Enteric methane mitigation and fermentation kinetics of forage species from Southern Mexico: in vitro screening

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Received: 2 April 2020 / Accepted: 28 November 2020 / Published online: 3 January 2021
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Abstract Enteric methane (CH₄) emission from ruminant livestock is one of the main sources of greenhouse gases from the agricultural sector worldwide. In tropical regions there is a wide variety of forage species that have the capacity to improve cattle diets and reduce enteric CH₄ emissions. A screening trial was conducted to investigate the nutrient and phytochemical composition, total gas and CH₄ production of fifteen tropical multipurpose forage species from Southern Mexico. The content of crude protein (CP), neutral detergent fiber (NDF) and gross energy fluctuated among species, from 99.07 to 264.4, from 275.19 to 614.35 g kg⁻¹ dry matter (DM) and from 15.65 to 20.92 MJ kg⁻¹ DM. In vitro digestibility of DM (IVDDM) was lower for the species containing condensed tannins (CT) and fluctuated between 447.44 and 709.94 g kg⁻¹ DM. Bursera simaruba showed the lowest CH₄ production (9.077 mg g⁻¹ degraded organic matter) with a CT content of 200 g kg⁻¹ DM. Results suggest that several plant

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species widely available in Southern Mexico present high potential for mitigating enteric CH$_4$ production and have a high nutritional quality. These species are suitable as additive or supplementary feed to improve diet quality and reduce CH$_4$ emissions in cattle raised under grazing conditions in the tropical regions of the world.

**Keywords** Livestock agroforestry · Lacandon rainforests · Multipurpose forages · Cattle · Tropics

**Introduction**

Methane (CH$_4$) is a gas produced mostly by ruminants during anaerobic fermentation of carbohydrates in the rumen which is eructated to the atmosphere and is one of the main greenhouse gases. Enteric methane contributes approximately 6% of global anthropogenic emissions (Beauchemin et al. 2020), and it is also considered an energy loss for the animal. However, investigations on enteric CH$_4$ emissions from ruminants have been focused during the last 20 years on its environmental impact and the search for mitigation strategies and less on energy metabolism (Beauchemin et al. 2020). In this context, one of the main alternatives investigated for the reduction of enteric CH$_4$ is the use of plant secondary metabolites.

Tropical regions worldwide display a high forage biomass production from trees, shrubs and herbaceous plants with adequate chemical composition (140 to 320 g crude protein [CP] kg$^{-1}$ dry matter [DM] and < 400 g neutral detergent fiber [NDF] kg$^{-1}$ DM) and with potential to be used as suitable feed alternative in sustainable livestock production systems. This diversity of species provides ecosystem services such as natural rehabilitation of degraded soils, N-fixation (in the case of legumes) and decreased CH$_4$ emissions by the improvement of diet quality, addition of secondary metabolites to the diet and the possibility to alter the rumen microbiome (Rao et al. 2015; Arango et al. 2020). Secondary metabolites contained in many plant species have the capacity to modify the population of microorganisms that synthesize or are related to the formation of CH$_4$ (Melesse et al. 2017; Albores-Moreno et al. 2018) in the rumen.

The search for local resources with high nutritional value is important to improve profitability and productivity of livestock systems in tropical regions of the world as well as to reduce their impact on the environment (Valencia-Salazar et al. 2018). Some mitigation alternatives are associated with improved efficiency of animal production given their advantages from the nutritional and environmental standpoints (Patra et al. 2012). In Southern Mexico, cattle producers claim that during the dry season animals browse a diversity of species present in native vegetation in the form of green fodder, dry pods and leaves from trees and shrubs (López Herrera et al. 2008; Albores-Moreno et al. 2018). Secondary vegetation in livestock systems has been scarcely studied and is being displaced by introduced pastures for the establishment of extensive grazing systems (Albores-Moreno et al. 2020). Extensive cattle production systems in tropical regions have resulted in severe impacts on soil and ecosystems and continue to promote land use change (Rao et al. 2015). Those systems are also less efficient due to their impact on ecosystems, low quality of the pastures and poor management. This essentially extractive production system induces high rates of deforestation that has generated lost of biodiversity and a higher contribution to greenhouse gas emissions from the sector. Pastures used in extensive ruminant livestock systems have high content of NDF, low soluble carbohydrates and CP that induce higher CH$_4$ emissions, leading to low productivity (Valencia-Salazar et al. 2018; Gaviria-Uribe et al. 2020). Therefore, a better management of the grazing system is required to use resources more efficiently. Nonetheless, forestry resources in the tropics with multiple uses and cultural importance can be studied for the mitigation of CH$_4$ and for the implementation of livestock agroforestry systems.

