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Abstract:	In tropical regions worldwide there is a variety of forage species that have the capacity to improve cattle diet and reduce CH 4 emissions. A screening study was conducted to investigate the nutrient quality, phytochemical composition, in vitro gas and CH 4 production of fifteen tropical multipurpose forage species from southern Mexico. The results indicated that the highest crude protein (CP) and in vitro digestibility was found in Gliricidia sepium with 264 g/kg -1 dry matter (DM) and 709 g/kg -1 DM respectively. Bursera simaruba had the lowest CH 4 production with 3.924 mg/g -1 incubated organic matter (IOM) and 9.077 mg/g -1 degraded organic matter (DOM), condensed tannin (CT) content of 20% and relative low digestibility of 471 g/kg -1 DM. Results found in this study indicate that several species in tropical regions in Mexico are potential candidates in mitigating CH 4 production and can be used as additive or supplementary feed to improve diet quality in cattle raised under grazing conditions in the tropics.
Response to Reviewers:	Dear Editor, The manuscript has been improved. The species has been cited in the material and methods section. The number of figures and some of the references were modified.

Enteric methane mitigation potential of forage species from Southern Mexico

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Abstract

In tropical regions worldwide there is a variety of forage species that have the capacity to improve cattle diets and reduce enteric CH_4 emissions. A screening trial was conducted to investigate the nutrient and phytochemical composition, *in vitro* gas and CH_4 production of fifteen tropical multipurpose forage species from Southern Mexico. The results indicated that the highest crude protein (CP) and *in vitro* digestibility was found in *Gliricidia sepium* with 264 g/kg^{-1} dry matter (DM) and 709 g/kg^{-1} DM respectively. *Bursera simaruba* had the lowest CH_4 production with 3.924 mg/g^{-1} incubated organic matter (IOM) and 9.077 mg/g^{-1} degraded organic matter (DOM), condensed tannin (CT) content of 20% and relative low digestibility of 471 g/kg^{-1} DM. Results found in this study indicate that several plant species widely available in Southern Mexico are potential candidates for mitigating enteric CH_4 production and can be used as additive or supplementary feed to improve diet quality in cattle raised under grazing conditions in the tropics.

Keywords: Agroforestry, greenhouse gases, Lacandon Rainforests, Mitigation strategies, Silvopastoral systems.

Introduction

Livestock production is an increasingly important issue in global research and development agendas (LGA 2016, FAO 2019). This activity provides 17% of the protein consumed worldwide, contributes to food security of almost 1,300 million people and more than 800 million poor people in the world subsist on animal husbandry (FAO 2016). However, extensive cattle production systems in tropical regions have generated severe impacts on soil and ecosystems and continue to promote land use change. Those systems are also less efficient from the productive standpoint due to the low quality of the pastures, poor management and its dependence on external inputs. This essentially extractive production system induces high rates of deforestation that has generated loss of biodiversity and a higher contribution to greenhouse gas (GHG) emissions. Pastures used in extensive livestock systems have high content of neutral detergent fiber (NDF), low soluble carbohydrates and crude protein (CP) that induce higher CH_4 productions with low production parameters (Kennedy and Charmley 2012), therefore, a better management of the grazing system is required to efficiently use solar energy. Nonetheless, tropical regions worldwide display a high forage biomass production from trees, shrubs and herbaceous plants with adequate chemical composition (14-32% CP and <400 g/kg NDF) 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 phytochemicals in the diet and the possibility to modify the rumen microbiome. Methane is a GHG produced by ruminants during anaerobic fermentation of carbohydrates in the rumen which is quantitatively eructated to the atmosphere. 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. 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 silvospastoral 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 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 the country (Jiménez-Ferrer et al. 2008), this potential allows a reconversion of livestock towards sustainable production systems through the use of available resources such as fodder trees, shrubs and herbaceous plants that have potential to reduce CH_4 emission from cattle and regenerate degraded areas. The objective of this study was to evaluate the enteric CH_4 mitigation potential and nutritional quality of different 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 taken 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 (García del Valle et al. 2015). The predominant formation is lower montane rainforest, montane rainforest occurs on the moist, cool upland slopes

and riparian zones in the low-lying areas with a unique ecological niche where pines intermingle with lowland, rain- forest species (Cook, 2016). Large areas have been extensively transformed by settlers, and timber and oil companies, creating a patchwork of secondary forests, cultivated fields, and *acahuales* (fallow fields) in various stages of secondary growth (Cook, 2016). 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 in the region have 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

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, Paz-Cortés 2010, Douterlungen, 2010). 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.*

Sampling

Leaves were collected from 5 to 9 individuals per species. During the sampling, the location points of each species were taken by a GPS (Garmin® Etrex 30x) 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 hours or until constant weight to determine dry matter (DM) content and ground in a Thomas Wiley® laboratory mill at a particle size of 1mm and stored in bags with airtight seal.

