Scientific Paper

In vitro methane production in forage resources of the high Andean tropic of Nariño

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Abstract

Objective: To evaluate the *in vitro* methane production in forage resources and their mixtures, used in cattle feeding in the high Andean tropic of Nariño, Colombia.

Materials and Methods: Three forage shrubs, from a forage bank of *Tithonia diversifolia* (Hemsl.) Gray, *Sambucus nigra* L. and *Smallanthus pyramidalis* (Triana) H.Rob (70 days old); two grasses, *Cenchrus clandestinum* (Chiov.) and *Lolium perenne* L. (37 and 28 days), and four mixtures of these forage shrubs and grasses, were evaluated. Representative samples of the forages and their mixtures were analyzed, at 24 and 48 hours of incubation, to evaluate the methane production, dry matter degradation and production of volatile fatty acids. The information was processed through a variance analysis and by Tukey's mean comparison test.

Results: The concentration of secondary metabolites and tannins was numerically higher in the forage species *S. nigra* (22,1 g/kg DM) compared with the grass *C. clandestinus* (8,9 g/kg DM). The highest DM degradation percentages (p< 0,01) were obtained in *S. nigra* (70,6 %), *T. diversifolia* (70,2 %) and *L. perenne* (71,6 %). *C. clandestinus* (87,2 mL) recorded the highest methane production and *T. diversifolia*, the lowest one (34 mL CH_4/g DM). In the mixture of *L. perenne* + *S. nigra* the highest degradation was found (78,3 %). The lowest methane production was for *C. clandestinus* + *S. nigra* (p < 0,01).

Conclusions: Higher concentration of secondary metabolites was found in the forage shrubs with regards to the grasses. When 20 % of the forage species was included in the mixtures, methane production decreased and substrate degradability increased.

Keywords: greenhouse effect, gases, metabolites

Introduction

In the milk production systems of the high Colombian tropic, cattle feeding is based on grasses, such as *Cenchrus clandestinus* (Chiov.) and *Lolium perenne* L. (Cardona-Iglesias *et al*., 2020; Quiñonez-Chillambo *et al*., 2020).

Cardona-Iglesias *et al.* (2019a) stated that the inadequate agronomic and grazing practices, and to the adverse edaphoclimatic conditions caused by the so-called forage seasonality, the nutritional quality of these pastures is not the adequate one, which provokes nutritional unbalances in the animals and the emission of greenhouse gases (GHG), such as methane (Cardona-Iglesias *et al*., 2019a).

In the agricultural sector of Colombia, 26 % of CH_4 production is ascribed to the enteric fermentation of cattle (Pulido-Guio *et al*., 2015). If it is considered that in grazing dairy cows average emissions of 55 kg CH_4/cow/year are reported, a production of up to 181 207 kg CH_4/year, from the 3 294 676 dairy cows, existing in the country, could be predicted (Cardona-Iglesias *et al*., 2017; DANE, 2019). Besides the environmental damage that can be caused by the accumulation of methane in the atmosphere, the formation of this gas in the rumen represents an energy expense for the animal, which can vary between 15 and 18 % of the digestible energy consumed in the diet (Cardona -Iglesias *et al*., 2017; Sandoval-Pelcastre *et al*., 2020).

The nutritional strategies based on the improvement of the compositional quality and digestibility of the diet, are the ones that have proven higher effectiveness in the methane decrease in animal husbandry systems (Pérez-Can, 2020; Sandoval-Pelcastre *et al*., 2020).

At present, in the Colombian high Andean tropic the use of perennial forage shrub species, such as *Tithonia diversifolia* (Hemsl.) Gray, *Sambucus nigra L.* and *Smallanthus pyramidalis* (Triana) H.Rob, is positioned for ruminant feeding. Their good contribution of nutrients, which improves the energy and protein balance in the rumen; the

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presence of secondary metabolites, such as tannins and saponins, besides the important decrease of methane production through *in vivo* and *in vitro* techniques (Cardona-Iglesias *et al.*, 2017; Quiñonez-Chillambo *et al.*, 2020) justify their utilization with this purpose.

From the above-stated facts, the objective of this study was to evaluate the *in vitro* methane production in forage resources and their mixtures, used in cattle feeding in the high Andean tropic of Nariño, Colombia.

