Journal of Agriculture and Food Research 18 (2024) 101450

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.sciencedirect.com/journal/journal-of-agriculture-and-food-research

Influence of nano-encapsulated *Yucca schidigera* extract on ruminal anaerobic gases of methane, carbon monoxide, and hydrogen sulfide production of different carbohydrate-based diets

Edwin Oswaldo Botia Carreño [a], Tonantzin Díaz Alvarado [b], Jorge Alfonso Diego Acosta [b], Pedro Enrique Hernández Ruiz [c], Mona M.M.Y. Elghandour [b], Oluwagbemiga A. Dada [d], Maximilian Lackner [e], Abdelfattah Z.M. Salem [b,*]

[a] Doctorado en Ciencias de la Producción y de la Salud Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México (UNAM), Av. Universidad 3000, Coyoacán, C.P. 04510, Ciudad de México, Mexico
[b] Facultad de Medicina Veterinaria y Zootecnia. Universidad Autónoma del Estado de México, El Cerrillo Piedras Blancas, C.P. 50295, Toluca, Estado de México, Mexico
[c] Escuela Superior de Medicina Veterinaria y Zootecnia No. 3, Universidad Autónoma de Guerrero (UAGro), Técpan de Galeana, Guerrero, Mexico
[d] Adeyemi Federal University of Education, Ondo, Nigeria
[e] Department of Industrial Engineering, University of Applied Sciences Technikum Wien, Hoechstaedtplatz 6, 1200, Vienna, Austria

ARTICLE INFO

Keywords:
Carbohydrates
Chitosan
Nano-encapsulated
Yucca schidigera
Ruminal gases

ABSTRACT

Saponins, the primary components of *Yucca schidigera* extract (YSE), are known to influence microbial activity in the rumen, which can affect various fermentation parameters. Moreover, encapsulating YSE with chitosan (CS) at varying carbohydrate levels (CHO) adds another layer of complexity that can provide valuable insights into the use of additives in mitigating greenhouse gases. This study investigated the impact of both crude and encapsulated forms of YSE on the production of ruminal anaerobic gases in cattle fed different levels of CHO-based diets. Ruminal contents were obtained from four slaughtered, crossbreed bulls (Charollais × Limousin) with a live weight of 400 ± 25 kg. The experimental design followed a completely randomized factorial arrangement, with factors including CHO level (25 %, 45 %, and 55 % DM), YSE forms (without extract, CS, crude, and nano-capsules), and dose of extract (0-, 0.25-, 0.5- and 1.0- mL/g of DM). Results showed that the type of extract significantly affected asymptotic total gas production, methane (CH_4), hydrogen sulfide (H_2S), carbon monoxide (CO), and dry matter digestibility. Nano-chitosan increased gas production and exhibited greater efficiency in reducing CH_4 production by up to 61.4 %. While crude YSE reduced CH_4 production by 38 %, nano-capsules increased production by 17.7 %–42.8 %. Furthermore, a significant interaction effect among CHO levels, type of extract, and extract dose was observed, particularly impacting H_2S and CH_4 production after 48 h of incubation, alongside an increase of about 25.3 % in metabolizable energy compared to the control. The use of CS and YSE improved ($p < 0.0001$) the CH_4 conversion efficiency by 71.3 % and 23.4 % respectively, and at some point, the encapsulation of YSE resulted in a significant reduction in efficiency by up to about 49.5 %. The study concluded that both CS and YSE have the potential to enhance digestibility in cattle and reduce CH_4 production and its conversion efficiency. However, encapsulating YSE with CS may reduce the efficiency of either compound in optimizing ruminal fermentation, unless other influencing factors are carefully considered. Therefore, the optimal efficacy of nano-encapsulated YSE depends on finding a balance between extract type, dosage, and dietary CHO level.

1. Introduction

Livestock has been identified as a significant contributor to climate change, accounting for 14.5 % of total anthropogenic greenhouse gas emissions [1,2]. Moreover, the use of antibiotics to reduce greenhouse gas emissions and boost animal production efficiency has also led to an increase in bacterial resistance to drugs [3]. As a result, plant extracts have emerged as natural alternatives to conventional antibiotics, with

* Corresponding author.
E-mail addresses: salem@uaemex.mx, asalem70@yahoo.com (A.Z.M. Salem).

https://doi.org/10.1016/j.jafr.2024.101450
Received 9 July 2024; Received in revised form 24 September 2024; Accepted 4 October 2024
Available online 5 October 2024

potential to exhibit similar or stronger effects against pathogenic organisms [4,5]. Many plant extracts under consideration are rich in saponins, phenolic compounds, tannins, terpenes, organic acids, flavonoids, complex carbohydrates, and more [6,7]. Besides *Quillaia saponaria*, *Yucca schidigera* (*i.e.,* YSE) is recognized as a significant commercial source of saponins [8]. This plant belongs to the Agavaceae family and is a tropical plant native to North America, particularly the Mexican desert [8]. *Yucca schidigera* contains bioactive compounds and is a rich source of steroidal saponins and polyphenols [9,10]. A series of studies [11,12] have reported that YSE significantly reduces methane (CH_4) and hydrogen sulfide (H_2S) production during rumen fermentation. However, its full potential remains unexplored due to the complexity of the ruminant digestive system. Nano-encapsulation is a delivery system for bioactive compounds that enhances their release and absorption at the target site, primarily the gastrointestinal tract, through active endocytosis [13]. One of such natural biological material is chitosan (CS), which has been extensively used in pharmacology to improve the absorption of bioactive substances through a nanoparticle delivery system [14]. Chitosan is preferred as an oral delivery vehicle due to its potential to increase retention time, allowing for prolonged release of bioactive compounds, its ultra-fine size, and its low toxicity [15,16]. Additionally, CS has been shown to scavenge free radicals by altering the oxidation process and exhibit antimicrobial effects against various bacteria, fungi, and yeast [17]. These antimicrobial properties have made CS a substance of interest in ruminant nutrition [18], with reports of improved fermentation [19]. Sudarshan et al. [20] suggested that the positively charged amine groups (NH3+) in glucosamine within chitosan interact with the negatively charged bacterial surfaces, causing leakage of intracellular contents and ultimately cell death. Another method to reduce enteric CH_4 emissions involves increasing carbohydrate availability in the rumen which enhances microbial capture of ammonia and reduces urinary nitrogen losses. Although it has been established that the type of extract and extract dose affects CH_4 production [21], their role under varying carbohydrate levels (*i.e.,* CHO) remains unclear [22].