Chemical Analysis

Chemical analysis and the *in vitro* gas production technique were carried out at the Laboratory of Animal Nutrition and Forage Quality at the International Center of Tropical Agriculture (CIAT), Cali, Colombia.

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 (AN 3001 FOSS; AOAC 1990: method 984.14), NDF and acid detergent fiber (ADF) content were 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 metabolite quantification was carried out 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 were carried out by the methodologies proposed by Domínguez (1979).

In vitro gas production technique

Cattle were treated in accordance to 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). For the screening, leaves and petioles of 15 species were used. Rumen liquor was obtained from three rumen cannulated Brahman bulls of 550 kg live weight, fed *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). 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 were incubated in bottles of 160 ml capacity by triplicate including blanks. Bottles were kept under constant flow of CO_2, sealed with a rubber stopper and aluminum ring and placed in a water bath at 39°C for 72 hours. 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. CH_4 concentration was quantified

using a gas chromatograph (GC-2014 Shimadzu, Japan). 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 was 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 was adjusted to the modified Gompertz model with the following equation:

$$y = ae^{-e^{b-cx}}$$

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) and Lag phase (LP or the microbial establishment, h). For its estimation the following formulas were used: TIP = b / c; GIP = a / e; MGPR = (a * c) / e; LP = ((b / c) - (1 / c)); where "e" is Euler's number, equivalent to ≈ 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. Treatments means were compared with the Tukey test with an Alpha of 0.05.

Results

Sampling and forage species

Plant species were identified and collected in Ocosingo Valley, Chiapas, Mexico in paddocks, *acahuales*, orchards and live fences. Species were mostly collected in induced grasslands, cultivated areas and mesophyll mountain forest as shown in Figure 1. Forage species were sampled between 683 and 1059 MASL. A total of 15 species were identified and collected (Table 1). From the species collected, 40% belonged to the *Fabaceae* family.

Chemical composition

Organic matter 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. The species with the lowest acid detergent fiber 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. CT were present in *B. variegata*, *C. obtusifolia*, *G. ulmifolia*, *S. mombin*, *A. pennatula*, *O. pyramidale*, *B. simaruba* and *M. pruriens* with 1.66, 13.27, 3.27, 0.99, 3.11, 3.95, 20.01 and 0.87% respectively.

In the phytochemical screening, presence of alkaloids was found in all species except for BS as shown 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* that was abundant (++).

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 observed in *B. simaruba* with 118.03

188 ml. *B. alicastrum*, *G. sepium*, *T. diversifolia*, and *B. variegata* had the highest MGPR with 9.72, 8.10, 7.77 and
189 7.46 ml/h.

190 Degradability at different hours (12, 24, 48 and 72 h) differed significantly ($P < 0.05$) among species (Table 5).
191 The highest degradability at 12, 24, 48 and 72 h were observed for *T. diversifolia* with 56.78, 69.04, 78.11 and
192 78.71% respectively. Dry matter degradability at 72 h ranged from 35.34% (*A. pennatula*) to 78.71% (*T.*
193 *diversifolia*).

194 Methane production

195 Distribution and differences in CH_4 produced at 48 h per degraded OM (mg/g-^{1}) from forage is shown in Figure
196 2. CH_4 production in mg/g^{-1} DOM (degraded OM) and mg/g^{-1} IOM (incubated OM) was different among
197 species ($P <.0001$). The lowest CH_4 production at 48 h was observed in *B. simaruba* with 3.924 mg/g^{-1} IOM
198 and 9.077 mg/g^{-1} DOM. At 48 h *B. alicastrum*, *G. sepium* and *M. pruriens* had the highest CH_4 production with
199 35.228, 20.713 and 19.977 mg/g^{-1} DOM respectively. Accumulated CH_4 at 48 h from different species is shown
200 in Figure 3. Lowest CH_4 production at 48 h were observed in *B. simaruba*, *P. aculeata*, *S. mombin* and *A.*
201 *pennatula* with 3.92, 4.10, 4.42 and 5.55 mg/g^{-1} IOM.

