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In vitro methane production from silages based on *Cenchrus purpureus* mixed with *Tithonia diversifolia* in different proportions

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ABSTRACT. Climate change (CC) affects food production, mainly those based on livestock systems. Producers must identify adaptation strategies to ensure the production, during periods of drought, and lack of forage. Besides contributing to CC, high emissions of ruminal methane (CH_4) are energy loss potentially usable for livestock production. The objective was to estimate *in vitro* ruminal gas production (RGP) and determine the CH_4 emissions from silages. Treatments were made with forage of *Cenchrus purpureus* mixed with *Tithonia diversifolia* T1= *C.purpureus* at 100%; T2= *C.purpureus/ T.diversifolia* in 33/67 percent ratio; T3= C.*purpureus/ T.diversifolia* 67/33; and T4= *T.diversifolia* at 100%. Samples of silages were analyzed, and they were inoculated with strains of *Lactobacillus paracasei* (T735); then they were fermented in vacuum-sealed bags for 67 days. RGP and CH4 were measured at 2, 4, 8, 12, 18, 24, 30, 36, and 48 hours. Additionally, modeling of CH4 production kinetics was conducted, using different equations. The results indicate that the highest cumulative CH4 production was for T1. This kinetics was represented using the Gompertz model. In conclusion, the inclusion of *T.diversifolia* to *C.purpureus* silages contributes to the decrease of methane at the ruminal level, which constitutes an adaptation practice at climate change.

Keywords: digestibility; greenhouse gasses; small ruminants; silvi-pastures.

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Introduction

Currently, livestock has become an activity of great importance within the agricultural sector of tropical areas and especially in the Colombian territory (Bettencourt, Tilman, Narciso, Carvalho, & Henriques, 2015). Within livestock activity, it is known that ruminants have a digestive system that can use fibrous material with a high content of structural carbohydrates and convert them into foods of high nutritional quality, such as meat and milk (Friedrich, 2014). However, different studies have suggested that this digestive system also produces methane (CH4), a potent greenhouse gas (GHG) that contributes significantly to global warming (Cárdenas & Flores, 2012).

With increasing pressure from the global community to reduce methane emissions and the inverse correlation between energy utilization and CH4 production (Olivo & Soto-Olivo, 2010), especially with ruminants, methane emissions studies have acquired great importance due to its negative effects on the environment (Ribeiro et al., 2016). It should be noted that the CH4 production at the enteric level is associated with the quality and quantity of the food ingested; diets with lower digestibility and higher content of structural carbohydrates, translate into increased gas production (Kulivand & Kafilzadeh, 2015). More gas production from enteric fermentation means a lower energy efficiency of animals and the emission of CH4 through belching, that is, the energy that the animal underutilizes, because it is not converted into livestock products.

Livestock products constitute some important high protein sources for global food security because they provide 17% of global energy consumption and 33% of global protein consumption (Rojas-Downing, Nejadhashemi, Harrigan, & Woznicki, 2017; Rosegrant, Fernandez, & Sinha, 2009). Thus, animal

production systems contribute to the livelihoods of one billion of the countryside population in the world (Hurst, Termine & Karl, 2007); mainly, the small ruminant production systems have been managed by small households from indigenous people and rural societies, some of them under extreme poverty conditions (Forero-Álvarez, 2013; Maluf, Burlandy, Santarelli, Schottz, & Speranza, 2016). In these production systems, the traditional grazing practices have been the most common cause of pasture degradation and consequently, environmental impacts by increasing greenhouse emissions (Mahecha-Ledesma, Angulo-Arizala, & Barragán-Hernández, 2017). Therefore, having livestock as a source, mainly in animal feeding practices (Verdecia et al., 2011).

Thus, feeding practices and strategic supplementation based on local forage resources available in tropical areas constitutes an eco-friendly technological option (Castaño, Grisalez, Navia, & Delgado, 2015). In this sense, the use of forage bushes constitutes a viable alternative, *Tithonia diversifolia* is a shrub whose forage is a good source of protein in the diets and other primary and secondary metabolites (Rivera, Naranjo, Cuartas, & Arenas, 2013); some of them have shown a tendency to decrease rumen methane synthesis. In fact, the supplementation of ruminant diets with *T. diversifolia* has been suggested as a promising dietary strategy (Ribeiro et al., 2016). Currently, *T. diversifolia* has been reported because it reduces methane output by 6-fold when compared to control due to the presence of secondary metabolites in the plant. These chemical characteristics of the *T. diversifolia* associated with its high consumption, high digestibility and high passage rate, are expressed in a significant decrease in methane production per unit of digested fodder (Meza et al., 2014). This means that the implementation of this fodder species in the feeding of ruminants has been shown to maintain an optimal ruminal balance that improves productivity and decreases methane production (Galindo et al., 2011).

