Archivos de Zootecnia
Journal website: https://www.uco.es/ucopress/az/index.php/az/

Effect of the source and concentration of saponins on *in vitro* and ruminal methane production

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Additional keywords
Greenhouse gases.
Secondary metabolites.
Tropical trees.
VFA's.
Tropic.

SUMMARY

The aim of the work was to assess the effect of incorporation of different sources of saponins on the *in vitro* total gas (TG) and ruminal methane (CH_4) productions, kinetics of fermentation, digestibility of dry matter (IVDDM) and organic matter (IVDOM). Treatments were interaction of three concentrations (3.5, 7.0 and 14.0 mg/g dry matter) of saponins from *Yucca schidigera* (YS), *Gliricidia sepium* (GS), *Enterolobium cyclocarpum* (EC) and a control (*Pennisetum purpureum* [PP] alone). A sample of 1.0 g of each treatment was incubated *in vitro* in a complete randomized design with factorial arrangement with three replicates per treatment. TG from PP was unaffected (P>0.05) by the inclusion of saponins from YS, GS and EC. Methane production was not affected (P>0.05) by any saponins from YS at any level of inclusion. When saponins from GS and EC were incorporated at concentrations of 7.0 and 14.0 mg/g DM, methane production was increased (P<0.05). IVDDM and IVDOM were significantly increased (P<0.05) with all sources of saponins, except with those from YS at concentration of 14.0 mg/g DM when IVDOM was reduced (P<0.05). It is concluded that saponins from different tropical plants did not reduce methane production under *in vitro* conditions.

Palabras clave adicionales
Gases de efecto invernadero.
Metabolitos secundarios.
Arboles tropicales.
AGV's.
Trópico.

Efecto de la fuente y concentración de saponinas en la producción in vitro y ruminal de metano

RESUMEN

El objetivo del trabajo fue evaluar el efecto de la incorporación de diferentes fuentes de saponinas sobre la producción total de gas in vitro (GT) y la producción de metano ruminal (CH4), la cinética de fermentación, la digestibilidad de materia seca (DIVMS) y digestibilidad de la materia orgánica (DIVMO). Los tratamientos tratamientos evauados fueron la interacción de tres concentraciones (3.5, 7.0 y 14.0 mg/g de materia seca) de saponinas de *Yucca schidigera* (YS), *Gliricidia sepium* (GS), Enterolobium cyclocarpum (EC) y un control (*Pennisetum purpureum* [PP] solo). Se incubó una muestra de 1,0 g de cada tratamiento in vitro en un diseño completamente al azar con arreglo factorial con tres repeticiones por tratamiento. GT de PP no se vio afectado (P> 0.05) por la inclusión de saponinas de YS, GS y EC. La producción de metano no fue afectada (P> 0.05) por ningún nivel de inclusión de saponinas de YS. Cuando las saponinas de GS y EC se incorporaron a concentraciones de 7.0 y 14.0 mg/g de MS, la producción de metano se incrementó (P <0,05). DIVMS y DIVMO aumentaron significativamente (P <0.05) con todas las fuentes de saponinas, excepto con las de YS a una concentración de 14 mg/g de MS cuando la DIVMO se redujo (P <0.05). Se concluye que las saponinas de las diferentes plantas tropicales no redujeron la producción de metano en condiciones in vitro.

Information
Cronología del artículo.
Recibido/Received: 13.02.2018
Aceptado/Accepted: 27.04.2019
On-line: 15.07.2019
Correspondencia a los autores/Contact e-mail:
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INTRODUCTION

Several nutritional strategies have been evaluated to mitigate methane emissions arising from rumen fermentation, among them: the use of lipids and by-products rich in oils, essential oils, the use of monensin, supplements rich in condensed tannins and saponins and compounds analog to CH4, animal selection with low residual feed intake, increasing of quality of forage, among several others (Bruocek, 2018; Brunes and Coeto,

2017; Piñero-Vázquez et al., 2017; Canul et al., 2014; Gerber et al., 2013; Ku-Vera et al., 2013).

In the tropical regions foliage of *Gliricidia sepium* production is 1.68 t DM/ha/year and is available during all year (Vizcaino et al., 2001). Pods of *Enterolobium cyclocarpum* are available during dry season when the availability of tropical grasses is low and their chemical composition is poor (crude protein below 7% and NDF above 80%). Those trees contain high levels of saponins which decrease rumen protozoa (Hess et al., 2003; Koenig et al., 2007) may alter the acetate:propionate ratio and increase the proportion of propionic acid in the VFA mixture, thus decreasing methane production (Anantosook et al., 2014; Patra and Yu., 2014).

