ^{aA}	9.95 ^{aA}	35.03 ^{aA}	76.75 ^{aA}	15.65 ^{aA}	7.61 ^{aA}	32.88 ^{aA}
	75	73.81 ^{aB}	16.42 ^{aA}	9.77 ^{aA}	34.81 ^{aA}	76.73 ^{abA}	15.84 ^{aA}	7.44 ^{aA}	33.33 ^{aA}
C. Argéntea	30	73.89 ^{aB}	16.78 ^{aA}	9.33 ^{aA}	33.35 ^{aA}	76.33 ^{abcA}	15.96 ^{aA}	7.71 ^{aA}	33.17 ^{aA}
	45	73.70 ^{aB}	16.44 ^{aA}	9.86 ^{aA}	32.88 ^{aA}	76.02 ^{abcdA}	16.26 ^{aA}	7.72 ^{aA}	26.41 ^{aA}
	60	73.46 ^{aA}	16.71 ^{aA}	9.83 ^{aA}	32.74 ^{aA}	75.42 ^{cdA}	16.38 ^{aA}	8.20 ^{aA}	36.21 ^{aA}
	75	74.22 ^{aA}	17.08 ^{aA}	8.71 ^{aA}	37.18 ^{aA}	75.10 ^{dA}	16.64 ^{aA}	8.26 ^{aA}	33.33 ^{aA}
SE		0.79	0.45	0.82	1.75	0.22	0.19	0.22	4.45
Forage		0.5218	0.7921	0.3599	0.4255	0.0006	0.0104	0.0200	0.6326
Ages		0.9306	0.7846	0.7960	0.7797	0.1155	0.2401	0.8582	0.5947
Forage × Ages		0.9547	0.6494	0.9872	0.3887	0.0007	0.1518	0.1631	0.8688

$^{a–d}$ The letters in lowercase superscript in the column make significant reference ($p < 0.05$) to the forage species and cutting ages.; ^{AB} The letters in uppercase superscript in the rows make a significant reference ($p < 0.05$) between dry and rainy seasons.; SE = standard error.

4. Discussion

4.1. Gas, CH_4 Production and Rumen pH

The nutritional value of plants varies according to the geographical area, seasonality of the year, forage species and regrowth age. The results obtained in the present study on in vitro gas production were influenced by the forage species, cutting age, interaction between forage species and cutting age, and the dry and rainy seasons (Table 1). This variability of responses could probably be due to the nutritional quality of the forage, associated with: (1) time of year (spring, summer, autumn and winter), (2) physiological maturity of the plant, which exerts changes on the components of the cell wall and protein [16,37,38] and, (3) presence of bioactive compounds (CT), which influence the rate of degradation and digestibility of the feed [39].

Under these concepts, the lower gas production evidenced in the rainy season in *T. diversifolia* and *C. argentea* compared to the dry season can probably be derived from the lower content of structural carbohydrates, influenced by the limited amount of sunlight available in the rainy season [13]. Contrary to this, the dry season, when presenting a greater intensity of solar radiation and temperature, could generate changes on the fibrous components in the plant, showing a greater amount of structural carbohydrates (cellulose and hemicellulose) [17,19,40]. In the same way, the moisture deficit in the dry season

probably negatively influences the growth of forages, decreasing the leaf area, increasing the amount of structural carbohydrates, decreasing the photosynthetic activity that affects the production and nutritional quality of the plants [41]. In addition, the gas production could be influenced by the low amount of N in the forages in the dry season, resulting in a decrease in microbial growth as an effect of the low availability of NH_3 in the rumen [42]. Or by the decrease in the N balance in animals, by increasing the amount of lignin in the plant and generating losses of endogenous and microbial N in the feces [19]. On the other hand, the results evidenced regarding the cut-off age on in vitro gas production (Table 1) are probably due to the greater deposit of structural carbohydrates in the plant, as the physiological development is prolonged [37,38].