Despite the high protein content of *T. diversifolia* (10.3-25.6%) (La et al., 2009), just a few silage production studies have been conducted (Holguín, Ortíz Grisalez, Velasco Navia, & Mora-Delgado 2015); therefore, it is necessary to determine the extent of incorporation of this plant and the possibility of blending it with grass to obtain the maximum benefit for animal nutrition and farmer's households.

On the other hand, in tropical areas, seasonal forage production has been a problem due to food shortage at certain times of the year associated with drought, and in other times by an overproduction caused to high rainfall (Miguel, Delagarde, & Ribeiro-Filho, 2019). Seen this way, the challenge is how to conserve fodder to defer its use between times of high production and shortages without losing quality; silage is an alternative, different studies (Florez Delgado, Capacho Mogollon, Quintero Muino, & Gamboa Vera, 2018; Galina, Ortiz-Rubio, Mondragón, Delgado-Pertíñez, & Elías, 2009) have shown silage of other non-legumes plants as protein sources. Therefore, it is necessary to investigate how silages with different proportions of grasses and protein forages could be an efficient alternative for production, and environmentally friendly (Steinfed et al., 2006). The present study aims to estimate *in vitro* ruminal gas production (RGP) and determine the CH4 emissions from silages used as diets for lambs. This was done through an experiment, based on the evaluation of four diets, prepared with a grass mixed with a high protein plant. Consequently, our goal is to produce a strategic knowledge for the small-ruminant farmers. The main result suggested that the lower cumulative CH4 production was for those diets in which the *T. diversifolia* was included.

Material and methods

Silage preparation

The forage used for silage production was harvested at the Experimental Center of the National University of Colombia - Palmira Headquarters (CEUNP) in the Department of Valle del Cauca. This area is located at an altitude of 1,000 meters above the sea level; 02°06' N and 65°03' W; it presents an annual average rainfall of 1,000 mm and an average temperature of 24°C. This area has been classified as a tropical dry forest (Holdridge, 1987). The forage of *Cenchrus purpureus* and *Tithonia diversifolia* was harvested at 60 and 90 days, respectively. The forage was pre-dried for 24 hours; then, it was chopped to reduce the particle size to 2 cm, in a three-blade mill, 7.5 HP, 1400 rpm, and 4.5 Amps Gaitan Brand. Once the forage was chopped separately for each species, a manual mixture was made. Simultaneously, using a hand pump, the forage was sprayed in layers with an inoculum based on *Lactobacillus paracasei* bacteria (T735), it was obtained from macerated leaves of *T. diversifolia*, following the protocol explained by Holguín, Grisalez,

Huertas, Fandiño and Delgado (2018), developed in the Diagnostic Laboratory Veterinarian of the University of Tolima. The concentration of the inoculum used was 30 x 107 CFU mL^{-1}. The silages were prepared using a silage bag packing machine and then they were stored in vacuum-sealed bags, as follows: Treatment 1 = 100% *Cenchrus purpureus* silage, Treatment 2 = *Cenchrus purpureus* silage in mixture with *Tithonia diversifolia*, with a proportion 33 and 67%, Treatment 3 = *Cenchrus purpureus* silage in mixture with *Tithonia diversifolia*, with a proportion 67 and 33%, respectively, and Treatment 4 = 100% *Tithonia diversifolia* silage. The silages were stored for 67 days.

Analytical phase

The bromatological analysis of the silages was carried out in the laboratory of Animal Ecophysiology at the University of Tolima, where proximal chemical analyses were performed. We followed the methods established by the Association of Official Analytical Chemists (AOAC, 1990) (Horwitz, Latimer, & AOAC International, 2010) for dry matter (DM), organic matter (OM), crude protein CP), ether extract (EE) and ash content (AC). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the protocol of Van Soest, Robertson and Lewis (1991). After that, the *in vitro* laboratory phase was conducted at the Agrosavia Nutrition Laboratory in Mosquera-Cundinamarca.