The effect of saponins on rumen fermentation and methane production are unclear, and is influenced by the concentrations, the chemical structure and the source of saponins used in the ration (Holtshousen et al., 2009; Castro-Montoya et al., 2011). Mao et al. (2010) using 3 g/day of steroidal saponins from tea leaves with Huzhou sheep found 27% methane ruminal reduction. Under *in vitro* conditions a reduction in methane production has been reported with 1.5 mg of steroidal saponins/g DM from *Yucca schidigera* due to a reduction in ruminal fermentation and feed digestion (Holtshousen et al., 2009). With concentrations of 0.5 to 1.25 mg triterpenoid saponins of *Quillaja saponaria*/ml ruminal liquor incubated with hay: concentrate (1:1) was found a reduction (25%) under *in vitro* conditions in methane production (Castro-Montoya et al., 2011; Castro-Montoya et al., 2012). On the other hand, on in vitro conditions Pen et al. (2006) using 7 g triterpenoid saponins/day of *Quillaja saponaria* did not find effect on ruminal methane reduction but with steroidal saponins of *Yucca schidigera* was reduced methane production for 42% compared to control, suggesting that type and source of saponins altered ruminal fermentation differently; and Zhou et al., (2012) with incorporation of saponins from *Ilex kudingcha* at concentration of 5mg *in vitro* or *in vivo* conditions found that the pattern of VFA rumen fermentation were unaffected; similarly, Xu et al. (2010) with saponins concentration 0.5 mg from *Yucca schidigera*/g DM incubed in vitro did not find an effect on lag phase and kinetics of rumen degradation. Similarly with incorporation of foliage native plants do not reported effect on methane production on in vitro condition (Vélez et al., 2017). The different responses found under *in vitro* conditions using different sources and concentrations of saponins to reduce rumen methane production suggest further research to elucidate their effects on different substrates used in tropical rations.

The aim of the present work was to assess the effect of the inclusion of increasing levels of saponins from a commercial powder from *Yucca schidigera* (YS), foliage of *Gliricidia sepium* (GS) and ground pods of *Enterolobium cyclocarpum* (EC) on *in vitro* total gas production, methane production and kinetics of rumen fermentation as substrates on tropical grass ration.

MATERIAL AND METHODS

The experiment was carried out at the Laboratory of Animal Nutrition of the Faculty of Veterinary Medicine and Animal Science, Autonomous University of the State of Mexico in Toluca, State of Mexico, Mexico.

Sources of saponins: Sources of saponins employed were foliage of *Gliricidia sepium* (GS) and the fruit of *Enterolobium cyclocarpum* (EC). As a standard source of saponins a commercially available product was used (Biopowder M® reported as containing 11 mg of saponins/g dry matter; Baja Agro International, Baja California, México) derived from *Yucca schidigera* (YS).

Foliage of GS was harvested from a farm in the Eastern region of the State of Yucatan, Mexico; with a hot sub-humid climate with summer rains (AW0) and annual rainfall of 1056 mm, distributed throughout the months from June to November and mean annual temperature of 24.7°C (García, 1981).

Pods of EC were collected manually during the months of March and April 2013 in the central region of the State of Yucatan. Foliages, forages and pods were dried in a forced air oven at 60°C until constant weight and milled at 1 mm particle size with a screen sieve adapted on an electrical mill and samples were obtained for chemical analysis. The content of saponins of GS, EC and the commercial standard of saponins, was determined by the method of haemolysis (Makkar et al., 2007). Chemical compositions of GS, EC and YS are given in **Table 1**.

Animals and sampling of rumen liquor: Two Holstein cows weighing 480±20 kg live weight and cannulated in the rumen were used as donors of rumen liquor. Cows were handled according to the Official Mexican Regulations (NOM-062-ZOO-1999) for the use of experimental animals. Cows were kept in paddocks of ryegrass (*Lolium perenne*) where they grazed every day and were supplemented with 2 kg of a concentrate containing soybean meal (20%), ground sorghum (48%), wheat bran (15%), deffated meal canola (15%) and mineral salts (2%). Prior to feeding (8:00 h), 500 ml of rumen liquor were obtained from each cow and introduced into a vessel at 39°C under anaerobic conditions for their transport to the laboratory. Once in the laboratory, samples of rumen liquor were mixed and filtered through three layers of cheesecloth under anaerobic conditions by fluxing CO_2.