The Lacandon rainforest is located to the East and Northeast of the state of Chiapas in Southern Mexico. One of the main economic activities of the indigenous maya and mestizo peasants of this region is extensive cattle production, and this has generated the greatest changes in land use in the history of the Lacandon rainforest (Covaleda et al. 2014). Currently, there are only 498,138 ha of forests and preserved forests left (Covaleda et al. 2014). The biodiversity of this region is one of the most important in Mexico (Jiménez-Ferrer et al. 2008), and this potential allows a reconversion of livestock toward sustainable production systems through the use of available resources such as fodder trees, shrubs and herbaceous plants that have potential to reduce CH$_4$ emissions from cattle.
and regenerate degraded areas. In this context, the objectives of this study were to evaluate the nutritional quality, fermentation parameters and enteric CH$_4$ mitigation potential of fifteen forage species recognized as suitable for livestock production in the Lacandon rainforest region of Mexico.

**Materials and methods**

**Description of the study area**

Samples of forage species were collected in October–November 2018 and February 2019 in the municipality of Ocosingo Valley, Chiapas, Mexico, which is part of the socio-economic region XII Lacandon Rainforest (Flores-González et al. 2018). The climate is warm-humid (23–27 °C) with an altitude that ranges from 10 to 900 MASL (meters above sea level) (García del Valle et al. 2015). Ocosingo covers the largest region of the Lacandon rainforest and has a predominantly Tzeltal and Chol indigenous population (Flores-González et al. 2018). Indigenous communities own highland and midland rainforest vegetation with patches of cloud forests and pine and oak forests, as well as acahuales (secondary vegetation), maize plots, vegetable cultivars (García del Valle et al. 2015) and grazing cattle.

**Selection of species and sampling**

Species were selected from studies carried out in the region for identification of forage species based on local knowledge and cultural importance for livestock producers (Soulard 2003; Pinto-Ruiz et al. 2005; Velasco-Pérez 2007; Douterlungen 2013; Paz-Cortés 2010). Plant species were identified and collected in paddocks, acahuales, orchards and live fences. Species were mostly collected in induced grasslands, cultivated areas and mesophyll mountain forest as shown in Fig. 1. Forage species were sampled between 683 and 1059 MASL. A total of 15 species in their vegetative stage were identified and collected for the screening (Table 1). The species collected were: *Gliricidia sepium*, *Bauhinia variegata*, *Cecropia obtusifolia*, *Guazuma ulmifolia*, *Erythrina goldmanii*, *Spondias mombin*, *Acacia pennatula*, *Parmentiera aculeata*, *Tithonia diversifolia*, *Liabum glabrum*, *Platymiscium dimorphandrum*, *Ochroma pyramidale*, *Brosimum alicastrum*, *Bursera simaruba* and *Mucuna pruriens*.

Leaves were cut from 5 to 9 individuals per plant species. During the sampling, the location points of each species were taken by a GPS (Garmin® Etrex 30×) to geographically locate the presence of the species. Species were firstly identified with the help of producers and botanical samples were taken for further verification at the herbarium of the Southern Border College (ECOSUR). For chemical analysis, samples were dried in a forced air oven at 55 °C for 48 h or until constant weight to determine dry matter (DM) content and were ground in a Thomas Wiley® mill to a particle size of 1 mm. Samples were pooled and stored in bags with airtight seal for transportation.

**Chemical analysis**

Chemical analysis and the in vitro gas production technique were carried out at the Laboratory of Animal Nutrition and Forage Quality of the International Center of Tropical Agriculture (CIAT) located at Palmira, Colombia, and certified by the FAO-IAG proficiency test of feed constituents 2017. Plant samples were analyzed by triplicate. Organic matter (OM) content of the samples was determined by combustion in a muffle furnace at 500 °C for 4 h (AOAC 2005: method 942.05), CP (CP = N × 6.25) by Kjeldahl method (AN 3001 FOSS; AOAC 1990: method 984.14), NDF and acid detergent fiber (ADF) content was determined using the method proposed by Goering and Van Soest (1970), adapted to an Ankom Fiber Analyzer AN 3805 (Ankom® Technology Corp. USA), and gross energy was determined with an adiabatic bomb calorimeter following the procedure described in ISO 9831.1998. Ether extract content was determined by the Soxhlet method. The two-stage in vitro technique (Tilley and Terry 1963) was used for the determination of digestibility. Secondary metabolites were quantified at the Bromatology Laboratory of the Southern Border College (ECOSUR). Tannin content of the species was determined by the vanillin extract assay (Hagerman and Butler 1978). Qualitative quantification of alkaloids, cyanogenic glycosides and saponins was carried out by the methodologies proposed by Domínguez (1979). Mayer, Draggendorff and Wagner reagents that identify only the presence of alkaloids in general were used. For the quantification of cyanogenic glycosides 0.2 g of the sample was
weighed, 1 ml of chloroform was added and a strip of Whatman No. 1 filter paper with freshly prepared sodium picrate (1 g of sodium carbonate, 100 mg of picric acid and 10 ml of water, moisten in the reagent and let the strips dry) was introduced in the solution. The tubes were capped and heated in a water bath at 37 °C for 3 h. Changes in the color of the filter paper from yellow to red or red to brown indicated that the sample was positive to cyanogenic glycosides.