In vitro incubation

The *in vitro* incubation procedure described by Schofield, Pell and Pitt (1994) was used. The ruminal liquor was obtained on an empty stomach of a fistulated sheep. The sheep were fed with kikuyo grass. The ruminal liquor was filtered using four layers of gauze and it was CO_2-gassing constantly. Then, 0.6 g of silage were weighed, and they were introduced into 60 mL-bottles, provided with butyl rubber stoppers and staples; then, 8 mL of buffer solution (pH 6.5) (Van Soest et al., 1991) and 2 mL of ruminal fluid were added to each bottle, maintaining a continuous gassing with CO_2. The four treatments and a control group, each with four repetitions, were incubated for 48 hours under a temperature of 37°C. Thus, a total amount of 20 bottles were incubated, 16 containing substrate and inoculum (4 treatments* 4 repetitions) and 4 corresponding to the control group, whose function was to correct the production of gas generated by the microorganisms.

In vitro digestibility of dry matter

The *in vitro* dry matter digestibility (INDDM) was determined at 48 hours of incubation. To do this, the silage content of each bottle was poured into a 50 mL tubes-Falcon brand, previously identified, and weighed. The not digestible (NDM) was determined by drying the filtered material at 60°C for 48 hours; consequently, digestible (DDM) was determined by the difference.

Gas production

Following the methodology of Theodorou, Williams, Dhanoa, McAllan and France (1994), the gas production generated by enteric fermentation was quantified for each treatment at 2, 4, 8, 12, 18, 24, 30, 36, and 48 hours. The gas quantification was made using a digital transducer (ASHCROFT®) which measures the amount of gas according to the pressure accumulated in the bottle. Thus, the total gas production was determined as the sum of the partial productions in the sampling hours. A sample of gas obtained at each hour of measurement was stored in vacutainer tubes (BD of 7 mL) under vacuum for subsequent determination of methane concentration. To convert the pressure (PSI) data into gas volume (mL), we use the equation:

$$Y = -0.1375 + (5.385 * X) + (0.0777 * X^2),$$

where: X is the pressure in PSI and Y is the gas volume in mL (Posada, Solano, & Vergara, 2006); gas production was expressed per gram of incubated dry matter (mL g^{-1} of DM).

Methane production

A sample of gas was taken from each treatment at each hour of measurement, which were stored in vacutainers (BD of 7 mL), under vacuum; subsequently, methane concentration was determined using a methane gas laser sight (CROWCON®).

Modeling methane production

To estimate the parameters of the fermentation kinetics, the cumulative methane gas production of the best treatment was adjusted to the models in table 1. The adjustment of the data to each model and the parameter estimation was performed using non-linear models through INFOSTAT® software version 2018 (Di Rienzo et al., 2008). Thus, the model that best represents the methane production kinetics was selected,the best values in the goodness-of-fit criteria BIC and AIC.

Statistical analysis

The statistical analysis for the dependent variables was done using a completely randomized design in each case. The dependent variables were: Cumulative gas production (mL); methane production per gram of dry matter (ppm); parameters obtained from the most adjusted model, and *in vitro* digestibility of DM (%). The first two variables were analyzed using repeated measures over time, using mixed models and heterogeneous variances. The linear model for the observations of this experiment is as follows:

$$Y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + s_k + \varepsilon_{ijk}$$

Y_{ijk}: represents the production of methane observed in the ith level of factor Silage and jth level of the factor Time for the kth subject; μ: represents the general average of the observed variable; τ_i: represents the effect of the ith level of silage factor; β_j: represents the effect of the jth level of the Time factor; ($\tau\beta_{ij}$): represents the interaction effect corresponding to the Silage and Time factor; s_k: represents the random effect corresponding to the k-th subject, where $s_k \sim N(0, \sigma_s^2)$; ε_{ijk}: represents the random error where $\varepsilon_{ijk} \sim N(0, \sigma_\varepsilon^2)$. It is also assumed that the two random terms s_k and ε_{ijk} are independent. The comparison of means was made using the Fisher LSD test. For the statistical analyzes, the INFOSTAT® software, version 2018 (Di Rienzo et al., 2008) was used.

Table 1. Models used to model the *in vitro* gas kinetics of *Cenchrus purpureus* silages in admixture with *Tithonia diversifolia*.