This study hypothesizes that the effectiveness of YSE in reducing greenhouse gas emissions can be optimized through encapsulation with CS and precise levels of CHO. Economically, crude YSE has been widely used and is competitively priced in the Mexican market, compared to other feed additives for ruminants, given its natural origin. No previous studies have explored the nano-encapsulation of YSE. Therefore, this study aimed to evaluate the combined effects of nano-encapsulation of YSE, dosage, and dietary carbohydrate levels on rumen fermentation characteristics and the reduction of ruminal greenhouse gases associated with environmental pollution.

2. Materials and methods

2.1. Nanoencapsulation of Yucca schidigera extract

The liquid extract (Bioliquid 3000®) obtained from *Yucca schidigera* stems was purchased from Baja Agro International S.A. de C.V. AGROIN® (Ensenada, Baja California, northwestern Mexico) and contains a 97.9 % concentration of *Y. schidigera*. This extract contains saponins, a group of high molecular weight glycosides with saccharide chain units (1–8 residues) linked to a steroidal aglycone moiety. Nano-encapsulation of the *Y. schidigera* extract was performed using chitosan as the encapsulating biopolymer. The process involved two separate systems that were subsequently combined. In the first system, a 1 % acetic acid solution (100 mL) was prepared, and 0.5 g of Pluronic F127® was gradually dissolved in 50 mL of this solution. After the Pluronic F127® was fully dissolved, 0.3 g of chitosan was added to act as the encapsulating polymer. In the second system, 0.1 g of sodium tripolyphosphate (Sigma-Aldrich®, Toluca, Mexico) was added to the remaining 50 mL of the 1 % acetic acid solution. Then, 0.18 mg of *Y. schidigera* liquid extract was combined with the corresponding part of the first system. The second system was then added to the first system, and mechanical stirring was performed at 600 rpm until complete mixing was achieved. Macroscopic observations were conducted for 72 h after nanoparticle formation to monitor changes in the mixture phases [23].

2.2. Determination of particle size and polydispersity index

For the evaluation of the determination of particle size and polydispersity (PDI) of the Chitosan + *Yucca schidigera* nanoemultion, it was characterized by photon correlation spectroscopy (PCS) using a Malvern laser particle size analyzer (Zetasizer Ver. 7.11, UK) at 25 °C, ensuring that the values (<1 μm) were within the nano range [20]. The values are presented in Table 2.

2.3. In vitro incubation

Incubation involved using *in vitro* measurement techniques to assess the effects of various additives and/or extracts on a sample of rumen fluid. The goal was to study the impact of gas production on animal energy expenditure and greenhouse gas emissions, including methane (CH_4), carbon monoxide (CO), and hydrogen sulfide (H_2S) over a specified incubation period. The evaluated treatments are composed of alfalfa hay, wheat grains, corn grains, bran, corn gluten, soybean meal, molasses, and vitamin and mineral mixture (see Table 1) at three carbohydrate level (25 % (CHO25), 40 % (CHO40) and 55 % (CHO55) with four different extract types [(negative control (without extract), positive control (chitosan), crude extract of *Y. schidigera*, nano-capsules of *Y. schidigera* extract)] included at four doses (0-, 0.25-, 0.5- and 1.0- mL), however, the total was 48 treatments.

The incubation was conducted in amber glass vials with a capacity of 120 mL, each containing 1 g DM of the substrate (diets with 25 %, 40 %, and 55 % dietary carbohydrate), using various volumes of the final product that the chitosan solution managed to encapsulate without showing phase separation after the 72 h of observation following the

Table 1
Ingredients and composition (% DM basis) of diets used as substrates with different levels of carbohydrates.

	Carbohydrate level (%)		
	25	40	55
Ingredients (%)			
Alfalfa hay	9.1	6.5	6.0
Wheat grains	25.0	22.0	12.0
Corn grains	25.0	40.0	55.0
Bran	13.9	11.0	7.0
Corn gluten	12.9	10.0	7.9
Soybean meal	2.0	3.0	6.0
Molasses	12.0	7.4	6.0
Vitamin and mineral premix[a]	0.1	0.1	0.1
Composition			
Crude protein (%)	14.66	14.31	14.21
Ether extract (%)	18.03	15.43	12.21
Acid detergent fiber	9.46	8.25	7.171
Neutral detergent fiber	24.51	22.38	18.67
Free nitrogen extract	66.41	69.63	72.70
Ca (gr/kg)	1.58	1.22	1.17
P (gr/kg)	3.75	3.64	3.43
Mg (gr/kg)	1.76	1.60	1.50
Na (gr/kg)	0.61	0.44	0.36
K (gr/kg)	9.47	7.73	7.36
Cl (gr/kg)	0.70	0.64	0.62
Zn (gr/kg)	22.83	22.18	20.51
Cu (gr/kg)	8.19	6.47	5.10
Fe (gr/kg)	123.26	103.87	79.62

[a] Vitamin and mineral premix provide the following per-kg: 240 g Ca; 30 g P; 20 g Mg; 80 g Na; 120 g Cl; 5 g K; 5 g S; 5 mg Cr; 4000 mg Mn; 2000 mg Fe; 5000 mg Zn; 100 mg I; 30 mg Se; 60 mg Co; 500000 UI A vitamin; 150000 UI D vitamin; 1000 UI E vitamin.

Table 2
Characterization of Chitosan + *Yucca schidigera* nanoparticles in terms of size, PDI, St Dev.

Size (d.nm)	St Dev (d.nm)	PDI[a]
244.8	15.85	0.212

[a] PDI = polydispersity.

formation of the of nano-capsules of *Y. schidigera* extract (0- (negative control), 0.25-, 0.5-, and 1.0- mL), 40 mL of nutrient solution, the nutrient solution contained buffer solution, resazurin, microminerals, macrominerals and distilled water. The nutrient solution was prepared according to the method described by Goering and Van Soest [24], along with 10 mL of rumen fluid. The same doses of chitosan were used as a positive control during the incubations.