The differences between the cutting ages on CH_4 production is probably associated with: (1) the highest fiber concentration, (2) variations in the passage rate and (3) the presence of secondary compounds (CT) (Table 2). According to Carmona et al. [43], the CH_4 emission rate is related to the physical-chemical characteristics of the forage. It is evident that, with a greater presence of structural carbohydrates, the production of CH_4 increases [44]. On the other hand, the lower production of CH_4 at a lower regrowth age is probably associated with a greater amount of highly soluble carbohydrates, showing a close relationship between the physiological maturity of the plant and the fiber content [37,38]. In such a way that the forage species, cutting age and time of year influence the production of CH_4, possibly due to the variability in its chemical composition. Previous studies have shown that the use of tropical tree or shrub plants containing secondary metabolites in ruminant feed can minimize CH_4 emission [30,45]. low percentages of fibrous fractions in the plant are associated with a lower production of CH_4 [46].

The ruminal pH evidenced in the present study (Table 1) is consistent with that reported by Vargas-Ortiz et al. [25], who consider positively benefit the activity of microorganisms that degrade cellulose.

4.2. Fermentation Profile

The results obtained in the production of VFA in this study are probably due to the interaction between the seasons studied (dry and rainy) and the nutritional composition of the plants, showing better results in the rainy season (Table 3). The higher production of acetic acid in the rainy season could probably be due to a rapid degradation of the forage species in relation to the cutting age, due to the contribution of nutrients of rapid fermentation in physiologically young plants [47]. In this context, Rodríguez et al. [48] mention that the VFA profile can be modified by the type of fermented substrate. The lack of effects on the VFA profile in the present study in the dry season is probably due to the moisture deficit, which negatively influences the growth, production and nutritional quality of the forage [13]. High temperature, evaporation and wind speed, and low humidity can also have detrimental effects on forage quality [41]. It has been shown that as the plant advances in its state of physiological maturity, the grasses and forages present a greater amount of structural carbohydrates, related to the control of the water balance in the plant, in such a way that, in conditions of moisture stress (water scarcity), especially in dry seasons, the stomata are closed and transpiration is reduced to prevent water loss, with subsequent decrease in dry matter content [49,50].

5. Conclusions

The use of shrub and tree species such as *Tithonia diversifolia*, *Erythrina poeppigiana* and *Cratylia argéntea* in ruminant production systems in the rainy and dry seasons, with an early regrowth age between 30 and 45 days, present a promising alternative to the reduction of CH_4, possibly becoming a sustainable strategy in the tropics to reduce enteric GHG production in ruminants and improve their productive performance.

Author Contributions: Conceptualization, G.M.-B., C.M.-B., J.A.-C., R.C.-C. and M.B.-R.; data tabulation, M.B.-R., R.V.-T., M.C.-C., W.V.-A. and F.I.-F.; writing—review and editing, M.B.-R., F.M.-B., A.Z.-S., R.B.-E., T.S.-B., L.M.-A. and W.G.-C. All authors have read and agreed to the published version of the manuscript.

Funding: The research was funded by the State Technical University of Quevedo, Ecuador through the FOCICYT-2016 project.

Institutional Review Board Statement: All procedures followed the recommendations of the ARRIVE Guide [Kilkenny et al., 2010] and were approved by the Bioethics Committee of the Faculty of Livestock and Biological Sciences of the State Technical University of Quevedo (resolution 20/2019).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this paper are available on request from the corresponding author.