Model	Equation	Parameter
Gompertz (1825)	$Y = \alpha * \exp(-\beta * \exp(-\gamma * t))$	Y, the accumulated gas production (mL g^{-1} DM incubated) α, the fermentation potential of the treatment under incubation conditions (asymptote of the curve, mL g^{-1} MS incubated) β, the specific gas accumulation rate (mL h^{-1}) γ, latency or delay phase (h) t, the incubation time (hours)
Richards	$Y = \alpha * (1 + \beta * \exp(-\gamma * t)\ ^(1/1 - \delta)$	Y, the gas produced at time t α, the maximum asymptotic growth this is when "t" tends to infinity γ, the curvature parameter that expresses how fast it reaches maximum growth (rate) β, an adjustment parameter that depends on the initial condition at t = 0 δ, the parameter of allometry The positive sign is used when M > 0 and the negative sign when 0 <M <1
Mitscherlich (Monomolecular)	$Y = \alpha * (1 - \beta * \exp(-\gamma * t))$	Y, the gas production of the DM at a time 't' α, the production of gases at 0 hours β, accumulated gas production at time 't' γ, gas production rate t, the incubation time (hours)
Logistic	$Y = \alpha/(1 + \beta * \exp(-\gamma * t))$	Y, the gas production of the DM at a time 't' α, the volume of gas corresponding to complete digestion (asymptote) β, gas production rate γ, delay time at the start of gas production (h) t, regressive variable (h)

Source: Plata Pérez, González Ramírez, and Calderón Sánchez (2017).

Results

Bromatological analyses

The bromatological analysis (table 2) shows higher CP and DM contents in the treatments in which the *T. diversifolia* was included in silage. On the contrary, ashes, NDF, and ADF were higher as the proportion of *C. purpureus* increases in the silage, the above related to a lower digestibility, and greater emission of methane (Barbosa et al., 2018).

Table 2. Bromatological analysis of *Cenchrus purpureus* silages in mixture with *Tithonia diversifolia*.

		T1		T2		T3		T4	
		Cenchrus purpureus (100%)		Cenchrus purpureus/ *Tithonia diversifolia* (33%/67%)		Cenchrus purpureus/ *Tithonia diversifolia* (67%/33%)		*Tithonia diversifolia* (100%)	
Variable		Mean	S.E	Mean	S.E.	Mean	S.E.	Mean	S.E.
DM	%	17,68 a	0,11	27,2 c	0,46	22,56 b	0,44	30,07 c	1,42
CP	%	4,81 a	0,16	12,73 c	1,99	6,88 ab	0,09	9,36 bc	0,18
NDF	%	64,06 c	0,64	50,02 a	0,58	54,79 b	0,37	49,17 a	0,68
ADF	%	50,4 c	0,63	41,77 b	0,63	48,95 c	0,78	38,17 a	0,63
Ash	%	16,35 c	0,34	11,45 a	0,16	13,49 b	0,38	10,56 a	0,29
Lignin	%	1,09 c	0,27	2,09 a	0,42	4,69 a	0,29	10,14 a	0,51

Source: The authors. A, b, c Means with different letters are statistically different, according to the Fisher LSD test($p < 0.05$).Abbreviations: DM = Dry matter, CP = Crude protein, NDF = Neutral detergent fiber, ADF = Acid detergent fiber.

In vitro digestibility of dry matter

In table number 3 it is possible to notice the *in vitro* digestibility of dry matter (IVDDM) at 48 hours of incubation. No statistical significance was found among the treatments studied. The positive interaction effect was confirmed in the treatment with 100% *Tithonia diversifolia* inoculated with *L. paracasei* which resulted in the best IVDDM ($p < 0.0001$).

Table 3. *In vitro* digestibility of dry matter from the vegetable silages of *Cenchrus purpureus* in mixture with *Tithonia diversifolia*.

		T1		T2		T3		T4	
		Cenchrus purpureus (100%)		*Cenchrus purpureus/ Tithonia diversifolia* (33%/67%)		*Cenchrus purpureus/ Tithonia diversifolia* (67%/33%)		*Tithonia diversifolia* (100%)	
Variable		Mean	S.E	Mean	S.E.	Mean	S.E.	Mean	S.E.
IVDDM	%	46,16 a	3,84	49,51 a	0,78	49,85 a	1,31	51,15 a	

Source: The authors. Equal letter values have no significant statistical difference according to the Fisher LSD test ($p > 0.05$).