Materials and Methods

Location. The study was conducted at the Obonuco Research Center, property of the Colombian Corporation of Agricultural Research (Agrosavia), located in the Obonuco village, Pasto municipality (Nariño-Colombia), with coordinates 1°11´29,6" N and 77°18´47,9" W and height of 2 865 m.a.s.l.

Climate conditions. The mean temperature is 10 ºC, mean annual rainfall is 841,57 mm and relative humidity, 83 %, which corresponds to the life zone low mountain dry forest (Holdridge, 1966).

Experimental design and treatments. A complete randomized design was applied, with factorial arrangement for the forages (five species by two evaluation times, 24 and 48 h) as well as for the four mixtures (two evaluation times, 24 and 48 h).

The treatments were three forage shrubs: *T. diversifolia, S. nigra,* and *S. pyramidales*, at the age of 70 days, harvested in a forage bank. The grasses *C. clandestinus* and *L. perenne*; besides four mixtures between forage species and grasses (*C. clandestinus* 80 % + *S. nigra* 20 %, *C. clandestinus* 80 % + *T. diversifolia* 20 %, *L. perenne* 80 % + *S. nigra* 20 % and *L. perenne* 80 % + *T. diversifolia* 20 %).

The inclusion percentages of the forage shrubs were determined according to a characterization study of feeding systems, conducted in the zone by Agrosavia (non-published data). Table 1 shows the characteristics of the evaluated forage resources.

Forage collection. The forage samples were collected once, in December, 2019. The forage shrubs came from a silvopastoral system (in forage bank). The edible part by the animals (leaves and fresh stems) was harvested and the grasses in 1,5-ha paddocks (monoculture). Through visual inspection the edible parts by the animal were harvested (Ramírez *et al.*, 2015).

Chemical composition of the forages. For each of the forage shrubs, as well as for the grasses, a 500-g sample of fresh forage was obtained, which was dried in a forced-air stove at 65 ºC during 72 h and analyzed in the Animal Nutrition Laboratory of the Tibaitatá Research Center (Agrosavia) in the Mosquera city (Cundinamarca). Through the near infrared reflectance spectroscopy (NIRS) technique (Ariza-Nieto *et al.*, 2018), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), non-structural carbohydrates (NSC), net lactation energy (NLE), total tannins (TT), condensed tannins (CT) and saponins (Sap.), were determined.

Methane production. The *in vitro* production technique (Mauricio *et al.*, 1999) was used in the Animal Research and Nutrition Laboratory (NUTRILAB), located in the University Research Campus (SIU, for its intials in Spanish), of the University of Antioquia, Medellín, Colombia, at 1 540 m.a.s.l. The incubation was carried out in glass flasks, with capacity for 100 mL. In each flask 0,5 g of dry substrate was added ground at 1 mm, and 45 mL of artificial saliva. An artificial saliva / rumen inoculant ratio of 9/1 (45/5 mL/mL) was used. The flasks with the artificial saliva remained in a forced-air ventilation stove at 39 ºC during 6 h, before the inoculation with the rumen fluid. The ruminal liquid was obtained from three Holstein cows with fistula, which consumed *C. clandestinus*, mineralized salt and water at will. In the laboratory, the inoculants

Table 1**.** Evaluated forage resources of the high tropic.

Forage resource	Category	Harvest age, days
T. diversifolia	Perennial forage shrub	70
S. nigra		
S. pyramidalis		
C. clandestinus	Grass	37
L. perenne		28
C. clandestinus (80 %) + *S. nigra* (20 %)	Grass + perennial forage mixture	Grass, 37 + forage shrub, 70
C. clandestinus (80 %) + *T. diversifolia* (20 %)		
L. perenne (80 %) + *S. nigra* (20 %)		Grass, 28 + forage shrub, 70
L. perenne (80 %) + *T. diversifolia* (20 %)		

were filtered through a cotton cloth, gassed with CO_2 and were preserved in Bain Marie during the inoculation process.