Treatments: Experimental treatments tested were the incubation of PP (ground through a sieve of 1 mm), as a control (without saponins) and three concentrations of saponins (3.5, 7.0 and 14.0 mg/g dry matter) from three sources: foliage of GS, fruits of EC and the commercial powder from YS. The grass was obtained from forage plots at the Faculty of Veterinary Medicine and Animal Science, University of Yucatan located at 21° 15´ N and 83° 32´ W in South Mexico; which has a hot sub-humid climate with summer rains, mean annual temperature of 25.8°C and mean annual rainfall of 983.8 mm (García, 1981). Samples of *P. purpureum* were oven dried at 60°C and milled at 1.0 mm particle size with an electrical mill and a screen sieve, and subsamples were stored until chemical analysis. Sources of saponins were mixed with PP used as a substrate until a quantity of 1.0 g was reached per each tube used to replicate treatments. Mixtures of PP and the sources of saponins were made assuming that YS contained 11

mg saponins/g DM, GS contained 17.0 mg saponins/g DM and EC contained 19.0 mg saponins/g DM. The YS was the reference for GS and EC to evaluate the effect of saponins level.

In vitro gas production: Kinetics of rumen fermentation was determined with the *in vitro* gas production technique of Theodorou et al. (1994). For each sample to be incubated, 1.0 g dry matter of the mixture were weighed along with the appropriate source of saponins, and these were incubated with 90 ml of buffer solution and 10 ml of rumen liquor (inoculum) in glass bottles of 150 ml capacity. Incubation media was prepared according to Theodorou et al. (1994). Bottles were incubated in a water bath at 39°C and the volume of total gas production was registered hourly during the first eight hours of incubation. Then, gas production was measured at hourly intervals up to 8 h, then at 12, 16, 20, 24, 28, 36, 44, 52, 72 and 96 hours incubation. Gas produced between 20 and 24 hours of incubation was measured to determine methane concentration by gas chromatography (Clarus 500 Perkin Elmer, Massachusetts, USA). Cumulative gas volume from each treatment was adjusted according to the mathematical model proposed by Jessop and Herrero (1996) with the software Grafit v3.

This model is described as:

$GP = a * (1-\exp(-ca + t)) + b * (1-\exp(-cb * (t - lag))) * (t > lag) \times -1$.

Where, GP= cumulative gas production (ml); a= gas production arising from fermentation (ml) of the soluble fraction from soluble carbohydrates; b= gas production (ml) from the insoluble but potentially degradable fraction; ca= rate of fermentation of fraction a; cb= rate of fermentation of fraction b; lag= time in h before the start of fermentation of the insoluble but potentially degradable fraction.

Chemical analyses: Chemical analysis of the diets and the substrates were carried out by triplicate. Ash content of the samples from each treatment was determined by complete combustion at 550°C for 5 h in a muffle furnace according to methodology #942.05 of AOAC (1999). NDF was assayed according to the method proposed by Van Soest et al. (1991), without use of amylase and results are reported including residual ash. Methane concentration was determined by gas chromatography (Elite –Q plot; Perkin Elmer, Massachusetts, USA), using N as a carrier gas. A capillary column of 30 x 0.32 µm was used and the temperature of the injector was kept at 50°C, while the detector was kept at an isothermic temperature of 250°C. *In vitro* digestibility of dry matter (IVDMD) and organic matter (IVDOM) were determined after 96 h incubation (Menke and Steingass, 1988).

Statistical analyses: Data of total gas production, methane concentration, IVDMD, IVOMD and kinetics of rumen fermentation were analyzed as a completely randomized design using the General Linear Model procedures of SAS (2006), analyzing each source of saponins independently. For the comparison of means, the Tukey test considered at 0.05% was used. Each source of saponins compared the control with the level of saponins (treatments) using three replicates per treatment with the following statistical model (Kaps and Lamberson, 2004):

$Yij = i + Ti + Aj + eij$

$i = 1,\ldots,a; j = 1,\ldots,b; k = 1,\ldots,n$

Where:

Yij= response to saponins concentration in the treatment i, in the replicate j.

Ti: effect of the treatment i.