A total of 432 bottles (3 bottles of each triplicate sample within each one of the 3 carbohydrate levels (*i.e.,* 25 %, 40 %, and 55 %), with 4 different extract types (negative control (without extract), positive control (chitosan), crude extract of *Y. schidigera*, nano-capsules of *Y. schidigera* extract) of 4 extract doses (*i.e.,* 0-, 0.25-, 0.5- and 1.0- mL) and each treatment was subjected to incubation in triplicate in each series of incubation to ensure the accuracy of the results. In addition, three blank (no substrate) and negative controls per inoculum were included, as well as chitosan (same doses of extracts used) as a positive control. This is to allow for proper correction of the readings and to minimize any external interference in the data obtained. Once all the bottles were filled, they were immediately closed with rubber stoppers, gently shaken manually every 1–2 h, and placed in the incubator with water at 39 °C for 48 h. The volume of gas produced, CH_4, CO, and H_2S production were recorded at 2, 4, 6, 24, 28, 30, and 48 h of inoculation [22,25–27].

The ruminal fluid was obtained from the contents of the rumen of four crossbreeds (Charollais × Limousin) bulls of 400 ± 25 kg of live weight, immediately after they were slaughtered at the municipal slaughterhouse of Toluca, State of Mexico, Mexico. The slaughtering procedure follows the Official Mexican Standard NOM-033-SAG/ZOO 2014, which establishes methods for the humane slaughter of domestic and wild animals. The contents of the rumen from each animal were transferred separately to a hermetic container to be taken to the bromatology laboratory of the Faculty of Veterinary Medicine and Zootechnics at the Autonomous University of the State of Mexico, located 30 min away. The fluid was then filtered through four layers of sterile cotton gauze (Model FN17100, Lab. Dibar, CDMX, Mexico) to obtain the ruminal fluid, eliminating coarse particles while allowing the passage of larger microorganisms, such as rumen protozoa. The final mixture was created by combining the filtered ruminal fluid. Before slaughter, the bulls were fed a diet of hay and commercial concentrate (Purina®, Toluca, State of Mexico, Mexico) in a 50:50 ratio and had continuous access to fresh water.

2.4. Ruminal total gas, CH_4, CO and H_2S productions

Treatments were dosed into vials and incubated at a constant temperature of 39 °C in a water bath for 48 h. Total gas production (measured in psi) from each bottle was recorded due to the rapid degradation of carbohydrates in the rumen at specific time intervals (*i.e.,* 2, 4, 24, 26, 28, 30, and 48 h post-inoculation), following the technique described by Theodorou et al. [28]. Simultaneously, measurements of CH_4, CO, and H_2S were taken from each bottle at these same time intervals using a 5 mL diffusion gas detector (Dräger Safety X-am 20500 MONITOR, Lübeck, Germany). After each recording, gas was dispersed inside each bottle using a syringe needle to avoid gas accumulation and to keep the pressure in the headspace of the bottle below 48 kPa. Each treatment was subjected to incubation in triplicate in each run of incubation to ensure the accuracy of results. In addition, three blank (no substrate) negative controls per inoculum as well as the chitosan (same doses of extracts used) as a positive control, were included to allow for proper correction of the readings and to minimize any external interference in the data obtained.

2.5. Ruminal pH and dry matter degradability

At the end of fermentation, the contents of the vials were filtered using filter bags with a porosity of 25 μm (Filter bags F57, ANKOM Technology Corp., Macedonia, NY, USA) to separate the diet that was not degraded from the liquid part. The filtrate was collected in beakers and used to measure the pH with a potentiometer (pH wireless electrode HALO® model HI11102, Hanna® Instruments, Woonsocket, RI, USA). The bags with the non-degraded diet were washed with plenty of tap water and dehydrated at 60 °C for 48 h to obtain the dry weight value. The dry matter degradability was obtained with the dry weight value.

2.6. Chemical analysis of the diet (substrate)

A representative sample was taken from each of the diets to be dried for 72 h at 60 °C, then they were ground using a hammer mill (Thomas Wiley®, model 4, Swedesboro, NJ, USA) for chemical analysis. Fiber fractions [29], including neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using an ANKOM200 Fiber Analyzer Unit (ANKOM Technology Corp., Macedon, NY) following the methodological guidelines of Van Soest et al. [30].

2.7. Calculations and statistical analysis

The production volumes (mL/g DM incubated) of total gas, CH_4, CO, and H_2S were used to estimate the asymptotic production, production rate, and lag phase time of each gas using the NLIN procedure of the Statistical Analysis System [31] and the model proposed by France et al. [32]. Metabolizable energy (ME; MJ/kg DM) was estimated using the equation proposed by Menke et al. [33], while short-chain fatty acids (SCFA; mmol 200/mg DM) were estimated according to Getachew et al. [34]. Additionally, the CH_4 conversion efficiency was evaluated through the production of CH_4 per unit of SCFA (CH_4: SCFA), ME (CH_4: ME), and MO (CH_4: MO) in mmol/mmol, g/MJ, and mL/g, respectively.

The experimental design was completely randomized with a factorial arrangement (3 × 4 × 4), where factor 1 was dietary carbohydrate levels (*i.e.,* 25 %, 40 %, and 55 %), factor 2 was the types of extracts used (negative control (without extract), positive control (chitosan), crude extract of *Y. schidigera*, nano-capsules of *Y. schidigera* extract), and factor 3 was the doses of each type of extract (*i.e.,* 0-, 0.25-, 0.5-, and 1.0- mL extract/g DM), with three repetitions for each. The data from the three repetitions of each treatment in each run were averaged, and these averages were used as the experimental unit for each treatment. Data analysis was performed using the GLM procedure of SAS [31] and the statistical model listed below:

$$Y_{ijk} = \mu + CH_i + TE_j + EX_k + (CH \times TE)_{ij} + (CH \times EX)_{ik} + (TE \times EX)_{jk} + (CH \times TE \times EX)_{ijk} + \varepsilon_{ijk}$$

where, Y_{ijk} is the response variable, μ is the general mean, CH_i is the effect of the dietary carbohydrate level, TE_j is the effect of the type of extract, EX_k is the effect of extract doses, $(CH \times TE)_{ij}$ is the effect of the interaction between the carbohydrate level and the type of extract, $(CH \times EX)_{ik}$ is the effect of the interaction between the carbohydrate level and the extract doses, $(TE \times EX)_{jk}$ is the effect of the interaction between the type of extract and the extract doses, $(CH \times TE \times EX)_{ijk}$ is the effect of the interaction between the carbohydrate level, the type of extract and the extract doses, and ε_{ijk} is the experimental error. The comparison of means was performed using Tukey's test, and they were considered significantly different when $p \leq 0.05$.