Gas production

The silages showed increases in gas production over time, with a significant effect for the interaction treatments and incubation time ($p = 0.0008$). The gas accumulation for the T1 samples is greater from hour 2 (12.44 mL g DM^{-1}), with no significant effect with T4 (Table 4). However, as of hour 8, a significant effect ($p = 0.043$) was observed in the gas production for T1 to the other treatments. Treatments 2, 3, and 4 show a similar behavior from hour 2 to hour 24, with a decrease in the gas production of T4 without significant differences between these treatments, but with T1 ($p = 0.032$). T4 treatment obviously maintains a decrease for the other treatments until 48 hours.

Methane production

Silages showed increases in methane production over time; a significant effect was observed for the interaction between treatments and hours ($p = 0.0001$). Thus, the accumulated methane production for T1 is greater since hour 2, showing a significant effect concerning the other treatments ($p = 0.0005$). This trend remains constant until hour 36; at that time, T3 also shows an increase concerning T1, with significant effects ($p = 0.008$) with respect to T2 and T4. For T2, methane production begins to decrease after 24 hours; T4 maintained a low methane production compared to T1 and T3, experiencing a slight increase at the hour 24. It was determined that the best treatments were T4 and T2, given the lower cumulative methane production. Therefore, we proceeded to perform the modeling of the kinetics of methane production with T2 treatment.

Modeling of methane production kinetics

The Mitscherlich and Gompertz models had the best goodness of fit, but between these two the second is the one that best predicts biologically the production of methane gas. That best prediction availability was verified by the CME, AIC, and BIC. In table 6, the equations that represent the potential of *in vitro* gas production of *T. diversifolia* for the different models are shown. The fermentation potential of the substrate under the incubation conditions (asymptote of the curve) in the Gompertz model corresponds to 311.97 mL gr^{-1} of DM (parameter α), with a latency or fermentation delay phase of 2, 85 h (parameter β) and a fermentation rate of 008 mL h^{-1} (parameter γ).

Also, in Figure 1 the equation of greater adjustment is described, showing an increase over time in gas production, a trend that is interpreted by France, Dijkstra, Dhanoa, Lopez and Bannink (2000) as an increase in microbial activity, although this does not imply any assumption about the constancy of microbial growth performance.

The gas higher production was observed in the treatment 4 (100% *T. diversifolia)*, compared to silages with lower inclusion of this species (T2 and T3) or in all-grass silage (T1).

Table 4. Production of *in vitro* gas accumulated (mL g^{-1} DM) in the silage mixture of *T. diversifolia* (T) / *C.purpureum* (P).

	Times (Hour)								
Treatments	2	4	8	12	18	24	30	36	48
1	12,44 ± 10,57 mnop	32,31 ± 11,14 lmn	70,36 ± 12,1 ijk	104,91 ± 11,24 fgh	134,19 ± 10,52 bcde	146 ± 9,57 abc	151,76 ±7,89 ab	156,96 ± 7,56 a	158,56 ± 8,59 a
2	0,26 ± 8,19 p	5,23 ± 8,63 nop	24,81 ± 9,37 mno	51,79 ± 8,7 jkl	93,33 ± 8,15 hi	120,3 ± 7,41 defg	134,11 ± 6,11 bcde	142,38 ± 5,86 abc	150,89 ± 6,66 ab
3	2,13 ± 6,92 op	9,31 ±7,29 nop	33,71 ± 7,92 lm	71,61 ± 7,36 ij	106,55 ± 6,88 fgh	125,83 ± 6,27 cdef	139,55 ± 5,17 abc	150,45 ± 4,95 ab	158,37 ± 5,7 a
4	7,14 ± 10,57 nop	17,82 ± 11,14 mnop	41,07 ± 12,1 klm	64,75 ± 11,24 jk	95,04 ± 10,52 ghi	111,92 ± 9,57 efgh	124,08 ± 7,89 cdef	131,81 ± 7,56 bcde	137,03 ± 8,59 abcd

Source: The authors. Different letters indicate significant differences in the interaction between treatment* time ($p < 0.05$). Treatments: 1: *Cenchrus purpureus* (100%); 2: *Cenchrus purpureus/Tithonia diversifolia* (33%/67%); 3: *Cenchrus purpureus/Tithonia diversifolia* (67%/33%); 4: *Tithonia diversifolia* (100%).

Table 5. Production of *in vitro* gas methane accumulated (ppm g^{-1} DM) in the silage mixture of *T. diversifolia* (T) / *C.purpureum* (P).