A total of 156 flasks were inoculated, 144 (12 substrates x 3 repetitions/treatment (inoculants) and 2 reading times x 2 replicas per time) and 12 corresponding to the blanks (2 times x 3 inoculants x 2 blanks per time). The flaks contained buffer solution and inoculant, but without substrate, with the function of correcting the gas production generated by rumen microorganisms. The pressure originated by the gases product of fermentation, measured in pounds per square inch (PSI), was quantified with a digital transducer, at 24 and 48 h of incubation, according to the description by Theodorou *et al.* (1994). To transform the pressure data (PSI) (x) into gas volume (mL), the equation $Y = -0{,}1375+ (5{,}1385X + 0{,}0777X^2)$, obtained by Posada *et al.* (2006), was used. The gas production was expressed as gram of degraded dry matter (dDM).

In vitro dry matter degradation. This determination, expressed in percentage, was carried out at 24 and 48 h. at the end of the incubation, the flask content was filtered with crucibles of known weight and a vacuum pump was used. The resulting residue was dried in an oven during 48 h at 65 ºC; then it was weighed and used to calculate DM degradability by gravimetry (García-González *et al*., 2008).

Determination of the fermentation profile. The concentration of volatile fatty acids (VFA), acetic, propionic and butyric, was determined in the resulting liquid from the filtration of the crucibles, at 24 and 48 h of incubation. This liquid was preserved through the addition of sulfuric acid (98 % v/v) until reaching pH of 2 approximately, and later it was centrifuged repeatedly at 4 000 rpm until there was no presence of precipitate (Ramírez *et al*., 2015). For the determination of VFA, the samples of 1 µl of liquid were injected in a Thermo Trace GC Ultra gas chromatograph, equipped with a FID detector and a column of 0 m; 0,32 mm and 0,25 µm. The concentration of VFA (mmol/L) was calculated from the concentration (ppm) determined by chromatography. A molar mass of 60,05; 74,08 and 88,11 g/mol was assumed for the acetic, propionic and butyric acid, respectively.

Statistical analysis of the information. The degradability, methane production and VFA concentration data were analyzed by a variance analysis through the statistical program R. V.3.5.1., accompanied by Tukey's mean comparison test ($p < 0{,}01$) for those variables that showed statistical differences.

Results and Discussion

Chemical composition. Table 2 shows the bromatological characterization of the forage resources. The shrub species showed higher quantity of CP, NSC and NLE and lower NDF and ADF, with regards to the grasses. This coincides with works conducted by Londoño *et al*. (2019), Cardona-Iglesias *et al*. (2019a; 2019b), where higher values were found in these fractions in forage shrubs compared with kikuyu (*Pennisetum clandestinum*; Hochst. ex Chiov.) and ryegrasses (*L perenne*). If it is taken into consideration that in this work the NDF and ADF were lower in the shrubs with regards to the grasses, in this type of species higher content of intracellular components and higher content of hemicellulose, carbohydrate of higher solubility and easily utilizable energy source in ruminants, could be expected (Portillo-López *et al*., 2019).

In this study, for *T. diversifolia*, at 70 days, 17,5 % of CP was obtained, which coincides with the report by Lezcano *et al*. (2012) in *Megathyrsus maximus* (Jacqs.) B.K. Simon & S.W.L. Jacobs (Guinea grass), at a similar age. For *S. nigra* 23,0 % of CP was recorded, which coincides with what was referred by Guatusmal-Gelpud *et al.* (2020), who at 60 days obtained 26,0 % CP. This could be due to the fact that the edaphoclimatic conditions under which both works were conducted were similar, as well as the cutting age.

Table 2. Chemical composition of five forage species of the high Andean tropic of Nariño.

Species	DM, %	CP, %	NDF, %	ADF, %	NSC, %	NLE, Mcal/ kg DM	TT, g/kg DM	CT, g/kg DM	Sap, g/kg DM
T. diversifolia	14,0	17,5	46,7	23,6	13,7	1,4	16,0	2,0	10,8
S. nigra	17,5	23,6	45,9	24,8	13,9	1,5	22,1	4,1	17,1
S. pyramidales	12,8	17,9	43,2	21,3	17,3	1,4	12,8	2,4	13,8
C. clandestinus	15,8	19,4	57,7	34,4	7,0	1,3	8,9	1,2	12,3
L. perenne	15,0	18,3	55,7	32,9	13,3	1,4	6,9	1,2	7,4

DM dry matter, CP crude protein, NDF neutral detergent fiber, ADF acid detergent fiber, NSC non-structural carbohydrates, NLE net lactation energy, TT total tannins, CT condensed tannins, Sap saponins.