202 **Discussion**

203 The search for local resources with high nutritional value is important to improve profitability and productivity
204 of the systems as well as to reduce the impact of livestock on the environment (Valencia-Salazar et al. 2018).
205 However, there is not enough information on the use of these resources on specific animal production systems
206 or for the design of silvopastoral systems. Incorporation of a variety of forage species improves diet quality and
207 enhance milk production and weight gain. Some mitigation alternatives are associated with improved efficiency
208 of animal production given their advantages from the nutritional and environmental standpoints (Patra et al.
209 2012). In Southern Mexico, cattle producers have observed that during the dry season animals consume many
210 species present in native vegetation in the form of green fodder, dried pods and leaves from trees and shrubs
211 (López-Herrera et al. 2008, Albores-Moreno et al. 2018). Secondary vegetation in livestock systems has been
212 scarcely studied and is being displaced by introduced pastures for the establishment of extensive grazing
213 systems (Albores-Moreno et al., 2020).

Nutritional quality of forage species especially CP content is of great relevance due to protein deficiencies in pastures used in tropical extensive livestock systems. In this experiment, CP contents were all above 120 g kg^{-1} DM with the exception of *B. simaruba* (99.07 g kg^{-1} DM) and *B. aliscastrum* (116.21 g kg^{-1} DM). However, these values can meet the requirements of CP for moderate levels of production of ruminants in tropical regions. Crude protein values of *C. obtusifolia* (187.48 g kg^{-1} DM), *G. ulmifolia* (150.72 g kg^{-1} DM) and *S. mombin* (126.95 g kg^{-1} DM) were similar to those obtained by López-Herrera el at. (2008) and Rodriguez et al. (2019); *C. obtusifolia* (165.4 g kg^{-1} DM), *G. ulmifolia* (137.8 g kg^{-1} DM) and *S. mombin* (148.0 g kg^{-1} DM). *T. diversifolia* and *G. sepium* had the highest content of CP and also the highest digestibility. Neutral detergent fiber content of *G. sepium* was similar to the value reported by Molina-Botero et al (2019) (575.4 g/kg DM), however, CP from *G. sepium* was much higher in the present trial. The content of CP and EE of *S. mombin* was similar to those obtained by Yusuf et al (2020); 145.1 and 60.0 g kg^{-1} DM, respectively. The greatest DM degradability was for the species that contained lower or no concentrations of secondary metabolites (*G. sepium*, *T. diversifolia* and *B. alicastrum*), these 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 been shown to have antimicrobial properties (Patra et al. 2012) which constitutes one of the strategies to decrease CH_4 production.

Low CH_4 production in *B. simaruba* and *A. pennatula* can be explained by their content of CT (20.01 and 3.11%) which is related to 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). No methane reduction per day was reported by Molina-Botero et al (2019) when *G. sepium* with 45.9 mg/g of CT was included in different levels mixed with pods of *Enterolobium cyclocarpum* in cattle rations. Piñeiro-Vasquez et al. (2017) found 28% reduction on CH_4 when included 30% of *B. simaruba* with 13.3% of CT and no reduction with *A. pennatula* with 18.9% of CT in sheep rations. However, although ruminant species have similar CH_4-synthesis pathways, they differ considerably in their level of feed intake, rumen morphology, and rumen physiology so results with the use of the same strategy to reduce methane production may vary depending on ruminant species (van Gastelen et al., 2019).

In general, CH_4 emissions from plant species that had some content of CT were below average (8.88 mg per incubated OM), except for *M. pruriens* with 11.26 mg CH_4/IOM and 0.87% 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). Methane 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 methane production and animal response is dependent on the metabolite source, type, dose and molecular weight (Aboagye and Beauchemin, 2019).

Cyanogenic glycosides from *A. pennatula* (++++) have the capacity to reduce methane synthesis as shown in the present study. Cyanogenic glycosides are nitrogen compounds that, when hydrolyzed, produce hydrogen cyanide that stops cellular respiration (Hassan, 2011) and inhibits cytochromes present in methanogens (Smith et al 1985). In many species of the *Acacia* genus, the high presence of cyanogenic glycosides is common, however, there is not much information on the use of this metabolite as an enteric methane mitigation strategy.

Saponin content was found abundant in *G. sepium* and in low abundance in *B. variegata*, *G. ulmifolia*, E. *goldmanii*, *S. mombin* and *O. pyramidale*. Lopez-Herrera et al. (2008) observed low abundance of saponins in *O. pyramidale* and Molina-Botero reported 17.0 mg/g of saponins leaves of *G. sepium*. CH_4 production of the plant species in relation to their saponin content was not consistent. Saponins can form complexes with the lipid membrane of the bacteria, which increases its permeability generating an imbalance and consequent lysis of the microorganism, 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 CH_4 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 CH_4 synthesis in the long term. 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 have not undergone a genetic

selection so differences in chemical composition, secondary metabolite content and effect on CH_4 synthesis may vary in comparison with other studies.