Aj: effect of the replicate j.

E ij: random error with mean and variance $\sigma 2=0$.

RESULTS

Kinetics of rumen fermentation: Kinetics of fermentation is given in **Tables I, II** and **IV**.The quickly degradable fraction (a) and the rate of fermentation (ca) were unaffected (P>0.05) by the incorporation of different concentrations of saponins from foliage ground of YS and pods meal of EC. While the soluble fraction (a) and the rate of fermentation (ca) were unaffected (P>0.05) by the incorporation of saponins from foliage ground from GS at 3.5 mg/g DM and 7.0 mg/g DM, the concentration at 14.0 mg/g DM increased (P<0.05) fermentation of the a fraction and its rate of fermentation (ca). The slowly fermentable fraction (b) was reduced (P<0.05) with respect to the control when saponins from the three sources were incorporated.

Both for YS as for GS, b fraction was significantly reduced (P<0.05) at concentrations of 14.0 mg/g dry matter; while for EC a significant reduction (P<0.05) was observed at concentrations of 3.0 and 14.0 mg

Table I. Chemical composition (%) of the substrates used in in vitro gas production experiments are given in dry matter basis (Composición química (%) de los sustratos utilizados en experimentos de producción de gas in vitro se dan en la base de la materia seca)

Substrates	DM	OM	FOM	NDF	Lig	Sap
Enterolobium cyclocarpum	95.7	95.4	533.8	30.1	8.9	3.2
Gliricidia sepium	95.3	91.5	528.0	35.7	19	1.7
Pennisetum purpureum	94	91.2	491.0	71	9.8	----
Yucca schidigera	94.3	73.5	402.0	8.5	22	2.3

DM: Dry mater ; OM: Organic matter; NDF: Neutral detergent fiber; Sap:Saponins; ::: without data; FOM: Fermentable organic matter

Table II. Effect of three concentrations of saponins from *Yucca schidigera* on kinetics of rumen degradation, total gas production, methane production, IVDDM, and IVDOM of tropical forages under *in vitro* conditions (Efecto de tres concentraciones de saponinas de Yucca schidigera en la cinética de la degradación del rumen, la producción total de gas, la producción de metano, el IVDDM y el IVDOM de forrajes tropicales en condiciones in vitro.)

Item	Control	Saponins of *Yucca schidigera* (mg/g DM)			
		3.5	7.0	14.0	SEM
a	13.5[a]	9.3[a]	24.7[a]	31.9[a]	7.1
ca	0.29[a]	0.28[a]	0.12[a]	0.2[a]	0.1
b	263.8[a]	246.9[a]	235.3[a]	192.9[b]	6.7
cb	0.03[a]	0.03[a]	0.03[a]	0.03[a]	0.002
lag	10.2[a]	10.1[a]	10.1[a]	9.7[a]	0.2
Total gas production (ml)	108.2[a]	98.5[a]	107.0[a]	102.7[a]	5.9
Gas production (ml/g FDM)	201.21[a]	153.5[b]	169.3[ab]	176.8[ab]	8.9
Gas production (ml/FOM)	211.2[a]	169.41[a]	191.4[a]	205.7[a]	9.8
Methane production					
ml	23.2[a]	19.0[a]	22.3[a]	22.8[a]	1.2
ml/g FDM	43.0[a]	29.6[b]	35.3[ab]	39.2[a]	2.2
ml/g ODM	45.2[a]	32.7[b]	39.9[ab]	45.7[a]	2.0
IVDMD (%)	53.8d	64.2[a]	63.2[b]	58.1[c]	0.01
IVOMD (%)	51.2[c]	58.2[a]	55.9[b]	49.9d	0.01

abcd: Different letters in a row differ significantly (P<0.05); a=Gas production after 4 hours of fermentation (ml gas/g DM), ca : Fermentation rate of the fraction a; b=Potential gas production from the insoluble but potentially degradable fraction (ml gas/g DM); cb= Fermentation rate of the fraction b; lag= time in hours before starting the fermentation of NDF; SEM= standard error of means. Methane and total gas production at 24 hours post incubation, FDM= fermentable dry matter.

Table III. Effect of increasing levels of *Gliricidia sepium* on the kinetics of rumen degradation, gas production, IVDMD, IVOMD and methane production from tropical forages under *in vitro* condition (Efecto del aumento de los niveles de gliricidia sepium en la cinética de la degradación del rumen, la producción de gas, IVDMD, IVOMD y la producción de metano a partir de forrajes tropicales en condiciones in vitro).