In vitro gas production technique

Animals used in this study were treated in accordance with the Colombian normative num. 84 of 1989 and following the protocol approved by the Ethics Committee of the International Center of Tropical Agriculture (CIAT). Gas production was determined using the in vitro technique proposed by Menke and Steingass (1988) as modified by Theodorou et al. (1994). Rumen liquor was obtained from three rumen cannulated Brahman bulls of 550 kg live weight grazing *Cynodon plectostachyus*. Rumen liquor was filtered through 10 layers of gauze and mixed in a 1:9 ratio with a reduced mineral solution (Menke and Steingass 1988). Additionally, ruminal solid and liquid content was liquefied and filtered to ensure the presence of microorganisms of both the liquid and solid phase in the inoculum. For each treatment, 1000 mg of samples was incubated in bottles of 160 ml capacity by triplicate including blanks. Bottles were kept under constant flow of CO₂, sealed with a rubber stopper and an aluminum ring and placed in a water bath at 39 °C for 72 h. Gas pressure and volume in the headspace of the bottles were measured with a pressure transducer connected to a digital reader (Sper Scientific®, USA) and a three-way valve connected to a hypodermic needle that was inserted into the bottles and a 60 ml syringe to measure gas volume. Gas pressure and volume were measured at 0, 4, 8, 12, 24, 36, 48, 56 and 72 h. Gas volume was stored in amber
bottles with a capacity of 125 ml from samples collected from the accumulated gas at 12, 24 and 48 h fermentation. CH4 concentration was quantified using a gas chromatograph (GC-2014 Shimadzu, Japan) with the following specifications: Column: Shimadzu: packed 1/8" stainless steel columns 1.0 m HayeSep T 80/100 mesh, 4 m HayeSep D 80/100, 1.5 P–N, 0.7 m Shimalite Q 100/180, column temperature: 80 °C, detector temperature: FID = 250 °C, ECD 325 °C, methanizer temperature: 380 °C, carrier gas: nitrogen, column flow: 30.83 ml/min and injection volume managed by a loop with a capacity of 2 ml. For the degradation of DM and OM, the content of the bottles was withdrawn from fermentation at different times (12, 24, 48 and 72 h) and filtered in crucibles with fiberglass filter. The crucibles with the fiberglass filter were then dried in a forced air oven at 65 °C for 48 h and weighed.

Data obtained from the pressure and volume of the experiments were used to generate the following polynomial equation for the correction of the volume of gas produced:

\[ y = 0.0209x^2 + 5.9023x - 2.984 \]

\[ R^2 = 0.9729 \]

Gas production data were adjusted to the modified Gompertz (Lavrenčič et al. 1997) model with the following equation:

\[ y = ae^{-eb-cx} \]

where \( y \) is equal to the cumulative gas production at a time \( x \), \( a > 0 \) is the maximum gas production, parameter \( b > 0 \) is the difference between the initial gas and the final gas at a time \( x \) and the parameter \( c > 0 \) describes the specific rate of gas accumulation. The practical application of this model requires the conversion of parameters \( a, b, c \) into parameters with biological significance. The parameters were: time at the inflection point (TIP, hours), gas at the inflection point (GIP, ml), maximum gas production rate (MGPR ml h\(^{-1}\)) and Lag phase (LP). For its estimation the following formulas were used: 

\[ TIP = b/c; \quad GIP = ae/c; \quad MGPR = (a * c/e; \quad LP = ((b/c) - (1/c)); \text{ where “e” is Euler’s number, equivalent to } \approx 2.718281828459. \]

Statistical analysis

For the statistical analysis, a randomized block design was used with 15 treatments, three replicates per hour and three different inoculums as a blocking factor. To
assess the behavior of the variables, the PROC GLM procedure of SAS® software, version 9.4 (SAS 2012) was used. The statistical model used for analysis was: $Y_{ij} = \mu + \tau_i + \beta_j + \epsilon_{ij}$, where $Y_{ij}$ is the observations of samples assigned to the treatment $i$ and block $j$; $\mu$: is the overall mean of the population; $\tau_i$: is the effect of $i$th treatment; $\beta_j$: is the effect of $j$th block; $\epsilon$: is the experimental error. Treatments means were compared with the Tukey test with an Alpha of 0.05.