The potential of forage trees, shrubs, and herbaceous plants lies in the wide biodiversity and traditional knowledge that exists in their agroecosystems (Jiménez-Ferrer et al. 2008). Native vegetation must be studied in order to be incorporated in the establishment of systems in association with grasses or management of the secondary succession to allow the increase and conservation of biodiversity (Rosales and Gil, 1997) and the existence of edible biomass in greater quantity and quality that remains during the critical seasons in grazing areas. Also, native species and their biodiversity have a great potential in the reduction of ruminant methane emissions in smallholds in tropical regions as demonstrated in this and other studies (Yusuf et al 2020, Albores-Moreno et al 2018). 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 methane mitigation strategy.

Conclusion

The use of native plant species from tropical regions in small farms may be considered as a viable, resource conservation and low-cost strategy accessible to producers to mitigate methane emissions and improve protein intake of ruminants grazing low-quality pastures. Species hereby described hold potential to be used in cattle farms as live-fences and the forage can be directly browsed or cut by hand for the animals. Future research should be aimed at the evaluation of biomass yields at different times of the year, propagation of species, yields in holistic systems, *in vivo* evaluations that include voluntary intake, bioeconomic parameters and methane production measurements in long-term studies with techniques such as SF_6 or respiration chambers.

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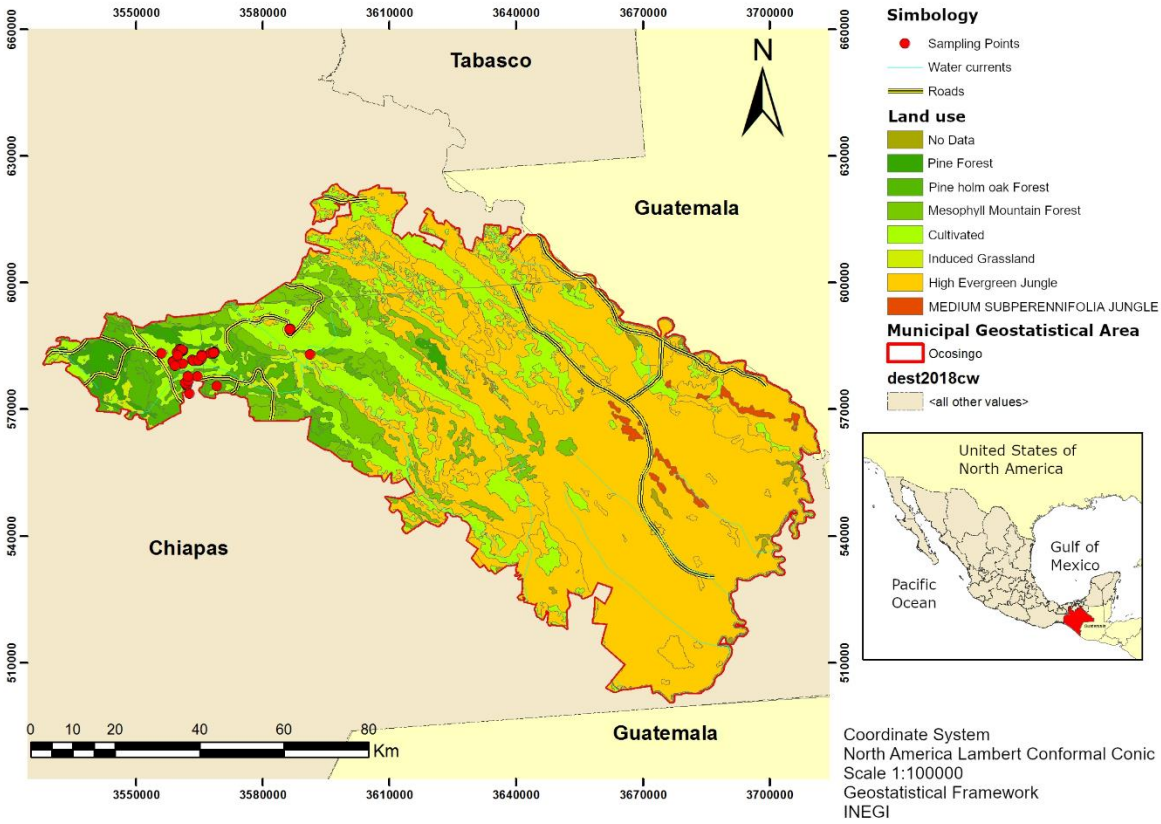