Item	Control	Saponins of *Gliricidia sepium* (mg/g DM)			SEM
		3.5	7	14	
a	13.5[ab]	11.1[b]	16.22[ab]	38.64[a]	5.7
ca	0.3[ab]	0.3[a]	0.2[ab]	0.1[b]	0.05
b	263.8[a]	253.6[a]	247.7[ab]	216.1[b]	7.3
cb	0.03[b]	0.04[ab]	0.04[ab]	0.04[a]	0.001
lag	10.2[a]	10.2[a]	10.1[a]	9.7[a]	0.2
Total gas production (ml)	108.2[a]	109.6[a]	111.6[a]	118.9[a]	8.0
Gas production (ml/g FDM)	201.2[a]	177.0[a]	175.9[a]	184.0[a]	8.9
Gas production (ml/FOM)	211.2 [a]	194.5 [a]	192.0 [a]	200.0[a]	9.8
Methane production					
ml	23.2[b]	25.3[ab]	25.1[ab]	28.3[a]	1.0
ml/g FDM	43.0 [a]	40.8 [a]	39.7 [a]	43.7 [a]	2.0
ml/g ODM	45.2 [a]	44.9 [a]	43.2 [a]	47.6 [a]	2.3
IVDMD (%)	53.8c	61.9[b]	63.4[ab]	64.7[a]	0.4
IVOMD (%)	51.2c	56.3[b]	58.2[ab]	59.6[a]	0.4

abcd: Different letters in a row differ significantly (P<0.05); a=Gas production after 4 hours of fermentation (ml gas/g DM), ca : Fermentation rate of the fraction a; b=Potential gas production from the insoluble but potentially degradable fraction (ml gas/g DM); cb= Fermentation rate of the fraction b; lag= time in hours before starting the fermentation of NDF; SEM= standard error of means. Methane and total gas production at 24 hours post:incubation, FDM= fermentable dry matter.

saponins per g dry matter. Rate of fermentation of the b fraction (cb) was similar between the control treatment (P>0.05) and the different concentrations of saponins used from YS; nonetheless, a significant increase (P<0.05) was observed for the rate of fermentation of b when the concentrations of saponins from GS and EC were increased. A tendency to increase b fraction was observed with 7.0 mg of EC saponins/g DM; however, with 3.5 and 14.0 mg of EC saponins/g DM, the b fraction tends to be reduced.

While for GS, con 14.0 mg/g DM of saponins increased (0.040) significantly cb compared to control. With respect to the time of start of fermentation the insoluble fraction (lag phase), no significant effects (P>0.05) were observed due to the incorporation of saponins from the different sources used.

Total gas and ruminal methane production: Total gas production was similar between treatments (P>0.05; **Tables II, III,** and **IV**). However, methane production was affected depending on the concentration of saponins and the source used. Inclusion of saponins up to concentrations of 14.0 mg/g dry matter from YS did not affect significantly (P>0.05) methane production, even when observe a tendency reduction of 17.0, 3.0 and 1.9% with respect to the methane production obtained with control. While when saponins from GS and EC were incorporated, an increment (P<0.05) in methane production was observed. Incorporation of 14.0 mg/g dry matter of saponins from GS induced a significant increase in the production of methane compared to that obtained with control (23.15 vs. 28.30 ml for control and for treatment with 14.0 mg saponins/g dry matter, respectively). With respect to EC, concentrations of saponins at 7.0 mg/g dry matter significantly (P<0.05) increased methane production, but at concentrations of 3.5 and 14.0 mg/g dry matter, methane production (28.94 and 26.45 ml, respectively) was similar (P>0.05) to control (23.15 ml).

In vitro dry matter and organic matter digestibilities: Inclusion of saponins from the different sources significantly (P<0.05) increased IVDDM and IVDOM with respect to the digestibility obtained for the control. The inclusion of saponins from YS at concentrations of 3.5 and 7.0 mg /g dry matter yielded greater (P<0.05) IVDMD and IVOMD, while at concentrations of 14.0 mg/g dry matter, lower IVDMD and IVOMD were obtained (58.12 and 49.96 respectively). With inclusion of saponins from GS, IVDMD and IVOMD were increased (P>0.05) as the level of incorporation in the ration was increased. With respect to the use of EC a significant increase (P<0.05) in IVDMD and IVOMD was observed, obtaining greater values at concentrations of 7.0 and 14.0 mg/g dry matter (62.18 and 61.74% for IVDMD; 56.87 and 56.10 for IVOMD, respectively).