3. Results

3.1. Total gas production

The main effect of CHO was significant ($p = 0.0140$) on asymptotic gas production and gas production (GP, mL gas/g DM incubated) at 4, 24, and 48 h of incubation (Table 3). Total gas production increased with carbohydrate level. Specifically, at CHO25, total gas production was 324.5 mL while at CHO55, it increased to 378.0 mL, representing a 16.5 % increase. The type of extract used (TE) also had a highly significant ($p < 0.0001$) effect on asymptotic total GP. The use of nano-CS increased the GP by 57 % compared to the control, while 38.9 % and 39.8 % increases were observed when compared to crude extract and nano-capsule respectively. The dose of extract significantly ($p < 0.0001$) influenced GP at 4 and 24 h, but not consistently across all carbohydrate levels. The interaction effect of CHO × TE × ED on GP was significant ($p < 0.0001$) only at 4 h of incubation, with 1 mL nano-chitosan producing the highest gas values, about 56.8 % more than the control. At 4 and 24 h of incubation, the use of nano-extract at increased dietary carbohydrate levels (*i.e.,* 40 % and 55 %) resulted in significantly ($p = 0.014$) lower GP compared to the control. This represents between 24.3 and 41.5 % reduction in GP compared to the negative control. A significant decrease in GP was also observed at 48 h of incubation when 0.5 mL of nano-extract was used at 40 % and 55 % carbohydrate levels.

Table 3
Effect of nanoparticles of *Y. Schidigera* at different doses of each extract (0.0-, 0.25-, 0.5- and 1.0- ml of extract/g dietary DM) on *in vitro* ruminal total gas production (ml/g DM) of diets with three percent levels of carbohydrate (25 (CHO25), 40 (CHO40, and 55 (CHO55) %) compared with nanoparticles of chitosan (as positive control (PC)) and the crude extract used male bulls as donor animals.

Carbohydrate level (%CHO)[a]	Type of extract	Dose (ml/g DM)	Gas production kinetics[b]			Gras production (ml gas/g DM incubated)		
			b	c	Lag	4h	24h	48h
CHO25	Without extract	0	324.5	0.03163	0.5315	60.9	183.2	308.4
	Nano-chitosan	0.25	479.4	0.02287	1.33767	170.8	303.7	450.7
		0.5	457.7	0.023	1.46213	169.1	300.0	432.5
		1	509.5	0.02377	1.2418	183.6	337.8	485.5
	Crude extract	0.25	341.0	0.0283	0.65303	103.1	229.7	327.7
		0.5	345.3	0.029	0.72597	111.0	235.9	331.3
		1	366.7	0.0287	1.34617	154.3	274.2	357.0
	Nano-extract	0.25	359.2	0.0279	0.07503	80.3	201.7	338.7
		0.5	364.4	0.02803	0.2541	86.6	205.9	343.7
		1	356.3	0.02753	0.3097	81.9	202.2	335.8
	Type of extract (TE)		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Extract dose (ED)		0.3139	0.8759	0.0873	0.0005	0.0683	0.2521
	TE X ED		0.5712	0.9261	0.0579	0.0021	0.4753	0.5631
	SEM		15.1989	0.0007	0.1264	4.9929	9.8142	14.3761
CHO40	Without extract	0	364.4	2.69443	0.62407	106.8	240.5	349.0
	Nano-chitosan	0.25	497.3	0.03727	0.75327	175.1	324.0	472.1
		0.5	540.0	0.02547	0.8607	179.5	357.5	516.7
		1	509.7	0.02833	0.97533	187.7	386.4	502.1
	Crude extract	0.25	358.4	0.02863	0.787	114.6	246.3	344.4
		0.5	409.6	0.02687	0.8692	148.6	277.6	391.5
		1	415.5	0.0281	1.23333	163.8	301.4	401.2
	Nano-extract	0.25	403.9	0.0566	1.2181	75.5	188.2	429.7
		0.5	362.9	0.02977	0.1165	77.9	208.1	342.6
		1	369.0	0.02547	0.22057	86.5	215.3	362.1
	Type of extract (TE)		0.0001	0.9999	0.0374	<0.0001	<0.0001	0.0021
	Extract dose (ED)		0.8279	0.9997	0.1947	0.0002	0.0191	0.9951
	TE X ED		0.5731	1.0000	0.0094	0.0196	0.8672	0.3908
	SEM		27.6452	0.2721	0.1687	5.3060	14.6898	29.0227
CHO55	Without extract	0	378.0	0.02947	0.24743	84.5	226.9	359.8
	Nano-chitosan	0.25	524.0	0.02277	1.1051	175.2	318.5	490.5
		0.5	503.4	0.02427	1.0192	171.8	322.7	477.0
		1	504.6	0.0237	1.2954	183.6	330.5	478.5
	Crude extract	0.25	394.4	0.0264	0.78293	119.1	245.4	373.4
		0.5	382.6	0.027	1.10803	128.7	249.0	363.9
		1	407.3	0.0305	0.23437	107.9	253.5	387.4
	Nano-extract	0.25	402.7	0.0615	1.13887	53.6	181.5	387.6
		0.5	285.7	0.02917	0.6528	68.0	176.8	271.1
		1	415.3	0.0304	0.69667	71.6	208.1	405.8
	Type of extract (TE)		<0.0001	0.1418	0.0013	<0.0001	<0.0001	<0.0001
	Extract dose (ED)		0.0148	0.4423	0.0472	0.2048	0.0809	0.0123
	TE X ED		0.0512	0.3435	0.0008	0.0101	0.6051	0.031
	SEM		20.5702	0.0038	0.1107	4.1403	7.0345	20.1798
SEM pooled [b,c]			21.2303422	0.092229	0.134919	4.848398	10.52698	21.028875
P value:								
Carbohydrate level (CHO)			0.0140	0.0281	0.9827	<0.0001	0.0002	0.0148
Type of extract (TE)			<0.0001	0.9991	<0.0001	<0.0001	<0.0001	<0.0001
Extract dose (ED)			0.2048	0.9993	0.5233	<0.0001	0.0003	0.1529
CHO X TE			0.8334	1.0000	<0.0001	0.0009	0.2090	0.9763
CHO X ED			0.2162	1.0000	0.0199	0.0107	0.3233	0.5409
TE X ED			0.2541	1.0000	0.0053	0.0112	0.8210	0.1631
CHO X ET X ED			0.3731	1.0000	0.0002	<0.0001	0.8107	0.3304