Regarding the NSC of *T. diversifolia*, contents of up to 33 % were reached in the pre-flowering stage (Ferreira, 2015), higher than those contributed by *C. clandestinus*. Vargas-Martínez *et al.* (2018) reported for this species a content of NSC similar to the one in this study. These authors state that the inadequate relation between the concentration of non-structural carbohydrates and protein in *C. clandestinus* limits the nutritional quality of this resource on its own.

The results in grasses coincide with the report in several studies conducted by Cardona-Iglesias *et al.* (2017) and Arce-Ramírez *et al.* (2020), who indicate at an age of 33-35 days of cutting of *C. clandestinus* and *L. perenne,* average CP of 20 % and a similar performance of NDF (higher than 50 %) and ADF (close to 30 %).

Regarding the NLE, for *L. perenne*, Castro-Rincón *et al*. (2019) found as average 1,4 Mcal/kg DM at 35 days in several localities of Nariño, similar value to the one in this study (1,38 Mcal/kg), but at 28 days. Urbano-Estrada *et al*. (2020) report for *C. clandestinus* 1,15 Mcal/kg DM of NLE as average, numbers below the values referred for forage shrubs. The NDF and ADF contents for *C. clandestinus* and *L. perenne* were 57,71 and 34,4 %; 55,7 and 32,9 %, respectively, similar to those obtained by Vargas-Martínez *et al*. (2018).

According to Vargas-Martínez *et al.* (2018), Cardona-Iglesias *et al*. (2019b) and Castro-Rincón *et al.* (2019) the high fiber values are the ones that limit grass nutritional quality and intake in the high tropic.

Regarding total tannins, concentrate tannins and saponins, the shrubs showed higher quantity of these metabolites compared with the grasses (table 2). The presence of these compounds in the forage plants allows to manipulate the rumen microbial ecology and, thus, reduce methane production (Cardona-Iglesias *et al.,* 2017).

In studies conducted by Fasuyi and Ibitayom (2011), 2,09 mg of tannins and 2,76 mg of saponins are reported in *T. diversifolia*. Meanwhile, Cardona-Iglesias *et al*. (2017) found 1,4 g of tannins and 4,53 g of saponins (kg/DM). Carvajal *et al.* (2012) recorded for *S. nigra* total tannin content of 0,08 mg and 0,6 mg/kg of CT (Cárdenas *et al.,* 2016). For the species *S. pyramidalis* the works of characterization of secondary metabolites are still scarce. Nevertheless, Apráez-Gerrero and Galvéz-Cerón (2019) refer absence of tannins and saponins for this species, contrary to the findings in this research.

Table 3 shows the statistically significant differences ($p < 0,01$) among the evaluated forage resources for methane production, DM degradation and volatile fatty acids (acetic, propionic and butyric).

L. perenne and *S. nigra* showed the highest degradation values (71,6 and 70,6 %; respectively). Castro-Hernández *et al*. (2017) found results of up to 71,0 % of degradation in *L. perenne* harvested at 28 days, value they related to higher efficiency in the synthesis of microbial protein in the rumen. Meanwhile, García-Morcote and Ortegón-Espejo (2014) referred for *S. nigra* 82,5 % of degradation at 48 h of incubation, higher value than the one in this study. Regarding this, Jaramillo-Benavides (2019) states that the *S. nigra* forage stands out for the high *in vitro* DM degradation and its nutritional quality.

The methane production was lower in the forage shrubs compared with the grasses ($p < 0,01$). *T. diversifolia* reached, as average, 34,0 mL/g DM/day, and it was followed by *S. pyramidalis* (41,2) and *S. nigra* (46,9 mL/g DM/day). Meanwhile, *C. clandestinus*

Table 3. Methane production, dry matter degradability and volatile fatty acids in five forage species.