DISCUSSION

Kinetics of rumen fermentation: Saponins contained in YS and EC did not affect fraction a and ca, which suggest that their association with PP did not increase the amount of quickly fermentable carbohydrates. Addi-

Table IV. Effect of increasing levels *Enterolobium cyclocarpum* on kinetics of rumen degradation, gas production, IVDMD, IVOMD and methane production from tropical forages under in vitro conditions (Efecto del aumento de los niveles enterolobium cyclocarpum en la cinética de la degradación del rumen, la producción de gas, IVDMD, IVOMD y la producción de metano a partir de forrajes tropicales en condiciones in vitro).

Item	Control	Saponins of *Enterolobium cyclocarpum* (mg/g DM)			SEM
		3.5	7	14	
a	13.5^{a}	26.1^{a}	37.3^{a}	21.7^{a}	9.7
ca	0.3^{a}	0.2^{a}	0.2^{a}	0.2^{a}	0.05
b	263.8^{a}	247.4^{b}	256.8^{ab}	241.4^{b}	3.6
cb	0.03^{c}	0.03^{a}	0.04^{b}	0.04^{ab}	0.0003
lag	10.2^{a}	10.1^{a}	10.2^{a}	10.5^{a}	0.15
Total gas production (ml)	108.2^{a}	121.3^{a}	123.7^{a}	109.7^{a}	5.3
Gas production (ml/g FDM)	201.2^{a}	201.8^{a}	199.0^{a}	178.0^{a}	8.9
Gas production (ml/FOM)	221.2 ^{a}	217.5 ^{a}	211.2 ^{a}	195.3 ^{a}	9.8
Methane production					
ml	23.2^{b}	28.9^{ab}	33.7^{a}	26.4^{b}	1.4
ml/g FDM	43.0^{b}	48.2^{ab}	54.3^{a}	42.8^{b}	2.1
ml/g ODM	45.2^{b}	52.8^{ab}	59.3^{a}	47.7^{b}	2.2
IVDMD (%)	53.8d	60.1^{c}	62.2^{a}	61.7^{b}	0.02
IVOMD (%)	51.2d	54.8^{c}	56.9^{a}	56.2^{b}	0.02

abcd: Different letters in a row differ significantly (P<0.05); a: Gas production after 4 hours of fermentation (ml gas/g DM), ca : Fermentation rate of the fraction a; b: Potential gas production from the insoluble but potentially degradable fraction (ml gas/g DM); cb: Fermentation rate of the fraction b; lag: time in hours before starting the fermentation of NDF; SEM: standard error of means. Methane and total gas production at 24 hours post:incubation.

tion of saponins (14.0 mg/g dry matter) from GS improved fraction a, which suggest an associative effect and could be explained by a smaller amount of NDF in the ration and an increase soluble carbohydrates as it has been pointed out by López et al. (2000) which render more readily available the quickly soluble fraction. Fraction b was reduced with the inclusion of 14.0 mg of saponins with either of the saponin sources used, however, the rate of fermentation of fraction b was increased, this could be due to the fact that inclusion of saponins could even favor the increase of the cellulolytic bacteria population (Patra and Yu, 2013; Patra and Yu, 2014; Narváez et al. 2013). On the other hand, it is possible that the quick generation of protozoa (9 to16 h) allowed a relatively good digestibility of this fraction since protozoa contribute to digestion of 19-28% of cellulose (Dehority et al., 1998). The inclusion of 3.0 and 14.0 mg of saponins from pods EC/g DM tends to reduce the b fraction fermentation. This is comprehensible because of higher fermentation rate during the first 5 hours of incubation, due to lower lignin content (Rodríguez et al., 2009). Comparably, high gas production substrates have lower microbial biomass production which can explain reduction of b fraction degradation by lower bacteria population of the incubation medium (Blummel et al., 1996). Lag time did not vary significantly (P>0.05) between treatments, which indicates the ratios have similar chemical characteristics which have not influence on the lag time.