[a] Basal diet illustrated in Table 1, with different carbohydrate levels of 25 % (CHO25), 40 % (CHO40) and 55 % (CHO55) of the total mixed ration.
[b] *b* = asymptotic total gas production (mL/g DM); *c* = rate of total gas production (mL/h); *Lag* = initial delay before total gas production begins (h).
[c] SEM = standard error of the mean.

3.2. Anaerobic gases of CH_4, CO and H_2S productions

Table 4 shows that CHO had a significant ($p \leq 0.0074$) effect on asymptotic methane (CH_4) production and the rate of CH_4 production but not significant ($p = 0.3066$) on lag time.

The CHO significantly ($p = 0.0183$) affected CH_4 production (mL gas/g DM incubated) at 4 and 48 h. However, the effect of CHO on ml CH_4/100 mL gas was not significant at any of the incubation hours. The TE significantly ($p < 0.0001$) affected CH_4 production at 4, 24, and 48 h of incubation. Nano-chitosan produced the least CH_4, followed by crude extract. While nano-extract produced more CH_4 than even the control. The extract dose (ED) affected ($p = 0.0079$) CH_4 production until 48 h of incubation. Apart from the crude extract, which led to a decrease in CH_4 production, it was observed that increasing the doses of other extracts did not lead to a further reduction in CH_4 output, though values were lower than the control. At CHO25, CHO40, and CHO55, the type of extract used resulted in significant differences in CH_4 production kinetics, with nano-extract producing the highest CH_4 and CS producing the least. However, ED did not affect the rate of CH_4 production and the lag, regardless of the CHO level. The CHO × TE × ED interaction effect was observed to be significant ($p = 0.0002$) on CH_4 production at 48 h of incubation, with a reduction in methane production. The only reduction effect of nano-extract on CH_4 production was observed using 1 mL at CHO55, which reduced CH_4 by 15.3 %. Comparatively, 1 mL of crude extract reduced methane production by 42.2 % at 48 h of incubation. In terms of CO production, CHO significantly ($p = 0.0053$) affected asymptotic CO production (Table 5).

Similarly, the effect of TE on asymptotic CO production and associated production kinetics was significant. Notably, 0.5 mL of the crude extract resulted in the lowest asymptotic CO and CO production (mL/g DM) at 48 h of incubation, while nano-chitosan generated the highest asymptotic CO gas. However, ED did not show any significant effect on CO production kinetics or CO production at any of the incubation hours. The interaction effect of CHO × TE was statistically significant for CO production at 4, 24, and 48 h. TE × ED had no significant effect on CO production parameters. However, the interaction effect of CHO × ET × ED on CO production was significant for asymptotic CO production and mL gas/g DM at 48 h of incubation, with nano-extract reducing CO production by 67.7 % compared to the control. Table 6 revealed that CHO did not affect the asymptotic H_2S production and production rate but did affect the lag time.

Similarly, CHO did not significantly affect H_2S production at any incubation hours. Type of extract significantly ($p < 0.0001$) affected H_2S production (asymptotic and mL gas/g DM incubated) at 4, 24, and 48 h of incubation. Nano-extract reduced H_2S gas the most by about 30.3 % compared to the control while nano-chitosan resulted in increased H_2S gas production. Extract dose (*i.e.,* ED) did not exert any significant ($p = 0.0139$) effect on H_2S production kinetics. CHO × TE, CHO × ED, and CHO × TE × ED had a significant ($p = 0.0139$) effect on asymptotic H_2S production. The interaction effect of CHO × TE × ED was also significant for H_2S production at 4 and 48 h.

3.3. Rumen fermentation profile and CH_4 conversion efficiency

An increase in the level of carbohydrates from 25 to 40 % resulted in a reduction in pH values (Table 7), although, this effect was not statistically significant ($p = 0.3205$). The CHO effect was significant ($p \leq 0.0004$) on short-chain fatty acid (SCFA) and metabolizable energy (ME), resulting in elevated SCFA and ME observed *in vitro*. Moreso, TE significantly ($P < 0.0001$) affected the pH of the rumen inoculum. Specifically, the crude extract treatment had the lowest pH values compared to the control, representing about a 17 % reduction. This represents between 10.08 and 20.5 % reduction in pH values compared to the negative control. The TE had a significant effect on DMD % ($p < 0.0001$), SCFA, ME, and CH_4: SCFA. The effect of ED was also significant ($p = 0.0006$) on pH. Extract dose significantly ($p = 0.0003$) affected SCFA and ME but did not have a significant effect on CH_4: ME, CH_4: OM, and CH_4: SCFA. CHO × TE and TE × ED interaction effects were observed to be significant ($p < 0.0001$) on rumen pH. The use of 1 mL of crude extract resulted in the lowest pH values irrespective of the CHO level in the diet. There was no interaction effect of CHO × TE on CH_4 conversion efficiency. However, the interaction effect of CHO × TE × ED was significant ($p = 0.0491$) on DMD% and CH_4: SCFA.