Acknowledgments: All authors thank the State Technical University of Quevedo, Ecuador, for financing this research and the Technical University of Ambato, Ecuador, for the collaboration through the Ruminology Laboratory of the Faculty of Agricultural Sciences.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Torres, O.P.N.; Rodriguez, M.B.; Sanchez, D.; Guishca-Cunuhay, C. Comportamiento productivo, degradación ruminal y producción de gas in vitro en ovinos alimentados con dietas a base de residuos pos-cosecha de *Chenopodium quinoa*. *Rev. Investig. Vet. Perú* **2018**, *29*, 765–773. [CrossRef]
2. Hawu, O.; Ravhuhali, K.E.; Mokoboki, H.K.; Lebopa, C.K.; Sipango, N. Sustainable use of legume residues: Effect on nutritive value and ensiling characteristics of maize straw silage. *Sustainability* **2022**, *14*, 6743. [CrossRef]
3. Lara, P.E.; Canché, M.C.; Magaña, H.; Aguilar, E.; Sanginés, J.R. Producción de gas in vitro y cinética de degradación de harina de forraje de morera (*Morus alba*) mezclada con maíz. *Rev. Cubana Cienc. Agríc.* **2009**, *43*, 273–279.
4. Gaviria, X.; Naranjo, J.F.; Barahona, R. Cinética de fermentación in vitro de *Leucaena leucocephala* y *Megathyrsus maximus* y sus mezclas, con o sin suplementación energética. *Pastos Forrajes* **2015**, *38*, 55–63.
5. Fulkerson, W.J.; Neal, J.S.; Clark, C.F.; Horadagoda, A.; Nandra, K.S.; Barchia, I. Nutritive value of forage species grown in the warm temperate climate of Australia for dairy cows: Grasses and legumes. *Livest. Sci.* **2007**, *107*, 253–264. [CrossRef]
6. Kurihara, M.; Magner, T.; McCrabb, H.; McCrabb, G. Methane production and energy partition of cattle in the tropics. *Brit. J. Nutr.* **1999**, *81*, 227–234. [CrossRef]
7. Hristov, N.A.; McAllister, T.A.; Van Herk, F.H.; Cheng, K.J.; Newbold, C.J.; Cheeke, P.R. Effect of *Yucca schidigera* on ruminal fermentation and nutrient digestion in heifers. *J. Anim. Sci.* **1999**, *77*, 2554. [CrossRef] [PubMed]
8. Steinfeld, H.; Gerber, P.; Wassenaar, T.; Castel, V.; Rosales, M.; de Haan, C. *Livestock's Longs Adow: Environmental Issues and Options*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2006. Available online: http://www.fao.org/3/a0701e/a0701e00.pdf (accessed on 15 September 2022).
9. Gerber, P.J.; Steinfeld, H.; Henderson, B.; Mottet, A.; Opio, C.; Dijkman, J.; Falcucci, A.; Tempio, G. *Tackling Climate Change Through Livestock—A Global Assessment of Emissions and Mitigation Opportunities*; Food and Agriculture Organization of the United Nations (FAO): Rome, Italy, 2013. Available online: http://www.fao.org/3/i3437e/i3437e.pdf (accessed on 15 September 2022).
10. Food and Agriculture Organization of the United Nations (FAO). *Global Livestock Environmental Assessment Model (GLEAM)*; FAO: Rome, Italy, 2021. Available online: https://www.fao.org/gleam/ (accessed on 15 September 2022).
11. Elghandour, M.M.Y.; Kholif, A.E.; Salem, A.Z.M.; de Oca, R.M.; Barbabosa, A.; Mariezcurrena, M.; Olafadehan, O.A. Addressing sustainable ruminal methane and carbon dioxide emissions of soybean hulls by organic acid salts. *J. Clean. Prod.* **2016**, *135*, 194–200. [CrossRef]
12. Sun, K.; Liu, H.; Fan, H.; Liu, T.; Zheng, C. Research progress on the application of feed additives in ruminal methane emission reduction: A review. *PeerJ* **2021**, *9*, e11151. [CrossRef]
13. Tjelele, T.J. Dry Matter Production, Intake and Nutritive Value of Certain Indigofera Species. Ph.D. Thesis, University of Pretoria, Pretoria, South Africa, 2007. Available online: https://repository.up.ac.za/bitstream/handle/2263/23754/Complete.pdf (accessed on 25 September 2022).
14. Stoldt, A.K.; Derno, M.; Das, G.; Weitzel, J.M.; Wolffram, S.; Metges, C.C. Effects of rutin and buckwheat seeds on energy metabolism and methane production in dairy cows. *J. Dairy Sci.* **2016**, *99*, 2161–2168. [CrossRef]
15. Salem, A.Z.; Kholif, A.E.; Elghandour, M.M.; Hernandez, S.R.; Domínguez-Vara, I.A.; Mellado, M. Effect of increasing levels of seven tree species extracts added to a high concentrate diet on in vitro rumen gas output. *Anim. Sci. J.* **2014**, *85*, 853–860. [CrossRef]