Total gas and ruminal methane production: Methane emissions and total gas production were not affected by the inclusion of different concentrations of saponins from GS, EC and YS, however, with YS a slight trend was observed towards a reduction in methane production with the increase in the concentration of saponins (17.85, 3.75 and 1.46%). A similar trend was reported for gas production and methane emission with YS by Holtshousen et al. (2009) with an extract from YS at levels of 0.9, 3.0 y 4.5 g/kg DM were able to reduce methane emissions by 8.5, 15.5 y 25.8%. Similarly with saponins from *Tribulus terrestris* at levels of inclusion 0.3, 0.6 and 0.9 g/liter of inoculated rumen liquor, a reduction in methane production has been reported by 23.4, 25.0 and 25.3%, respectively (Feng et al., 2012). In the same way, with purified saponins Bharathidhasan et al. (2013) reported a reduction in total gas and methane production with levels of 1.55, 3.10 and 4.55 mg/30 ml of inoculated rumen liquor. On the other hand, Makkar et al. (1998) using saponins from *Quillaja saponaria* reported a lack of effect on total gas production. According with the above, González et al. (2007) reported a reduction in total gas production when they incubated *Pennisetum purpureum* with saponins from *Sapindus saponaria*.

The results obtained in this work regarding methane production (21 to 24% methane) are similar to those reported with tropical grasses could be explained by the presence of secondary metabolites. At this respect Hu et al. (2005) found a reduction 24.4 and 26.4% of methane production with 6.0 and 8.0 mg of saponins per 200 mg of fermentable substrate incubated with ruminal liquor of sheep fed hay of ryegrass and a concentrate (ratio 60:40; forage:concentrate). Geerken et al. (1980) reported values between 15 to 30% of methane production when they incubated *in vitro* forage of Bermuda grass (*Cynodon dactylon*) at different stages of maturity.

On the other hand, the increase in methane production with EC and GS was probably due to the high content of fermentable OM in both sources of saponins (534 and 528 g FOM/kg OM, respectively) respect to PP (491 g FOM/kg OM), which favours ruminal fermentation and increase methane production (Torres-Salado et al., 2018; Orskov et al., 1968); tis data are similarly to repodted by torres-Salado et al. (2018) with pods of *E. cyclocarpum* with mayor volumen gas production

Patra and Saxena (2009) proposed that steroidal and terpenoid saponins may affect different magnitude in the response in terms of methane mitigation due to the different mode of action on the microbial population. Moses et al., (2014) suggested that *Yucca schidigera* has part of steroidal saponins and another part is terpenoid, this chemical composition could be exerted effect in the reduction methane production at low doses (3.4 mg/g DM) and increasing methane production in high doses (7.0 y 14.0 mg/g DM). Hess et al., (2003) working with three different sources of tropical trees found that these exerted different extent of reduction in methane production *in vitro* Rusitec system. However in the present work, the three sources of saponins have the same type of saponins namely terpenoid.

In vitro dry matter and organic matter digestibilities: The increase in IVDMD and IVOMD were probably due to the higher population of fiber digesting bacteria and to the greater content of fermentable carbohydrate in the ration by interaction between tropical trees added with young forage (Soliva et al., 2005; Narváez et al 2013). These results are comparable to those reported by Kumar-Sirohi et al. (2014) who reported that inclusion of extracts of saponins from *Albizia lebbeck* increased digestibility of DM and OM. Similarly, by using surfactant saponins in doses of 5 to 20 µL/kg dry matter, apparent digestibility of the dry matter was also increased (Wang et al 2011).

CONCLUSIONS

Currents results indicate that effect of saponins contained in the foliage and pods from different tropical plants did not reduce methane production, nor increase it, but it may be feasible to increase the production of ruminants in the tropics.

On the other hand, incorporation of foliage from *Gliricidia sepium* and pods of *Enterolobium cyclocarpum* did increase methane production probably as a result of the high *in vitro* digestibility of dry matter and organic matter. Different like *Yucca schidigera*, that not reduced methane production probably by an increased lignin concentration and a lower amount of fermentable organic matter concentration. No change in kinetics of fermentation of DM was observed. Further work is required using higher concentrations of saponins from tropical trees, to fully assess their potential as

a tool to mitigate methane production in ruminants under *in vivo*, practical conditions.

ACKOWLEGMENTS

The senior author is indebted to Consejo Nacional de Ciencia y Tecnología of Mexico (CONACYT) for the scholarship to carry put PhD studies at the University of Yucatan, Mexico.

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