Results

Chemical composition

Organic matter content of the species ranged from 821.28 to 934.51 g kg$^{-1}$ DM. Species with the highest content of OM were M. pruriens, P. dimorphandrum and A. pennatula (Table 2). Crude protein content was higher for T. diversifolia, G. sepium, L. glabrum with 289.54, 261.41 and 240.16 g kg$^{-1}$ DM, respectively. B. simaruba had the lowest CP content (99.07 g kg$^{-1}$ DM). The lowest NDF contents were 275.19, 298.17, 305.88 g kg$^{-1}$ DM for B. variegata, B. alicastrum and T. diversifolia, respectively. Species with the lowest ADF content were SM, MP and GS with 171.18, 180.65 and 190.83 g kg$^{-1}$ DM, respectively. In vitro digestibility of DM was highest for G. sepium (709.94 g kg$^{-1}$ DM), T. diversifolia (704.03 g kg$^{-1}$ DM), M. pruriens (700 g kg$^{-1}$ DM) and B. variegata (698.27 g kg$^{-1}$ DM). A. pennatula had the lowest in vitro digestibility (447.44 g kg$^{-1}$ DM). Gross energy content of the species ranged from 15.65 to 20.92 MJ kg$^{-1}$ DM. Condensed tannins were present in B. variegata, C. obtusifolia, G. ulmifolia, S. mombin, A. pennatula, O. pyramidale, B. simaruba and M. pruriens with 1.66, 132.7, 32.7, 9.9, 31.1, 39.5, 200.1 and 8.7 g kg$^{-1}$ DM, respectively. In the phytochemical screening, the presence of alkaloids was found in all species except for B. simaruba as presented in Table 3. Cyanogenic glycosides were found highly abundant only in A. pennatula. Saponins were found in G. sepium, B. variegata, G. ulmifolia, E. goldmanii, A. pennatula and O. pyramidale with a low abundance (+) except for G. sepium in which these were abundant (++).

| Species                  | g kg$^{-1}$ DM | MJ kg$^{-1}$ DM |
|--------------------------|----------------|-----------------|
| Acacia pennatula         | 505.42         | 924.85          | 492.56         | 210.34 | 192.69 | 39.25 | 447.44 | 31.1 | 20.92 |
| Bauhinia variegata       | 384.04         | 854.41          | 275.19         | 194.57 | 146.45 | 28.72 | 698.27 | 16.6 | 17.92 |
| Brosimum alicastrum      | 489.18         | 821.28          | 298.17         | 269.22 | 116.21 | 29.92 | 686.38 | 0.00 | 15.65 |
| Bursera simaruba         | 356.71         | 900.82          | 354.37         | 249.23 | 99.07  | 25.05 | 471.37 | 200.1 | 18.92 |
| Cecropia obtusifolia     | 295.95         | 897.12          | 437.00         | 214.00 | 187.48 | 35.10 | 498.94 | 132.7 | 19.12 |
| Erythrina goldmanii      | 297.68         | 888.16          | 417.38         | 271.05 | 206.78 | 41.40 | 595.97 | 0.00 | 19.06 |
| Gliricidia sepium        | 242.83         | 890.78          | 43.56          | 190.83 | 261.41 | 30.12 | 709.94 | 0.00 | 19.48 |
| Guazuma ulmifolia        | 398.35         | 886.42          | 480.45         | 260.29 | 150.72 | 32.02 | 516.88 | 32.7 | 19.06 |
| Liabum glabrum           | 182.26         | 868.04          | 443.00         | 334.97 | 240.16 | 53.30 | 531.11 | 0.00 | 19.01 |
| Mucuna pruriens          | 248.35         | 934.51          | 386.68         | 180.65 | 228.31 | 29.90 | 700.19 | 8.70 | 19.44 |
| Ochroma pyramidale       | 217.03         | 882.84          | 369.39         | 232.65 | 195.85 | 21.70 | 565.31 | 39.5 | 18.36 |
| Parmentiera aculeata     | 308.95         | 874.47          | 614.35         | 268.81 | 183.17 | 13.85 | 548.37 | 0.00 | 18.04 |
| Platymiscium dimorphandrum| 279.54        | 931.01          | 539.15         | 324.83 | 236.32 | 18.72 | 616.47 | 0.00 | 20.45 |
| Spondias mombin          | 259.75         | 852.29          | 307.77         | 171.18 | 126.95 | 44.78 | 638.59 | 9.90 | 16.25 |
| Tithonia diversifolia    | 190.50         | 853.53          | 305.88         | 337.81 | 289.94 | 21.25 | 704.03 | 0.00 | 18.31 |

DM, dry matter; OM, organic matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; CP, crude protein; EE, ether extract; IVDDM, in vitro digestibility of dry matter; CT, condensed tannins
In vitro gas fermentation and degradability

Maximum gas production (a), time at the inflection point (TIP), gas inflection point (GIP), maximum gas production rate and lag phase differed significantly (P < 0.05) among the forage species evaluated (Table 4). Maximum in vitro gas production was obtained in *B. alicastrum*, *T. diversifolia*, *M. pruriens*, and *S. mombin* with 256.7, 232.4, 225.7 and 216.5 ml. The lowest gas production was recorded for *B. simaruba* with 118.03 ml. *B. alicastrum*, *G. sepium*, *T. diversifolia*, and *B. variegata* had the highest MGPR with 9.72, 8.10, 7.77- and 7.46-ml h⁻¹.