16. Barros-Rodríguez, M.; Oña-Rodríguez, J.; Mera-Andrade, R.; Artieda-Rojas, J.; Curay-Quispe, S.; Avilés-Esquivel, D.; Guishca-Cunuhay, C. Degradación ruminal de dietas a base de biomasa pos-cosecha de *Amaranthus cruentus*: Efecto sobre los protozoos del rumen y producción de gas in vitro. *Rev. Investig. Vet. Perú* **2017**, *28*, 812–821. [CrossRef]
17. Hassen, A.; Tessema, Z.K.; Tolera, A. Seasonal variations in chemical composition, in vitro digestibility and ruminal degradation of browse species in the Rift Valley of Ethiopia. *Livest. Res. Rural. Dev.* **2017**, *29*, 112.
18. Mwangi, F.W.; Charmley, E.; Adegboye, O.A.; Gardiner, C.P.; Malau-Aduli, B.S.; Kinobe, R.T.; Malau-Aduli, A.E. Chemical composition and in situ degradability of *Desmanthus* spp. forage harvested at different maturity stages. *Fermentation* **2022**, *8*, 377. [CrossRef]
19. Ravhuhali, K.E.; Msiza, N.H.; Mudau, H.S. Seasonal dynamics on nutritive value, chemical estimates and in vitro dry matter degradability of some woody species found in rangelands of South Africa. *Agroforest. Syst.* **2022**, *96*, 23–33. [CrossRef]
20. Muir, J.P.; Pitman, W.D.; Foster, J.L.; Dubeux, J.C.J. Sustainable intensification of cultivated pastures using multiple herbivore species. *Afr. J. Range Forage Sci.* **2015**, *32*, 97–112. [CrossRef]
21. Naumann, H.D.; Cooper, C.; Bow, J.R.; Muir, J.P. Condensed tannin characteristics of Limpopo, South Africa arboreal species retaining leaves in the dry season. *Afr. J. Ecol.* **2017**, *55*, 168–175. [CrossRef]
22. Ramírez, R.G.; Foroughbackhch, R.; Hauad, L.; Alba-Avila, J.; García-Castillo, C.G.; Espinosa-Vázquez, M. Seasonal dynamics of dry matter, crude protein and cell wall digestion in total plant, leaves and stems of common buffelgrass (*Cenchrus ciliaris*). *J. App. Anim. Res.* **2001**, *19*, 209–218. [CrossRef]
23. Beauchemin, K.A.; Ungerfeld, E.; Abdalla, A.; Álvarez, C.; Arndt, C.; Becquet, P.; Benchaar, C.; Berndt, A.; Mauricio, R.; McAllister, T.; et al. INVITED REVIEW: Current enteric methane mitigation. *J. Dairy Sci.* **2022**. [CrossRef]
24. Ku-Vera, J.C.; Jiménez-Ocampo, R.; Valencia-Salazar, S.S.; Montoya-Flores, M.D.; Molina Botero, I.C.; Arango, J.; Solorio Sánchez, F.J. Role of secondary plant metabolites on enteric methane mitigation in ruminants. *Front. Vet. Sci.* **2020**, *7*, 584. [CrossRef] [PubMed]
25. Vargas-Ortiz, L.; Andrade-Yucailla, V.; Barros-Rodríguez, M.; Lima-Orozco, R.; Macías-Rodríguez, E.; Contreras-Barros, K.; Guishca-Cunuhay, C. Influence of *Acacia Mearnsii* Fodder on Rumen Digestion and Mitigation of Greenhouse Gas Production. *Animals* **2022**, *12*, 2250. [CrossRef] [PubMed]
26. Jamarun, N.; Zain, M.; Arief, P.R.; Pazla, R. Populations of rumen microbes and the in vitro digestibility of fermented oil palm fronds in combination with tithonia (*Tithonia diversifolia*) and elephant grass (*Pennisetum purpureum*). *Pak. J. Nutr.* **2017**, *17*, 39–45. [CrossRef]
27. Pazla, R.; Adrizal, S.R. Intake, nutrient digestibility and production performance of Pesisir cattle fed *Tithonia diversifolia* and *Calliandra calothyrsus*-based rations with different protein and energy ratios. *Adv. Anim. Vet. Sci.* **2021**, *9*, 1608–1615. [CrossRef]