Figure 1. Geographic location of the study area

Table 1. Forage species from the Lacandon Rainforest region of Chiapas, Mexico

Family	Growth habit	Scientific name	Common name	Uses	MASL
Fabaceae	Tr	*Gliricidia sepium*	*Cocoite, matarratón*	1, 2, 3, 5	854 - 1014
Fabaceae	Tr	*Bauhinia variegata*	*Pata de vaca*	2, 3, 4	877 - 911
Moraceae	Tr	*Cecropia obtusifolia*	*Guarumbo*	1, 3, 4, 6, 7, 8	859 - 1047
Sterculiaceae	Tr	*Guazuma ulmifolia*	*Wasil, wasim, pixoy, caulote*	1, ,3, 4, 5, 6, 7	878 - 897
Fabaceae	Tr	*Erythrina goldmanii*	*Mote, Colorín, Pichoco*	1, 4, 5, 7	826 - 888
Anacardiaceae	Tr	*Spondias mombin*	*Jobo, lulúy, jujup*	1, 3, 5, 7	866 - 912

Fabaceae	Tr	*Acacia pennatula*	*Acacia, chimay*	6	881 - 891
Bignoniaceae	Tr	*Parmentiera aculeata*	*Cuajilote*	1, 3, 4, 5, 6, 7	683 - 685
Asteraceae	Sh	*Tithonia diversifolia*	*Árnica*	1, 3, 4, 5, 6, 7	882 - 1025
Asteraceae	Sh	*Liabum glabrum*	*Tsuy*	5	869 - 1058
Fabaceae	Tr	*Platymiscium dimorphandrum*	*Judío, hormiguillo*	3, 9	878 - 887
Malvaceae	Tr	*Ochroma pyramidale*	*Corcho, balso*	3	854 - 888
Moraceae	Tr	*Brosimum alicastrum*	*Ash, ramón, ox*	1, 3, 4, 5, 6, 7, 9	683 - 890
Burseraceae	Tr	*Bursera simaruba*	*Mulato, chakah*	1, 3, 4, 6, 7	794 - 895
Fabaceae	He	*Mucuna pruriens*	*Nescafé*	5	865 - 1059

Tr: Tree; Sh: Shrub; He: Herbaceous; Uses: 1: Live fences, 2: Wood, 3: Construction, 4: Firewood, 5: Edible, 6: Medicinal, 7: Shadow, 8: Wooden posts, 9: elaboration of tools/instruments.

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Table 2. Chemical composition, condensed tannin content and *in vitro* digestibility of multipurpose forage species from the Lacandon Rainforest region, Chiapas, Mexico

	g kg^{-1} DM								
Species	**DM**	**OM**	**NDF**	**ADF**	**CP**	**EE**	**IVDDM**	**MJ kg^{-1} DM**	**%CT**
Gliricidia sepium	242.83	890.78	431.56	190.83	261.41	30.12	709.94	19.48	0.00
Bauhinia variegata	384.04	854.41	275.19	194.57	146.45	28.72	698.27	17.92	1.66
Cecropia obtusifolia	295.95	897.12	437.00	214.00	187.48	35.10	498.94	19.12	13.27
Guazuma ulmifolia	398.35	886.42	480.45	260.29	150.72	32.02	516.88	19.06	3.27
Erytrhina goldmanii	297.68	888.16	417.38	271.05	206.78	41.40	595.97	19.06	0.00
Spondias mombin	259.75	852.29	307.77	171.18	126.95	44.78	638.59	16.25	0.99
Acacia pennatula	505.42	924.85	492.56	210.34	192.69	39.25	447.44	20.92	3.11
Parmentiera aculeata	308.95	874.47	614.35	268.81	183.17	13.85	548.37	18.04	0.00
Tithonia diversifolia	190.50	853.53	305.88	337.81	289.94	21.25	704.03	18.31	0.00
Liabum glabrum	182.26	868.04	443.00	334.97	240.16	53.30	531.11	19.01	0.00
Platymiscium dimorphandrum	279.54	931.01	539.15	324.83	236.32	18.72	616.47	20.45	0.00
Ochroma pyramidale	217.03	882.84	369.39	232.65	195.85	21.70	565.31	18.36	3.95
Brosimum alicastrum	489.18	821.28	298.17	269.22	116.21	29.92	686.38	15.65	0.00
Bursera simaruba	356.71	900.82	354.37	249.23	99.07	25.05	471.37	18.92	20.01
Mucuna pruriens	248.35	934.51	386.68	180.65	228.31	29.90	700.19	19.44	0.87