Species	Incubation time, h	DM degradation, %	CH_4/g/mL DDM	Acetic, Mmol/L	Propionic, Mmol/L	Butyric, Mmol/L
C. clandestinus	24	40,6e	40,9d	80,9e	33,5de	12,2c
	48	59,9b	87,2a	98,8bcd	42,9bcd	13,5abc
L. perenne	24	47,3cd	28,5f	80,0e	39,6cde	13,5abc
	48	71,6a	74,2b	128, a	59,5a	16,6a
T. diversifolia	24	48,5c	21,2g	76,e	33,9de	11,9c
	48	70,2a	34,0e	97,9bcd	33,6de	11,7c
S. nigra	24	57,8b	22,3g	85,1de	32,3e	13,1bc
	48	70,6a	46,9c	111,2b	41,2bdce	16,2ab
S. pyramidales	24	44,2de	24,6fg	99,9bc	47,1bc	13,0bc
	48	50,7 c	41,2d	112,b	50,2ab	12,6 c
SE ±		0,61	0,67	2,40	0,58	0,57
P - value		0,0001	0,0001	0,0001	0,0001	0,0102

CH_4: methane, DDM: degraded dry matter, Mmol: milimol, SE: standard error of the meana
Means with different letters in the columns differ among them, according to Tukey's test ($p < 0,01$)

showed the highest value of methane production (87,22 ml/gdDM), and it was followed by *L. perenne* (74,2 mL/g DM/day).

The results obtained here are in correspondence with the report in a research conducted by Cardona-Iglesias *et al.* (2017), where higher methane production was reported in *C. clandestinus* and lower concentrations in *T. diversifolia.* The methane content in *C. clandestinus* and *L. perenne* was higher than the report by Restrepo (2016) who indicated values of 35,9 and 35,8 mL/g DM/day, at 37 and 30 cutting days, respectively. In a study carried out by Marín *et al*. (2014) methane productions were found for *C. clandestinus* that varied between 109,7 and 204,1 mL/g DM/day, values that differed depending on the zones where the study was conducted. For *Lolium* sp methane values of up to 329 mL/g DM/day have been reported (Tabla-Rojas, 2019).

For *T. diversifolia*, Pérez-Can *et al.* (2020) reported methane production of 20,17 mL/g DM/day; while Cardona-Iglesias *et al*. (2017), at 70 days of cutting, reported values of 19,3 mL/g DM/day, also at 48 days of incubation. Rivera *et al*. (2011) observed low production of this gas in the forages *S. nigra* and *T. diversifolia* compared with other forages.

Benaouda *et al*. (2017) state that the use of forage plants like *T. diversifolia* can influence the decrease of methane production at rumen level, and that this action depends on the species, age of the plant and content of secondary metabolites. In this regard, some authors sustain that this potential in the decrease of methane emissions is due to the fact that they modify rumen fermentation and the population of methanogens and protozoans (Galindo *et al*., 2014).

In general, this study proved that forage shrubs show less methane production compared with the evaluated grasses, results that can be associated to the higher content of secondary metabolites, such as tannins and saponins.

According to Bayat and Shingfield (2012), Cardona-Iglesias *et al*. (2017) and Galindo-Blanco *et al*. (2018), secondary metabolites inhibit methanogenesis through certain mechanisms, such as the decrease of hydrogen formation in the rumen, inhibition of functional enzymes in methanogens and reduction in the population of protozoans. That is why forage resources with presence of secondary metabolites have potential for decreasing enteric methane (Pérez-Can, 2019).

In this study, the production of volatile fatty acids (VFA) was higher at 48 h of incubation with regards to the time of 24 h. This can be due, as stated by Ramírez *et al*. (2015), to the fact that in the *in vitro* gas production technique there is no removal of VFA via absorption, for which their concentration depends on the fermentation rate and can be gradually increased.

The molar proportion of acetic acid was higher in all the species compared with the propionic and butyric fraction, result that is explained because the evaluated species constitute sources of forage origin with moderate to high fiber contents. Regarding this, Cardona-Iglesias *et al*. (2017) indicate that the VFA profile is modified according to the nutritional composition of the substrate, the acetic fermentation being higher in forages.

In a study conducted by Vélez-Ruiz *et al*. (2017) higher acetic and lower propionic and butyric fermentation was also reported for *L. perenne* and *C. clandestinus*. The acetic acid content was higher in *L. perenne* (117,2 mmol/L) compared with the other forages.