28. Barwani, D.K.; Bacigale, S.B.; Kibitok, N.K.; Webala, A.W.; Gicheha, M.G.; Katunga, D.M.; Osuga, I.M. Nutritional characterization of eight trees and shrubs used as livestock feeds in the Eastern Democratic Republic of the Congo. *Livest. Res. Rural Dev.* **2022**, *34*, 10. Available online: http://www.lrrd.org/lrrd34/10/3486issa.html (accessed on 24 October 2022).
29. Jiménez-Ferrer, G.; Mendoza-Martínez, G.; Soto-Pinto, L.; Alayón-Gamboa, A. Evaluation of local energy sources in milk production in a tropical silvopastoral system with *Erythrina poeppigiana*. *Trop. Anim. Health Prod.* **2015**, *47*, 903–908. [CrossRef]
30. Aragadvay-Yungán, R.G.; Barros-Rodríguez, M.; Ortiz, L.; Carro, M.D.; Marcos, C.N.; Elghandour, M.M.M.Y.; Salem, A.Z.M. Mitigation of ruminal methane production with enhancing the fermentation by supplementation of different tropical forage legumes. *Environ. Sci. Pollut. Res.* **2021**, *29*, 3438–3445. [CrossRef]
31. Fagundes, G.M.; Benetel, G.; Carriero, M.M.; Sousa, R.L.M.; Muir, J.P.; Macedo, R.O.; Bueno, I.C.S. Tannin-rich forage as a methane mitigation strategy for cattle and the implications for rumen microbiota. *Anim. Prod. Sci.* **2020**, *61*, 12. [CrossRef]
32. Menke, K.H.; Steingass, H. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Anim. Res. Dev.* **1988**, *28*, 7–55.
33. Theodorou, M.K.; Williams, B.A.; Dhanoa, M.S.; Mcallan, A.B.; France, J. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminants feeds. *Anim. Feed Sci. Techol.* **1994**, *48*, 185–197. [CrossRef]
34. Elghandour, M.M.Y.; Rodríguez-Ocampo, I.; Parra-Garcia, A.; Salem, A.Z.M.; Greiner, R.; Marquez-Molina, O.; Barros-Rodriguez, M.; Barbabosa-Pilego, A. Biogas production from prickly pear cactus containing diets supplemented with *Moringa oleifera* leaf extract for a cleaner environmental livestock production. *J. Clean. Prod.* **2018**, *185*, 547–553. [CrossRef]
35. Krishnamoorthy, U.; Soller, H.; Steingass, H.; Menke, K.H. A comparative study on rumen fermentation of energy supplements in vitro. *J. Anim. Physiol. Anim. Nutr.* **1991**, *65*, 28–35. [CrossRef]
36. Ryan, J.P. Determination of volatile fatty acids and some related compounds in ovine rumen fluid, urine, and blood plasma, by gas-liquid chromatography. *Anal. Biochem.* **1980**, *108*, 374–384. [CrossRef]
37. Ortiz-Tirado, P.; Barros-Rodriguez, M.; Mayorga-Paredes, S.; Chay-Canul, A.; Guishca-Cunuhay, C.; Romero-Herrera, R.; Reyes, H. Influence of cutting age on chemical composition, rumen degradation kinetics and in vitro digestibility of green hydroponic fodder of *Avena sativa*. *Trop. Subtrop. Agroecosyst.* **2019**, *22*, 819–825. Available online: https://www.revista.ccba.uady.mx/ojs/index.php/TSA/article/view/3082 (accessed on 26 September 2022).
38. Andino, M.; Barros-Rodriguez, M.; Burgos, J.V.; Andrade-Yucailla, V.; Acosta-Lozano, N.; Aragadvay-Yungan, R.; Mayorga-Paredes, S. Effect of the cutting age of *Brachiaria decumbens* on rumen functions and in vitro gas production. *Trop. Subtrop. Agroecosyst.* **2019**, *22*, 803–809. Available online: https://www.revista.ccba.uady.mx/ojs/index.php/TSA/article/view/3080 (accessed on 26 September 2022).