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

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Table 3. Phytochemical screening of multipurpose forage species from Southern Mexico

Species	Alkaloids			Cyanogenic glycosides	Saponins
	Mayer	Draggendorff	Wagner		
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	-	-	-	-	-
Mucuna pruriens	++	++	-	-	-

- (No presence); + (low abundance); ++ (abundant); +++ (moderately abundant); ++++ (very abundant)

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

	Parameters						
Species	**a**	**b**	**c**	**TIP (h)**	**GIP (ml)**	**MGPR (ml/h)**	**LP**
GS	200.078$^{(f, g, e, d, c)}$	1.070$^{(a)}$	0.111$^{(a)}$	9.903$^{(d, e)}$	73.590$^{(f, g, e, d, c)}$	8.1033$^{(b, a)}$	0.204$^{(a)}$
BV	201.269$^{(f, b, e, d, c)}$	0.865$^{(b, a)}$	0.102$^{(b, a)}$	17.287$^{(d, e)}$	74.027$^{(f, b, e, d, c)}$	7.4633$^{(b, a)}$	-1.957$^{(b, d, a, c)}$
CO	214.405$^{(b, e, d, c)}$	0.837$^{(b, a)}$	0.048$^{(d, e)}$	8.613$^{(b)}$	78.860$^{(b, e, d, c)}$	3.8167$^{(e, c, d)}$	-3.363$^{(e, b, d, a, c)}$
GU	188.402$^{(f, g, e, d)}$	0.742$^{(b)}$	0.051$^{(d, e)}$	14.573$^{(c, b)}$	69.297$^{(f, g, e, d)}$	3.5533$^{(e, c, d)}$	-5.123$^{(e, d, f, c)}$
EG	168.493$^{(g, h)}$	0.668$^{(b)}$	0.063$^{(b, d, e, c)}$	10.637$^{(c, d, e)}$	61.970$^{(g, h)}$	3.8967$^{(e, c, d)}$	-5.290$^{(e, d, f)}$
SM	216.597$^{(b, d, c)}$	0.836$^{(b, a)}$	0.058$^{(d, e, c)}$	14.573$^{(c, b)}$	79.663$^{(b, d, c)}$	4.5800$^{(e, c, d)}$	-2.833$^{(e, b, d, a, c)}$
AP	146.883$^{(i, h)}$	0.679$^{(b)}$	0.056$^{(d, e, c)}$	12.453$^{(c, d)}$	54.023$^{(i, h)}$	2.9933$^{(e, d)}$	-6.090$^{(e, f)}$
PA	184.457$^{(f, g, e)}$	0.763$^{(b)}$	0.028$^{(e)}$	27.253$^{(a)}$	67.843$^{(f, g, e)}$	1.9033$^{(e)}$	-8.307$^{(f)}$
TD	232.433$^{(b, a)}$	0.832$^{(b, a)}$	0.092$^{(b, d, a, c)}$	9.157$^{(d, e)}$	85.490$^{(b, a)}$	7.7733$^{(b, a)}$	-2.077$^{(b, d, a, c)}$
LG	174.429$^{(f, g, h)}$	0.899$^{(b, a)}$	0.088$^{(b, d, a, c)}$	10.207$^{(d, e)}$	64.153$^{(f, g, h)}$	5.6667$^{(b, c, d)}$	-1.150$^{(b, a)}$
PD	185.037$^{(f, g, e, d)}$	0.806$^{(b, a)}$	0.085$^{(b, d, a, c)}$	9.477$^{(d, e)}$	68.057$^{(f, g, e, d)}$	5.7900$^{(b, c)}$	-2.303$^{(e, b, d, a, c)}$
OP	196.539$^{(f, g, e, d, c)}$	0.928$^{(b, a)}$	0.056$^{(d, e, c)}$	16.573$^{(b)}$	72.287$^{(f, g, e, d, c)}$	4.0433$^{(e, c, d)}$	-1.287$^{(b, a, c)}$

BA	256.729[(a)]	0.951[(b, a)]	0.103[(b, a, c)]	9.240[(d, e)]	94.427[(a)]	9.7200[(a)]	-0.473[(b, a)]
BS	118.030[(i)]	0.956[(b, a)]	0.081[(b, d, a, c)]	11.883[(c, d)]	43.410[(i)]	3.5067[(e, c, d)]	-0.697[(b, a)]
MP	225.745[(b, a, c)]	0.665[(b)]	0.089[(b, d, a, c)]	7.430[(e)]	83.030[(b, a, c)]	7.4267[(b, a)]	-3.767[(e, b, d, c)]
MSE	10.49507	0.09600	0.0149	1.3507	3.8597	0.8964	1.2962
P-Value	<.0001	0.0004	<.0001	<.0001	<.0001	<.0001	<.0001