4. Discussion

4.1. Total gas production

Saponins, the primary component of YSE, are known to influence microbial activity in the rumen, which can affect various fermentation parameters. Moreover, the encapsulation of YSE with CS at varying carbohydrate levels adds another layer of complexity that can potentially provide a better understanding of its application in greenhouse gas mitigation. The main effect of increasing CHO from 25 to 40 % resulted in a 12.2 % increase in asymptotic GP. However, a 3.7 % increase in GP was only observed when CHO was further increased to 55 %. This suggests that the relationship between dietary carbohydrates and asymptotic GP is not linear. The findings indicate that the benefit of increasing CHO on GP is not constant and may become marginal at higher levels of CHO. The highest gas produced at 40 % CHO might be a reflection of optimum microbial activity and fermentation. This suggests that CHO levels that are too low or high can either limit the fermentation process or result in a less efficient breakdown. The high GP values recorded with the use of nano-chitosan indicates its potential to enhance microbial fermentation in the rumen, especially in cattle. A previous study by Belanche et al. [35] suggested that CS improves fermentation patterns by decreasing metabolic hydrogen production. Del Valle et al. [36] and Zanferari et al. [37] have also reported improved feed efficiency in cows due to the use of CS. The observed increase in GP as a result of YSE is consistent with previous findings on the potential of saponin to improve rumen fermentation. However, contrary to the report of Besharati et al. [38] who reported reduced GP with nano-CS encapsulation of flaxseed oil in an *in vitro* experiment, the encapsulation of YSE with nano-CS in this study produced more gas compared to the control. This suggests the existence of a synergistic relationship between the tested additives. Nonetheless, the results of this study suggest that using CS as an encapsulating agent for YSE may not confer additional benefits on rumen gas production (mL gas/g DM incubated), unless there is a balance between dietary carbohydrate levels and extract dosage, as increased GP (comparatively to control) were obtained at CHO40 and CHO55 using 0.25 and 1.0 ml of nano-extract respectively.

4.2. Ruminal total gas, CH_4, CO and H_2S productions

The effect of increasing dietary energy levels on methane production is that more CH_4 will be produced per gram of DM incubated. The type and level of CHO can affect the dissolved hydrogen, which is capable of altering the fermentation pathway and ultimately CH_4 emissions [39]. The observed reduction in CH_4 production as a result of YSE inclusion is consistent with the findings of Zeid et al. [11]. According to Ref. [21], saponin which is the main component of YS possesses the potential to reduce the number of protozoa, subsequently resulting in a reduction of hydrogen ions available to methanogens for methane production. It is also possible for saponins to produce similar results by weakening the activity of methane-producing genes, while the population of methanogens remains unchanged [40]. However, using 0.25 mL of nano-chitosan proved to be more effective in reducing the asymptotic CH_4 production kinetics and associated parameters at 4, 24, and 48 h of incubation compared to other extracts tested in this study. This improvement is likely due to the potential of CS to inhibit H_2 production or encourage alternative metabolic pathways since methanogenesis is a primary biochemical mechanism of removing H_2 from the rumen. The

Table 4
Effect of nanoparticles of *Y. Schidigera* at different doses of each extract (0.0-, 0.25-, 0.5-, and 1.0- ml of extract/g dietary DM) on *in vitro* ruminal methane production (CH_4, ml/g DM) of diets with three percent levels of carbohydrate (25 (CHO25), 40 (CHO40, and 55 (CHO55) %) compared with nanoparticles of chitosan (as positive control (PC)) and the crude extract used male bulls as donor animals.