Regarding the production of propionic acid, it was higher ($p < 0,01$) for *L. perenne* (59,5 mmol/L), value that exceeds the one recorded by Vélez- Ruiz *et al*. (2017). According to Cardona *et al*. (2017) and Castro-Rincón *et al*. (2019), propionic fermentation is generally related to age and sugar content in the plant.

Figures 1 and 2 show the results of DM degradation and *in vitro* methane production, by mixing *T diversifolia* and *S. nigra* with the two grasses.

Regarding DM degradation (fig. 1), the results indicated significant differences $p < 0,01$) among the forage mixtures. The highest values were recorded at 48 h of incubation. There was higher degradation percentage in the mixtures of *S. nigra* (78,9 %) and *L. perenne* (78,3 %) with *C. clandestinus.* The lowest degradation percentage (65,0 %) corresponded to the mixture *C. clandestinus* + *T. diversifolia*.

Generally, lower DM degradation of the grasses was recorded, when analyzed separately (table 3). However, this fraction increased by mixing them with 20 % of the forage shrubs. In this study, degradability surpassed the finding for the mixture *T. diversifolia* (5 %) with *C. clandestinus* (95 %), reported by Cardona-Iglesias *et al*. (2017) which showed degradability (48 h) of 61,1 and 60,1 % for *T. diversifolia* and *C. clandestinus*, respectively.

The DM degradability is associated to the species, phenological state and nutritional composition of the plant. In general, higher degradability in the diet increases forage intake (Cardona-Iglesias *et al*., 2019b; Argüello-Rangel *et al*., 2020). A factor that has incidence on ruminal degradability is the NDF and

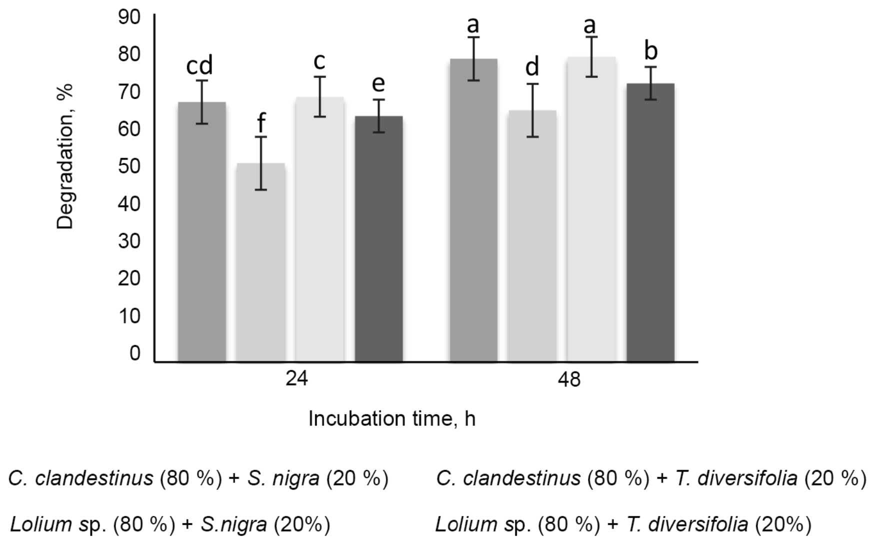

Figure 1. DM degradation percentage of four forage mixtures.
Vertical bars represent confidence intervals of 95 % and different letters show significant differences, according to Tukey's test (p < 0,01)..