Degradability at different hours (12, 24, 48 and 72 h) differed significantly (P < 0.05) among species (Table 5). The highest degradability at 12, 24, 48 and 72 h was observed for *T. diversifolia* with 567.8, 690.4, 781.1 and 787.1 g kg⁻¹ DM, respectively. Dry matter degradability at 72 h ranged from 353.4 (A. pennatula) to 787.1 g kg⁻¹ DM (*T. diversifolia*).

Methane production

Distribution and differences in CH₄ produced at 12, 24 and 48 h per degraded OM (mg g⁻¹) from evaluated species are shown in Fig. 2a, b, c. Methane production in mg g⁻¹ DOM was different among species (P < 0.0001). *C. obtusifolia, A. pennatula, P. aculeata* and *O. pyramidale* had the lowest CH₄ productions at 12 h with 1.58, 2.25, 1.78 and 1.52 mg g⁻¹ DOM, respectively. Lowest CH₄ production at 48 h was observed in *B. simaruba* with 9.077 mg g⁻¹ DOM. At 48 h, *B. alicastrum, G. sepium* and *M. pruriens* had the highest CH₄ production with 35.228, 20.713 and 19.977 mg g⁻¹ DOM, respectively. Accumulated CH₄ at 48 h from different species is shown in Fig. 2d. Lowest CH₄ production at 48 h was observed in *B. simaruba, P. aculeata* and *S. mombin* with 9.07, 9.78 and 9.44 mg g⁻¹ DOM, respectively.

Discussion

Nutritional quality of forage species especially CP content is of great relevance due to protein deficiencies in pastures used in tropical extensive cattle production systems. In the current study, CP contents were all above 120 g kg⁻¹ DM with the exception of *B. simaruba* (99.07 g kg⁻¹ DM) and *B. alicastrum* (116.21 g kg⁻¹ DM). However, these values can meet

Table 3 Phytochemical screening of multipurpose forage species from Southern Mexico

| Species                 | Alkaloids | Cyanogenic glycosides | Saponins |
|-------------------------|-----------|-----------------------|----------|
|                         | Mayer     | Draggendorff          | Wagner   |
| *Acacia pennatula*      | –         | ++                    | +++      | +        |
| *Bauhinia variegata*    | –         | +++                   | ++       | +        |
| *Brosimum alicastrum*   | +++       | +                     | –        | –        |
| *Bursera simaruba*      | –         | –                     | –        | –        |
| *Cecropia obtusifolia*  | –         | +++                   | +        | –        |
| *Erythrina goldmanii*   | +++       | +                     | ++       | –        | +        |
| *Gliricidia sepium*     | +         | +++                   | +        | –        | ++       |
| *Guazuma ulmifolia*     | +         | +                     | ++       | –        | +        |
| *Liatum glabrum*        | ++        | ++++                  | +++      | –        | –        |
| *Mucuna pruriens*       | ++        | ++                    | –        | –        | –        |
| *Ochroma pyramidale*    | –         | +++                   | –        | –        | +        |
| *Parmentiera aculeata*  | ++++      | +++                   | +++      | –        | –        |
| *Platymiscium dimorphandrum* | ++ | ++++                | –        | –        | –        |
| *Spondias mombin*       | –         | +                     | ++       | –        | –        |
| *Tithonia diversifolia* | +++       | ++++                  | +++      | –        | –        |

– (No presence); + (low abundance); ++ (abundant); +++ (moderately abundant); ++++ (highly abundant)
the requirements of CP for moderate levels of production of ruminants in tropical regions. Crude protein values presented in this study were similar to those reported by other authors (López Herrera et al. 2008; Rodriguez-Villanueva et al. 2019; Yusuf et al. 2020).