	Times (Hour)								
Treatments	2	4	8	12	18	24	30	36	48
1	68,02 ± 9,13 nop	95,33 ± 10,39 n	152,88 ± 13,68 kl	206,31 ± 20,47 hij	262,08 ± 19,92 defg	312,77 ± 25,96 abcde	329,33 ± 25,43 abc	346,79 ± 19,84 ab	346,79 ± 21,53 ab
2	21,14 ±7,07 rst	46,74 ± 8,05 opq	60,09 ± 10,59 op	100,77 ± 15,85 nm	158 ± 15,43 jkl	193,7 ± 20,11 ijk	228,59 ± 19,7 fghi	264,69 ± 15,37 def	283,35 ± 16,68 cde
3	5,57 ± 7,9 t	26,45 ± 9 qrs	61,98 ± 11,84 nop	136,17 ± 17,73 lm	212,06 ± 17,25 ghi	254,89 ± 22,48 efgh	316,14 ± 22,03 abcd	353,33 ± 17,18 ab	363,29 ± 18,65 a
4	5,58 ± 7,07 st	18,8 ± 8,05 st	41,59 ± 10,59 pqr	80,55 ± 15,85 no	158,52 ± 15,43 jkl	228,6 ± 20,11 fghi	270,87 ± 19,7 cdef	310,6 ± 15,37 bcde	330,84 ± 16,68 ab

Source: The authors. Different letters indicate significant differences in the interaction between treatment* time ($p < 0.05$). Treatments: 1: *Cenchrus purpureus* (100%); 2: *Cenchrus purpureus/Tithonia diversifolia* (33%/67%); 3: *Cenchrus purpureus/Tithonia diversifolia* (67%/33%); 4: *Tithonia diversifolia* (100%).

Table 6. Statistical equations and estimators of the modeling of methane gas production kinetics for *Cenchrus purpureus* silages in admixture with *Tithonia diversifolia*.

Model	Equation	MSE	AIC	BIC
Gompertz (1825)	$Y = 311{,}97 * \exp(-2{,}85 * \exp(-0{,}08 * t))$	417,57	404,15	411,38
Richards	$Y = 311{,}97 * (1 - 0{,}34 * \exp(-0{,}07 * t)\hat{}(\frac{1}{1} - 7{,}12)$	428,15	406,19	415,23
Mitscherlich	$Y = 426{,}94 * (1 - 1{,}01 * \exp(-0{,}03 * t))$	406,90	408,59	415,82
Logistic	$Y = 289{,}5(1 + 9{,}36 * \exp(-1{,}40 * t))$	453,35	407,85	415,08

Source: The authors.

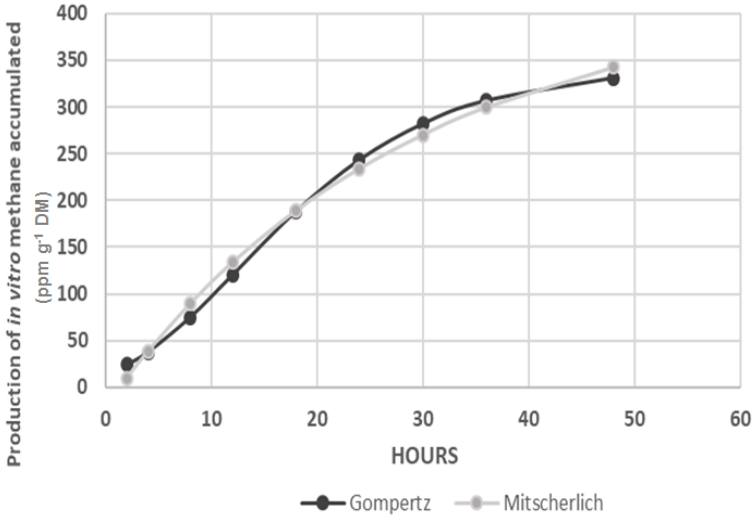

Figure 1. Modeling the *in vitro* production of methane accumulated (mL g^{-1} DM) in the silage mixture of *T. diversifolia* (T) / *C. purpureus* (C).
Source: The authors.

Discussion

The present study provides information about the biochemical characteristics of silages prepared with *T. diversifolia*, which allows us to appreciate the use of this species in the feeding of ruminant animals.