	Type of extract	Extract dose (ml/g DM)	CH_4 production kinetics[b]			CH_4 production (ml gas/g DM incubated)			CH_4 (ml CH_4/100 ml gas)		
Carbohydrate level (% of diet CHO)[a]			b	c	Lag	4h	24h	48h	4h	24h	48h
CHO25	Without extract	0	41.5	0.012	0.869	0.81	8.12	41.62	6.50	22.05	67.06
	Nano-chitosan	0.25	24.2	0.014	1.223	1.06	3.58	24.19	3.08	5.86	26.97
		0.5	25.7	0.014	1.209	1.07	3.78	25.84	3.17	6.22	29.56
		1	27.2	0.014	1.210	1.13	3.95	27.28	3.08	5.86	28.08
	Crude extract	0.25	46.0	0.012	0.491	2.61	12.86	46.33	12.33	27.89	70.67
		0.5	37.0	0.011	0.697	2.23	7.86	37.15	10.00	16.67	55.83
		1	31.7	0.009	0.389	2.57	8.72	31.61	8.33	15.83	44.17
	Nano-extract	0.25	49.5	0.009	0.333	2.29	13.07	49.61	14.17	32.22	73.06
		0.5	61.4	0.011	0.509	3.23	16.51	60.94	18.67	40.06	77.67
		1	61.9	0.010	0.409	2.90	16.88	62.07	17.83	41.72	81.61
	Type of extract (TE)		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Extract dose (ED)		0.8976	0.4269	0.4051	0.6669	0.8704	0.9209	0.7405	0.8631	0.3545
	TE x ED		0.0388	0.2959	0.7759	0.3572	0.0191	0.0339	0.1171	0.0026	0.0155
	SEM		3.5698	0.0006	0.1085	0.2409	1.0429	3.5451	1.1165	1.9651	4.0237
CHO40	Without extract	0	48.3	0.010	0.554	1.54	11.02	48.49	7.17	23.00	69.39
	Nano-chitosan	0.25	26.5	0.014	1.240	1.08	3.80	26.51	3.08	5.86	28.08
		0.5	29.7	0.012	1.062	3.54	27.67	29.92	9.58	14.33	37.00
		1	60.4	0.012	1.263	3.09	23.30	60.70	7.83	16.92	64.58
	Crude extract	0.25	45.5	0.010	0.306	3.36	13.40	45.56	14.67	27.16	66.06
		0.5	49.6	0.010	0.532	3.69	15.27	49.52	12.33	27.33	63.17
		1	39.1	0.010	0.213	3.76	13.02	38.97	11.50	21.50	48.44
	Nano-extract	0.25	61.2	0.011	0.933	2.86	15.71	61.00	19.00	42.61	71.67
		0.5	63.7	0.010	0.488	2.64	15.61	63.56	16.50	37.33	75.11
		1	69.0	0.009	0.565	2.95	14.98	68.97	16.67	34.72	72.22
	Type of extract (TE)		<0.0001	0.0010	<0.0001	0.4165	0.8394	<0.0001	0.0005	<0.0001	0.0002
	Extract dose (ED)		0.0469	0.2668	0.4088	0.5067	0.4954	0.0452	0.9052	0.9275	0.5510
	TE x ED		0.0087	0.5995	0.1853	0.6965	0.6428	0.0084	0.4127	0.2514	0.0115
	SEM		4.9971	0.0008	0.1233	0.6831	5.0173	5.0250	2.1193	3.6071	6.1485
CHO55	Without extract	0	53.1	0.010	0.543	1.63	13.84	52.87	9.67	30.50	73.56
	Nano-chitosan	0.25	31.1	0.013	1.107	1.28	5.35	31.16	3.67	8.39	31.72
		0.5	27.1	0.013	1.107	1.17	4.72	27.10	3.42	7.31	28.42
		1	28.5	0.012	0.818	1.74	6.43	28.54	4.75	9.75	29.75
	Crude extract	0.25	56.6	0.009	0.143	4.57	19.77	56.52	19.17	40.00	75.55
		0.5	38.2	0.010	0.334	3.74	12.20	38.04	14.50	24.50	52.28
		1	32.9	0.010	0.274	1.99	11.28	32.87	9.17	22.22	42.50
	Nano-extract	0.25	62.5	0.009	0.746	1.11	10.45	62.47	10.17	28.78	67.28
		0.5	45.0	0.008	0.568	1.40	9.65	44.85	10.00	27.78	68.22
		1	62.2	0.008	0.976	2.21	14.29	103.50	15.33	34.22	69.00
	Type of extract (TE)		0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Extract dose (ED)		0.0514	0.898	0.9706	0.4384	0.02	0.0129	0.4182	0.0141	0.0003
	TE x ED		0.2293	0.7516	0.0803	0.0001	0.0007	0.0005	0.0018	0.0004	<0.0001
	SEM		4.4760	0.0005	0.1027	0.2954	0.9007	4.7351	1.3416	1.7248	2.4416
SEM pooled [c]			4.38	0.0006	0.1117	0.410	2.315	4.468	1.539	2.390	4.245
P value:											
Carbohydrate level (CHO)			0.0074	0.0002	0.3066	0.0183	0.1029	0.0048	0.1324	0.0665	0.1488
Type of extract (TE)			<0.0001	<0.0001	<0.0001	<0.0001	0.1478	<0.0001	<0.0001	<0.0001	<0.0001
Extract dose (ED)			0.2951	0.2054	0.7379	0.6166	0.7534	0.0079	0.8460	0.3537	0.3581
CHO x TE			0.6306	0.5099	0.0021	0.0390	0.0996	0.1857	0.0038	0.0005	0.0258
CHO x ED			0.0114	0.5763	0.5255	0.5434	0.4356	0.0193	0.8571	0.4062	0.0675
TE x ED			0.0012	0.7923	0.1430	0.2439	0.3501	<0.0001	0.0084	0.0003	<0.0001
CHO x ET x ED			0.1218	0.3893	0.2471	0.2429	0.7740	0.0002	0.1784	0.0353	0.1097

[a] Basal diet illustrated in Table 1, with different carbohydrate levels of 25 % (CHO25), 40 % (CHO40) and 55 % (CHO55) of the total mixed ration.
[b] *b* = asymptotic CH_4 production (mL/g DM); *c* = rate of CH_4 production (mL/h); *Lag* = initial delay before CH_4 production begins (h).
[c] SEM = standard error of the mean.

Table 5
Effect of nanoparticles of *Y. Schidigera* at different doses of each extract (0.0-, 0.25-, 0.5-, and 1.0- ml of extract/g dietary DM) on *in vitro* ruminal carbon monoxide (CO, ml/g DM) of diets with three percent levels of carbohydrate (25 (CHO25), 40 (CHO40, and 55 (CHO55) %) compared with nanoparticles of chitosan (as positive control (PC)) and the crude extract used male bulls as donor animals.