Titonia diversifolia and G. sepium had the highest content of CP and also the highest DM digestibility. Neutral detergent fiber content of G. sepium was similar to the value reported by Molina-Botero et al. (2019) (575.4 g kg\(^{-1}\) DM); however, CP from G. sepium was much higher in the present trial. All species showed to be a good source of gross energy. The greatest DM degradability was for the species that contained lower concentrations of secondary metabolites (G. sepium, T. diversifolia and B. alicastrum), and it is likely that those species were more fermentable and susceptible to bacterial attack. The majority of secondary metabolites that have been studied for the reduction in CH\(_4\) synthesis in the rumen have shown to have antimicrobial properties (Patra et al. 2012); this, in consequence, can directly reduce DM degradability. NDF contents were below 550 g kg\(^{-1}\), with the exception of P. aculeata (614.35 g kg\(^{-1}\) DM); in this sense, species in this trial can be considered with a high and medium nutritional quality. Maximum gas production was obtained with G. sepium, B. alicastrum and T. diversifolia in agreement to their relatively low NDF and high CP content. Crude protein and carbohydrate content is directly associated with the degradation of dry matter, production of total gas and CH\(_4\). In a trial carried out by Molina-Botero et al. (2020) it was shown that there is a higher rate of gas production when degradation of the substrate is greater, and this is obtained when the incubated forage is rich in soluble carbohydrates. Likewise, Seresinhe et al. (2012) showed that protein content and gas production are moderately correlated (0.66). The above results are an improvement in the balance between nutrients that render the microorganisms more efficient in the degradation of the substrate and therefore produce more gas.

Table 4  Gompertz model parameters for in vitro gas production measured in forage species from Southern Mexico

| Species          | Parameters | TIP (h) | GIP (ml) | MGPR (ml/h) | LP      |
|------------------|------------|---------|----------|-------------|---------|
| A. pennatula     | a: 146.883 | b: 0.679 | c: 0.056 | d: 12.453   | e: 54.023 |
| B. alicastrum    | a: 256.729 | b: 0.951 | c: 0.103 | d: 9.240    | e: 94.427 |
| B. simaruba      | a: 118.030 | b: 0.956 | c: 0.081 | d: 11.883   | e: 43.410 |
| B. variegata      | a: 201.269 | b: 0.865 | c: 0.102 | d: 17.287   | e: 74.027 |
| C. obtusifolia   | a: 214.405 | b: 0.837 | c: 0.049 | d: 8.613    | e: 78.860 |
| E. goldmanii     | a: 168.493 | b: 0.668 | c: 0.063 | d: 10.637   | e: 61.970 |
| G. sepium        | a: 200.078 | b: 1.070 | c: 0.111 | d: 9.903    | e: 73.590 |
| G. ulmifolia     | a: 188.402 | b: 0.742 | c: 0.051 | d: 14.573   | e: 69.297 |
| L. glabrum       | a: 174.429 | b: 0.899 | c: 0.088 | d: 10.207   | e: 64.153 |
| M. pruriens      | a: 225.745 | b: 0.665 | c: 0.089 | d: 7.430    | e: 83.030 |
| O. pyramidalis   | a: 196.539 | b: 0.928 | c: 0.056 | d: 16.573   | e: 72.287 |
| P. aculeata      | a: 184.457 | b: 0.763 | c: 0.028 | d: 27.253   | e: 67.843 |
| P. dimorphandrum | a: 185.037 | b: 0.806 | c: 0.085 | d: 9.477    | e: 68.057 |
| S. mombin        | a: 216.597 | b: 0.836 | c: 0.056 | d: 14.573   | e: 79.663 |
| T. diversifolia  | a: 232.433 | b: 0.832 | c: 0.092 | d: 9.157    | e: 85.490 |
| MSE              | 10.49507  | 0.09600 | 0.0149   | 1.3507      | 3.8597   |
| P value          | < 0.0001  | 0.0004  | < 0.0001 | < 0.0001    | < 0.0001 |

Means in the same column with different superscript are significantly different (P < 0.05) according to Tukey test; a, maximum gas production; b, difference between the initial gas and the final gas at time x; c, specific rate of gas accumulation; TIP, time at the inflection point (hours); GIP, gas inflection point (ml); MGPR, maximum gas production rate (ml/h); LP, lag phase
Low CH$_4$ production in B. simaruba and A. pennatula can be attributed to their content of CT (200.1 and 31.1 g kg$^{-1}$) which is related to their ruminal degradability and CH$_4$ production (Soltan et al. 2012). Condensed and hydrolyzed tannins play an important role in mitigation of CH$_4$ emissions (Melesse et al. 2019; Rira et al. 2019). In general, CH$_4$ emissions from plant species that had some content of CT were below average (16.54 mg per DOM), except for M. pruriens with 19.97 mg CH$_4$ g$^{-1}$ IOM and 8.7 g kg$^{-1}$ CT; the lowest concentration. Condensed tannins are the polyphenolic compounds most abundant in plants that can effectively decrease CH$_4$ directly by inhibiting methanogenic archaea and indirectly by reducing hydrogen production as a result of decreased fiber digestion and protozoan population in the rumen (Patra et al. 2017). However, Bhatta et al. (2013) found no relation between dietary tannins and protozoa population in a screening test of 38 plant species. CH$_4$ mitigation with the use of CT can be very variable between species and experimental methods. A few in vivo experiments have shown that the effect of CT on CH$_4$ production and animal response is dependent on the metabolite source, type, dose and molecular weight (Aboagye and Beauchemin 2019).