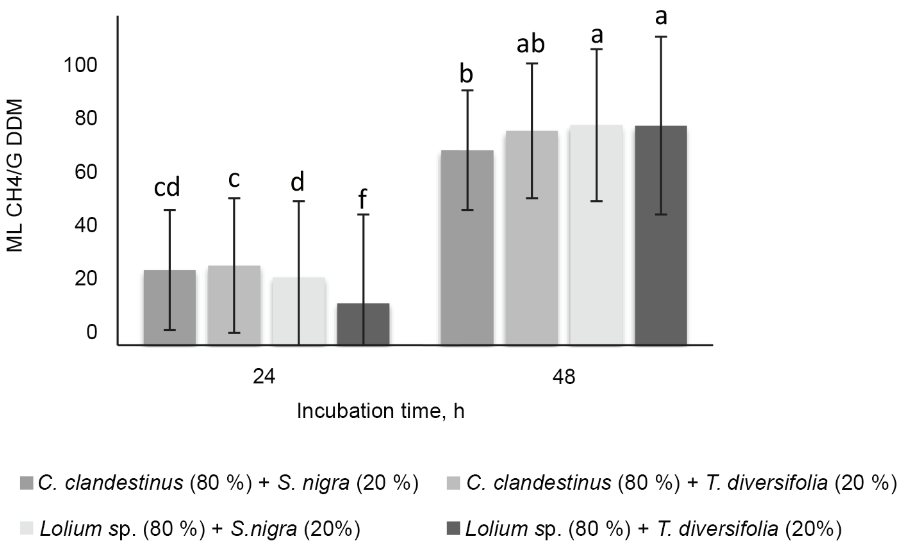

Figure 2. *In vitro* methane production from four mixtures.
Vertical bars represent confidence intervals of 95 % and different letters show significant differences, according to Tukey's test (p < 0,01)

ADF of the ration (Castro-Rincón *et al*., 2019). In this work, the grasses showed higher fiber contents than the shrubs (table 2). Nevertheless, by adding *S. nigra*, the total degradability of the mixture increased up to 18,4 and 7,2 % for the mixtures with *C. clandestinus* and *L. perenne*, respectively.

In a study conducted by Tabla-Rojas (2019) lower DM degradability was reported in *S. nigra* compared with grass species such as *L. perenne*. This response is ascribed to the physiological status of the plant, because it was evaluated in preflowering and flowering. According to the author, in these stages structural carbohydrates increase, at the expense of the degradability and utilization of nutrients by the animal.

The methane production at 48 h was lower ($p < 0,01$) for the mixture *C. clandestinus* + *S. nigra*

(66,3 mL/g DM/day), and higher for *L. perenne* (80 %) + *S. nigra* (20 %) and *L. perenne* (80 %) + *T. diversifolia* (20 %), with 74,8 and 74,0 mL/g DM/day, respectively. Authors like Quiñonez-Chillambo *et al*. (2020) argue that *C. clandestinus* has higher concentration of secondary metabolites with regards to *L. perenne*, and in mixture with tanniniferous shrubs lower methane production could be expected, with regards to the mixtures with *L. perenne.* On the contrary, Vélez-Ruiz *et al*. (2017) state that *C. clandestinu*s produces up to 14,3 % more methane than *L. perenne,* because it represents higher proportion of cellulose and lignin as it is a C4 species, which coincides with the record in this work (table 3).

The mixture of *L. perenne* plus *S. nigra* had higher methane production at 48 h (74,7 mL/g DM/day) compared with the mixture of *C. clandestinus* and *S. nigra* (66,3 mL/g DM/day). The above-stated fact could be due to a better response of *C. clandestinus* in the decrease of methane, when it was mixed with species that contain secondary metabolites such as *S. nigra*. It can also be explained because the tannin concentration in *C. clandestinus* was higher than in *L. perenne*, and by the nutritional balance provided by the mixture with perennial forages, because the nutritional efficiency is improved and the energy losses in methane synthesis decrease (Carro-Travieso *et al*., 2019; Pérez-Can, 2019).

Conclusions

Higher concentration of secondary metabolites was found in the forage shrubs with regards to the grasses. When 20 % of the forage plants was included in this mixture, the methane production decreased, and the substrate degradability increased.

It is necessary to continue the evaluation of the rumen dynamics and interaction among the different nutritional components to establish the best inclusion percentages of forage shrubs in grass-based diets, as strategy to improve the nutritional quality and decrease the methane emissions in animal husbandry systems of the Colombian high tropic.

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Authors' contribution

- Juan Leonardo Cardona-Iglesias. Devised the idea of the research and its methodology, as well as participated in the data processing, manuscript writing and final revision.
- María Fernanda Urbano-Estrada. Conducted the research, data processing and writing the original draft.
- Edwin Castro-Rincón. Supervised the research, writing and final revision of the manuscript.
- Carolina Guatusmal-Gelpud. Conducted the research, participated in the search for bibliography and in writing the original draft.
- Lina Marcela Ríos-Peña. Conducted the research, participated in the search for bibliography and in writing the original draft.

Conflict of interests

The authors declare that there is no conflict of interests among them..

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