Species: Means in the same column with different superscript are significantly different (P<0.05) according to Tukey test; *Gliricidia sepium* (GP), *Bauhinia variegata* (BV), *Cecropia obtusifolia* (CO), *Guazuma ulmifolia* (GU), *Erythrina goldmanii* (EG), *Spondias mombin* (SM), *Acacia pennatula* (AP), *Parmentiera aculeata* (PA), *Tithonia diversifolia* (TD), *Liabum glabrum* (LG), *Platymiscium dimorphandrum* (PD), *Ochroma pyramidale* (OP), *Brosimum alicastrum* (BA), *Bursera simaruba* (BS), *Mucuna pruriens* (MP); TIP: time at the inflection point (hours); GIP: Gas inflection point (ml); MGPR: Maximum gas production rate (ml/h); LP: Lag phase

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Table 5. DM degradability at different hours of incubation of forage species from Southern Mexico

Species	**12 h**	**24 h**	**48 h**	**72 h**
GS	40.402[(c, d, e, f)]	51.050[(b, d, e, f, g)]	58.043[(a, f, g, h, i, j, k)]	59.507[(a, c, d)]
BV	37.797[(e, h, k, n, o, p)]	47.778[(e, h, k, l, m, n, o)]	54.433[(e, i, n, r, w, z, 1, 2, 3]	55.242[(f, g, j, k, l)]
CO	29.013[(u, x, 2, 6, 7, 8)]	40.218[(w, z, 3, 5, 7)]	52.039[(j, o, s, x, z, 3, 4, 5, 6)]	53.913[(h, j, m, n)]
GU	25.456[(1, 5, 8, 10, 11)]	35.377[(1, 4, 6, 7, 8, 9)]	46.263[(6, 9, 11, 14, 17, 18)]	49.436[(r, s, u)]
EG	31.261[(p, r, s, w, x, y, z, 1)]	42.121[(n, r, u, x, 2, 3, 4)]	47.504[(2, 4, 7, 10, 11, 12, 13)]	49.893[(p, q, s, t)]
SM	34.053[(j, m, o, q, s, t, u, v)]	42.221[(m, q, t, x, y, z, 1)]	46.876[(5, 8, 10, 14, 15, 16)]	46.730[(t, u, v)]
AP	29.346[(v, y, 3, 6, 9, 10)]	30.770[(9, 10)]	34.546[(20)]	35.348
PA	25.472[(z, 4, 7, 9, 11)]	32.141[(8, 10)]	41.941[(13, 16, 18, 19, 20)]	45.256[(v)]
TD	56.781[(a)]	69.045	78.111	78.719
LG	37.923[(d, g, k, l, m)]	46.919[(g, j, l, p, t, u, v, w)]	55.715[(c, g, l, q, r, s, t)]	56.705[(d, e, g, h, i)]
PD	39.795[(c, g, h, i, j)]	47.585[(f, i, k, p, q, r, s)]	51.824[(k, p, t, y, 1, 3, 7, 8, 9)]	53.546[(I, k, m, o, p)]
OP	30.867[(t, w, 2, 3, 4, 5)]	49.589[(c, d, h, i, j)]	55.607[(d, h, m, q, w, x, y)]	57.907[(b, c, e, f)]
BA	52.917[(a, b)]	59.729[(a)]	60.907[(a, b, c, d, e)]	61.358[(a, b)]
BS	36.873[(f, i, l, n, q, r)]	41.892[(o, s, v, y, 2, 5, 6)]	43.321[(12, 15, 17, 19)]	51.202[(l, n, o, q, r)]
MP	47.550[(b)]	56.431[(a, b, c)]	56.951[(b, f, l, m, n, o, p)]	66.320[(t, u, v)]
MSE	1.948	2.254	2.315	1.228
P-value	<.0001	<.0001	<.0001	<.0001

Means in the same column with different superscript are significantly different (P<0.05). *Gliricidia sepium* (GS), *Bauhinia variegata* (BV), *Cecropia obtusifolia* (CO), *Guazuma ulmifolia* (GU), *Erythrina goldmanii* (EG), *Spondias mombin* (SM), *Acacia pennatula* (AP), *Parmentiera aculeata* (PA), *Tithonia diversifolia* (TD), *Liabum glabrum* (LG), *Platymiscium dimorphandrum* (PD), *Ochroma pyramidale* (OP), *Brosimum alicastrum* (BA), *Bursera simaruba* (BS), *Mucuna pruriens* (MP).