Low CH$_4$ and gas production recorded for A. pennatula can be explained by its content of cyanogenic glycosides, as shown in other studies. Cyanogenic glycosides are nitrogen compounds which, when hydrolyzed, produce hydrogen cyanide that stops cellular respiration (Hassan Adeyemi 2011) and inhibits cytochromes present in methanogens (Smith et al. 1985). Zavaleta et al. (2019) found linear CH$_4$ reductions when pure limarin (cyanogenic glycoside present in Manihot esculenta) was added at five different levels in an in vitro trial. Vongsamphanh et al. (2018) also found reductions in CH$_4$ when cassava leaves were incubated and they attributed this to concentrations of secondary metabolites such as cyanogenic glycosides, trypsin inhibitors, oxalates, phytate and tannins. In many species of the Acacia genus, the high presence of cyanogenic glycosides is common; however, more in vitro and in vivo studies on the use of this metabolite, its source and mode of action on CH$_4$ synthesis are needed.

Saponin content was found abundant in G. sepium and in low abundance in B. variegata, G. ulmifolia, E. goldmannii, S. mombin and O. pyramidale. López Herrera et al. (2008) observed low presence of saponins in O. pyramidale and Molina-Botero et al.

| Table 5 DM degradability (g kg$^{-1}$ DM) at different hours of incubation of forage species from Southern Mexico |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Species                        | 12 h            | 24 h            | 48 h            | 72 h            |
| A. pennatula                   | 293.46$^{(v,y,3,6,9,10)}$ | 307.70$^{(9,10)}$ | 345.46$^{(20)}$ | 353.48          |
| B. alicastrum                  | 529.17$^{(a,b)}$ | 597.29$^{(a)}$  | 609.07$^{(a,b,c,d,e)}$ | 613.58$^{(a,b)}$ |
| B. simaruba                    | 368.73$^{(f,l,m,q,r)}$ | 418.92$^{(x,y,2,5,6)}$ | 433.21$^{(12,15,17,19)}$ | 512.02$^{(a,n,q,r)}$ |
| B. variegata                   | 377.97$^{(e,h,k,n,p)}$ | 477.78$^{(e,h,k,l,m,n,o)}$ | 544.33$^{(e,i,n,r,w,z,1,2,3)}$ | 552.42$^{(d,g,h,i,k)}$ |
| C. obtusifolia                 | 290.13$^{(u,x,2,6,7,8)}$ | 402.18$^{(w,z,3,5,7)}$ | 520.39$^{(j,o,x,z,3,4,5,6)}$ | 539.13$^{(b,h,m,n)}$ |
| E. goldmannii                  | 312.61$^{(p,r,x,w,y,z,1,2,3)}$ | 421.21$^{(t,u,x,y,2,3,4)}$ | 475.04$^{(2,4,7,10,11,12,13)}$ | 498.93$^{(b,o,q,t)}$ |
| G. sepium                     | 404.02$^{(e,d,f)}$ | 510.50$^{(d,e,f,g)}$ | 580.43$^{(a,f,g,h,i,k)}$ | 595.07$^{(a,c,d)}$ |
| G. ulmifolia                   | 254.56$^{(1,5,8,10,11)}$ | 353.77$^{(1,4,6,7,8,9)}$ | 462.63$^{(6,9,11,14,17,18)}$ | 494.36$^{(s,u,v)}$ |
| L. glabrum                     | 379.23$^{(d,g,k,l,m)}$ | 469.19$^{(g,j,l,p,t,u,v,w)}$ | 557.15$^{(c,g,l,q,rs,t)}$ | 675.05$^{(s,e,g,h,i)}$ |
| M. pruriens                   | 475.50$^{(b)}$ | 564.31$^{(a,b,c)}$ | 569.51$^{(b,f,i,m,n,o,p)}$ | 663.20$^{(u,v)}$ |
| O. pyramidale                 | 308.67$^{(z,t,w,2,3,4,5)}$ | 495.89$^{(c,d,h,i,j)}$ | 556.07$^{(d,h,m,q,w,x,y)}$ | 579.07$^{(b,c,e,f)}$ |
| P. aculeata                   | 254.72$^{(z,4,7,9,11)}$ | 321.41$^{(3,10)}$ | 419.41$^{(13,16,18,19,20)}$ | 452.56$^{(v)}$ |
| P. dimorphandrum              | 397.95$^{(c,g,h,i,j)}$ | 475.85$^{(u,i,k,q,r,s)}$ | 518.24$^{(k,p,t,y,1,3,7,8,9)}$ | 535.46$^{(a,k,m,o,p)}$ |
| S. mombin                     | 340.53$^{(j,m,q,t,u,v)}$ | 422.3$^{(m,q,t,x,y,z,1)}$ | 468.76$^{(5,8,10,14,15,16)}$ | 467.30$^{(u,v)}$ |
| T. diversifolia               | 567.81$^{(a)}$ | 690.45 | 781.11 | 787.19 |
| MSE                           | 19.48           | 22.54           | 23.15           | 12.28           |
| P value                       | < 0.0001        | < 0.0001        | < 0.0001        | < 0.0001        |