39. Canul-Solis, J.; Campos-Navarrete, M.; Piñeiro-Vázquez, A.; Casanova-Lugo, F.; Barros-Rodríguez, M.; Chay-Canul, A.; Cárdenas-Medina, J.; Castillo-Sánchez, L. Mitigation of rumen methane emissions with foliage and pods of tropical trees. *Animals* **2020**, *10*, 843. [CrossRef]
40. Hassen, A.; Rethman, N.F.G.; van Niekerk, W.A.; Tjelele, T.J. Influence of season/year and species on chemical composition and in vitro digestibility of five Indigofera accessions. *Anim. Feed Sci. Technol.* **2007**, *136*, 312–322. [CrossRef]
41. Alarcon-Zuniga, B.; Ramirez-Gonzalez, J.; Quijano-Hernandez, S.; Cervantes-Martinez, T. Yield components of three *Brachiaria* species harvested at two cutting frequencies in hueytamalco, puebla, mexico. In *Molecular Breeding of Forage and Turf*; Budak, H., Spangenberg, G., Eds.; Springer: Cham, Switzerland, 2015. [CrossRef]
42. Warly, L.; Fariani, A.; Ichinohe, T.; Abdulrazak, S.A.; Fujihara, T. Comparative rumen degradability of some legume forages between wet and dry season in West Sumatra, Indonesia. *Asian-Aust. J. Anim. Sci.* **2004**, *17*, 1107–1111. Available online: https://www.animbiosci.org/upload/pdf/17_179.pdf (accessed on 26 September 2022).
43. Carmona, J.C.; Bolívar, D.M.; Giraldo, L.A. El gas metano en la producción ganadera y alternativas para medir sus emisiones y aminorar su impacto a nivel ambiental y productivo. *Rev. Colomb. Cienc. Pecu.* **2005**, *18*, 49–63.
44. Vanegas, J.L.; González, J.; Carro, M.D. Influence of protein fermentation and carbohydrate source on in vitro methane production. *J. Anim. Physiol. Anim. Nutr.* **2017**, *101*, e288–e296. [CrossRef]
45. Lascano, C.E.; Cárdenas, E. Alternatives for methane emission mitigation in livestock systems. *Rev. Bras. Zootec.* **2010**, *39*, 175–182. [CrossRef]
46. Kreuzer, M.; Hindrichsen, I.K. Methane mitigation in ruminants by dietary means: The role of their methane emission from manure. *Int. Congr. Ser.* **2006**, *1293*, 199–208. [CrossRef]
47. Gallego-Castro, L.A.; Mahecha-Ledesma, L.; Angulo-Arizala, J. Potencial forrajero de *Tithonia diversifolia Hemsl*: A gray en la producción de vacas lecheras. *Agron. Mesoam.* **2014**, *25*, 393–403. [CrossRef]
48. Rodríguez, R.; Fondevila, M.; Castrillo, C. In vitro ruminal fermentation of *Pennisetum purpureum* CT-115 supplemented with four tropical browse legume species. *Anim. Feed Sci. Technol.* **2009**, *151*, 65–74. [CrossRef]
49. Herrera, A.; Tezara, W.; Marín, O.; Rengifo, E. Stomatal and non-stomatal limitations of photosynthesis in trees of a tropical seasonally flooded forest. *Physiol. Plant.* **2008**, *134*, 41–48. [CrossRef]
50. Villalobos-González, L.; Alarcón, N.; Bastías, R.; Pérez, C.; Sanz, R.; Peña-Neira, Á.; Pastenes, C. Photoprotection is achieved by photorespiration and modification of the leaf incident light, and their extent is modulated by the stomatal sensitivity to water deficit in grapevines. *Plants* **2022**, *11*, 1050. [CrossRef] [PubMed]