In short, it was identified that the treatments in which the *T. diversifolia* was included to silage, showed better nutritional characteristics compared with treatments without the inclusion of the species. Similar results to those found in this study were described by Donney's et al. (2015). Also, La O et al. (2012) reported a protein content between 18.26 and 26.40% in different ecotypes of this species, and Mahecha, Escobar, Suárez, and Restrepo (2007), found contents of 16.73% protein.

Despite not finding statistical significance among the treatments for the IDIVDM variable, we were able to verify that the treatments in which the species *T. diversifolia* was included show higher values of digestibility. However, Naranjo and Cuartas (2011) reported similar values but with significant differences depending on the ADF contents since as it is known, the digestibility of the forages is inversely related to the content of these fibers, which also depends on the internal composition and its structure (Moreira, Leonel, Vieira, & Pereira, 2013). In this regard, Holguín et al. (2015) found that the application of an inoculum based on *Lactobacillus paracasei* resulted in better digestibility compared to non-inoculated silage but was similar to silage inoculated with SilAll ($p = 0.0060$).

Also, in the present study (Table 4), we observed that the treatment 4 (100% *T. diversifolia*) maintains a decrease in gas production concerning other treatments until the last measurement hour, the above is directly related to the nutritional composition of the silage, it means that forages with a lower content of fibrous carbohydrates and a greater amount of soluble carbohydrates produce less gas (Rivera et at., 2015; Kulivand & Kafilzadeh, 2015). The study conducted by Molina Botero, Cantet, Montoya, Correa Londoño and Barahona Rosales (2013) with the species *Leucaena leucocephala* and *Gliricidia sepium* blended with *Megathyrsus maximus* and *Dichantium aristatum* grasses, reached 48 hours of maximum gas production (around 115 mL of gas g^{-1} DM); similar values, although higher, were found in our experiment.

On the other hand, we found that the accumulated methane production for T1 is greater since hour 2, showing a significant effect concerning the other treatments, a trend that remains constant until hour 36. For treatment 2, the methane production begins to decrease after 24 hours, which is because the gas and methane production of soluble fractions of high-quality forages may be higher during the first hours of fermentation (Freire et al., 2017); while silages prepared with greater inclusion of the species *T. diversifolia* maintained a low methane production. However, from the animal production point of view, it is not convenient to produce silage based on a 100% protein source, given the problems in the low acidification of the medium in the silo and the metabolic problems that could be caused in the animal at the rumen level. Therefore, it is suggested that silage based on a mixture of *T. diversifolia* and grass is the one that could be recommended for use as a supplement, especially in times of scarcity due to the climatic seasonality of tropical areas.

The gas higher production was observed in the treatment 4 (100% *T. diversifolia)*, compared to silages with lower inclusion of this species. The authors suggest that this was due to the presence of secondary metabolites in the species *T. diversifolia* such as condensed tannins and saponins (Noguera, Saliba, & Mauricio, 2004). This follows a greater digestibility of the silage with a greater proportion of *T. diversifolia* (Holguín et al., 2015). On the other hand, La et al. (2009) explain that these high values in the gas production in silage with the greater inclusion of *T. diversifolia* may be due to the concentration of easily fermentable carbohydrates. It also becomes clear that the optimization of microbial fermentation occurs in the presence of this protein forage in the incubation medium. In a recent experiment, Terry et al. (2016) demonstrated that *in vitro* VFA concentration increased when *T. diversifolia* was supplemented at 15.2% DM replacing fresh sugarcane and concentrates; also, they reported an increase in CH_4 production with increasing concentrations of *T. diversifolia*. However, in the *in vivo* experiment, there was no effect ($p= 0.82$) of the inclusion of *T. diversifolia* on total VFA and as such, no increase in production parameters. Delgado et al. (2012) found that *T. diversifolia* had methane reducing properties when supplemented at 30% into a star grass (*Cynodon nlemfuensis*) based diet.

Conclusion

The inclusion of *T. diversifolia* in the silage improves the quality of a grass-based diet, due to its high protein content, high ruminal degradability, and low fiber content. Besides, the lower production of methane in silage mixtures containing *T. diversifolia*, represents a lower energy loss and therefore greater production of volatile fatty acids.

Considering the fact that in the current study, the presence of secondary metabolites, which presents as a difficulty when assessing their effects on CH_4 production, was not evaluated, we recommend doing this study to better understand that component of *T. diversifolia* in the CH_4 production at the ruminal level.

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