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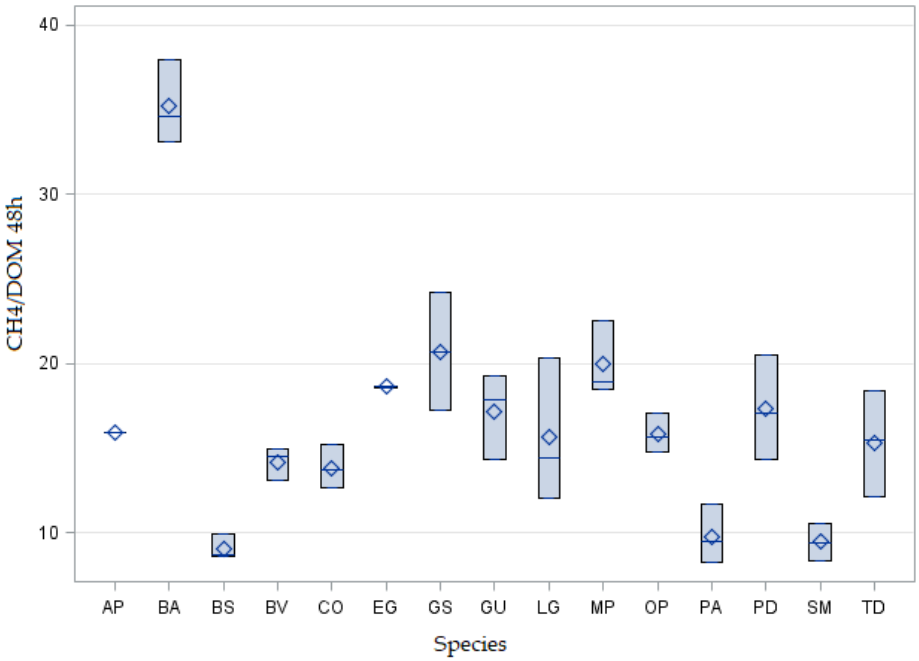

Figure 2. Distribution and differences of CH_4 produced at 48 h per degraded OM (mg/g-^{1}) from forage species from Southern Mexico

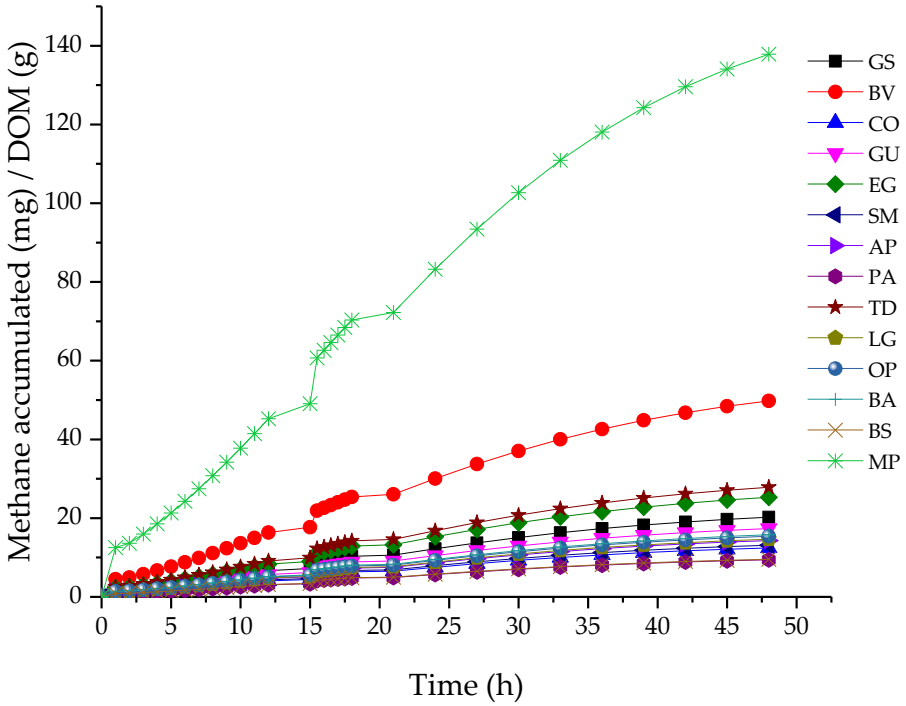

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Figure 3. Accumulated CH_4 at 48 h from different plant species from Southern Mexico