Means in the same column with different superscript are significantly different ($P < 0.05$)
(2019) reported 17.0 mg g⁻¹ of saponins in leaves of *G. sepium*. Methane production of plant species in relation to their saponin content was not consistent. Saponins can form complexes with the lipid membrane of bacteria, which increases its permeability generating an imbalance and consequent lysis of the microorganisms, and most of the saponins also have a similar effect on protozoa population (Makkar et al. 1995). The effect of any of the secondary metabolites on CH₄ synthesis varies considerably depending on the characteristics of the metabolite, as well as the type of diet (Patra et al. 2017). In addition, long-term studies on animal performance must be carried out to verify the effect of secondary metabolites on CH₄ synthesis. Secondary metabolite biosynthesis and accumulation depend on genetic, ontogenic, morphogenic and environmental factors such as light irradiation, temperature, soil water, soil fertility and salinity (Yang et al. 2018). Likewise, nutritional composition of a forage is also dependent on
environmental factors, plant phenological stage and management methods. Plant species collected in this study had not undergone genetic selection, so differences in chemical composition, secondary metabolite content and effect on CH$_4$ synthesis may vary in comparison with other studies.

Chemical composition and its correlation with fermentation kinetics of plants are a useful criterion for the selection of species with feeding potential (Albores-Moreno et al. 2018) and CH$_4$ mitigation in ruminants. Among the species evaluated in this trial B. alicastrum, M. pruriens, T. diversifolia and G. sepium showed a high potential for ruminant feeding due to their good chemical composition, digestibility, degradability and high gas production. For the mitigation of CH$_4$, A. pennatula, S. mombin, P. aculeata and B. simaruba showed the highest potential due to their content of secondary metabolites. Content of CT in B. simaruba is of high interest for the reduction of CH$_4$; however, the level of inclusion in the diet must be evaluated to prevent reductions in intake and fiber digestibility.

The presence of most of the species evaluated in this study has been reported in other countries in the tropical regions of the world. Screening of plant species is a practical first step for the discovery of new compounds and their development as feed additives to mitigate methanogenesis (Bhatta et al. 2013). Furthermore, native plant species have great potential for mitigation of CH$_4$ emissions in smallholds in tropical regions as demonstrated in this and other studies (Albores-Moreno et al. 2018; Yusuf et al. 2020). However, for the implementation of CH$_4$ mitigation strategies several aspects must be taken into consideration such as: effects on animal performance, safety for the ruminant and the consumer alike, and economic viability (Martin et al. 2010). Additionally, socioeconomic, cultural and agroecological aspects are also determinants in the selection of an appropriate CH$_4$ mitigation strategy.

**Conclusion**

Results reported in this study established that tropical plant species that contain secondary metabolites have potential to suppress enteric CH$_4$ synthesis and they can be of relevance in the development of nutritional additives for this matter. Although some species with secondary metabolites showed high nutritional quality, the level of inclusion of these species in a diet for ruminants must be determined to prevent low digestibilities and negative effects on animal performance. The use of native plant species from tropical regions in small farms can be considered as a viable, resource conservation and low-cost strategy available to producers to mitigate CH$_4$ emissions and improve protein intake of ruminants grazing low-quality pastures. Future research may be aimed at the evaluation of biomass yields at different times of the year, propagation of species, animal production, in vivo evaluations that include voluntary intake and CH$_4$ production measurements in long-term studies.

**Acknowledgements** We thank all donors that globally support the work of the CRP programs through their contributions to the CGIAR system. A special thanks to the University of the Jungle, Ocosingo, for facilitating their spaces for the handling of the samples, to the anthropologist Lorenzo Hernandez for his collaboration in fieldwork and translations in Tzeltal and to the chemist Johana Mazabel from the Laboratory of Animal Nutrition and Forage Quality of CIAT for making available the personnel and laboratory equipment for analysis.

**Funding** This study is part of the LivestockPlus project funded by the CGIAR Research Program (CRP) on Climate Change, Agriculture and Food Security (CCAFS). In addition, this work was also done as part of the Livestock CRP. Thanks to National Science and Technology Council (CONACYT, Mexico) for the funding of the project “Quantification of enteric methane and nitrous oxide emissions in cattle grazing and the design of strategies for its mitigation in Southern Mexico” (SEP-CONACYT CB 2014 No. 242541).

**Compliance with ethical standards**

**Conflict of interest** The authors declare no conflict of interest.

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