Carbohydrate level (% of diet CHO)[a]	Type of extract	Extract dose (ml/g DM)	CO production kinetics[b]			CO production (ml/g DM incubated)		
			b	c	Lag	4h	24h	48h
CHO25	Without extract	0	0.129660	0.000129	0.000023	0.001024	0.009714	0.064710
	Nano-chitosan	0.25	0.616083	0.000105	0.000024	0.005220	0.020039	0.309450
		0.5	0.544788	0.000106	0.000024	0.005204	0.020187	0.272892
		1	0.573828	0.000112	0.000024	0.005498	0.021449	0.288380
	Crude extract	0.25	0.093567	0.000099	0.000022	0.002461	0.009915	0.046672
		0.5	0.085330	0.000100	0.000022	0.002478	0.009598	0.042503
		1	0.100698	0.000094	0.000021	0.004585	0.013962	0.050163
	Nano-extract	0.25	0.158977	0.000098	0.000022	0.002322	0.017044	0.080255
		0.5	0.159688	0.000094	0.000022	0.001880	0.015139	0.080472
		1	0.139482	0.000093	0.000022	0.001453	0.014758	0.070094
	Type of extract (TE)		<0.0001	0.0363	<0.0001	<0.0001	<0.0001	<0.0001
	Extract dose (ED)		0.2205	0.9927	0.1951	0.1045	0.3601	0.2155
	TE x ED		0.2699	0.7476	0.1735	0.0072	0.2979	0.2658
	SEM		0.0148	0.0000	0.0000	0.0003	0.0012	0.0074
CHO40	Without extract	0	0.238224	0.000102	0.000023	0.004779	0.024679	0.118643
	Nano-chitosan	0.25	0.592359	0.000092	0.000024	0.006391	0.026381	0.296547
		0.5	1.182591	0.000109	0.000024	0.006781	0.033582	0.592364
		1	1.932138	0.000112	0.000024	0.009272	0.047674	0.967231
	Crude extract	0.25	0.111174	0.000093	0.000022	0.003201	0.013040	0.055525
		0.5	0.113423	0.000090	0.000021	0.005950	0.016835	0.056632
		1	0.142962	0.000102	0.000022	0.005639	0.019284	0.071085
	Nano-extract	0.25	0.184358	0.000120	0.000023	0.001435	0.015243	0.092908
		0.5	0.135032	0.000119	0.000022	0.001674	0.013500	0.067980
		1	0.099063	0.000098	0.000022	0.001627	0.009074	0.048038
	Type of extract (TE)		<0.0001	0.0701	<0.0001	<0.0001	0.0078	<0.0001
	Extract dose (ED)		0.1135	0.8480	0.3228	0.2437	0.5559	0.1156
	TE x ED		0.0634	0.1166	0.0771	0.5956	0.6429	0.0633
	SEM		0.1202	0.0000	0.0000	0.0008	0.0053	0.0603
CHO55	Without extract	0	0.195338	0.000088	0.000022	0.002789	0.021705	0.097623
	Nano-chitosan	0.25	0.643885	0.000100	0.000025	0.003069	0.008429	0.322075
		0.5	0.383970	0.000127	0.000025	0.002430	0.006914	0.191924
		1	0.496067	0.000113	0.000025	0.002787	0.007109	0.248356
	Crude extract	0.25	0.122041	0.000095	0.000021	0.004391	0.015778	0.061196
		0.5	0.114083	0.000093	0.000021	0.006252	0.017613	0.056946
		1	0.099422	0.000104	0.000022	0.002606	0.010123	0.049789
	Nano-extract	0.25	0.124531	0.000123	0.000023	0.000672	0.007947	0.062892
		0.5	0.062844	0.000105	0.000022	0.000989	0.006928	0.031514
		1	0.121849	0.000111	0.000023	0.001624	0.012583	0.072016
	Type of extract (TE)		<0.0001	0.1566	<0.0001	<0.0001	0.0003	<0.0001
	Extract dose (ED)		0.0374	0.9347	0.1143	0.3096	0.8656	0.0382
	TE x ED		0.1342	0.3643	0.3102	0.0339	0.0204	0.1243
	SEM		0.0315	0.0000	0.0000	0.0005	0.0015	0.0165
SEM pooled [c]			0.055403	0.000007	0.000000	0.000545	0.002614	0.028014
P value:								
Carbohydrate level (CHO)			0.0053	0.8217	0.7472	<0.0001	0.0008	0.0058
Type of extract (TE)			<0.0001	0.0139	<0.0001	<0.0001	0.0017	<0.0001
Extract dose (ED)			0.1995	0.8847	0.1059	0.2698	0.6002	0.1903
CHO x TE			0.0001	0.1990	0.0002	<0.0001	0.0002	0.0001
CHO x ED			0.0513	0.9902	0.1551	0.1983	0.7555	0.0559
TE x ED			0.1180	0.0905	0.3183	0.3588	0.6978	0.1235
CHO x ET x ED			0.0139	0.5654	0.0789	0.1709	0.5448	0.0132

[a] Basal diet illustrated in Table 1, with different carbohydrate levels of 25 % (CHO25), 40 % (CHO40) and 55 % (CHO55) of the total mixed ration.
[b] *b* = asymptotic CO production (mL/g DM); *c* = rate of CO production (mL/h); *Lag* = initial delay before CO production begins (h).
[c] SEM = standard error of the mean.

CHO × TE interaction effect significantly affected the proportion of CH_4 per 100 mL of gas produced at 4, 24, and 48 h. At CHO25 and CHO40, 0.25 mL of nano-chitosan produced the least asymptotic CH_4, while, a better effect was achieved at CHO55 with 0.5 mL of the same extract. The CHO × ED interaction effect revealed that increasing dietary energy levels will require a higher dosage of nano-chitosan to achieve similar or better reductions in asymptotic CH_4 production during ruminal fermentation. The CHO × TE × ED interaction significantly impacted on CH_4 production at 48 h of incubation. Methane was reduced by 49.0 % at CHO55 using 0.5 mL of nano-chitosan, while the crude extract and nano-extract reduced CH_4 production by 28.1 and 15.2 % respectively. The interaction effect observed in this study confirms the findings of Patra and Saxena [21] regarding the influence of saponins on ruminal fermentation characteristics as a function of the type of saponin, dosage, and dietary composition. Carbon monoxide (CO) is produced when organic matter (OM) is degraded by ruminal microbiota, and its production is closely related to microbial activity and the fermentation processes [41]. According to Techtmann et al. [42], CO can be oxidized to CO_2 and H_2 in the presence of water, with methanogens subsequently using these products to produce CH_4. The consistently low levels of CO observed with the nano-extract in this study may result from its oxidation and conversion into other gases, particularly CH_4.

Nano-extract and crude-extract reduced e asymptotic H_2S production, while the use of nano-chitosan resulted in elevated H_2S production. This suggests that different extracts might favour different groups of microorganisms, leading to varying effects on gas production. The

Table 6
Effect of nanoparticles of *Y. Schidigera* at different doses of each extract (0.0-, 0.25-, 0.5-, and 1.0- ml of extract/g dietary DM) on *in vitro* ruminal hydrogen sulfide (H_2S, ml/g DM) of diets with three percent levels of carbohydrate (25 (CHO25), 40 (CHO40, and 55 (CHO55) %) compared with nanoparticles of chitosan (as positive control (PC)) and the crude extract used male bulls as donor animals.

Carbohydrate level (% of diet CHO)[a]	Type of extract	Extract dose (ml/g DM)	H_2S production kinetics[b]			H_2S production (ml/g DM incubated)		
			b	c	Lag	4h	24h	48h
CHO25	Without extract	0	0.457830	0.000116	0.000023	0.005847	0.044631	0.228183
	Nano-chitosan	0.25	1.884323	0.000112	0.000023	0.030572	0.183028	0.940614
		0.5	1.643427	0.000103	0.000023	0.029750	0.147743	0.819970
		1	2.097693	0.000098	0.000023	0.028788	0.199987	1.048509
	Crude extract	0.25	0.398590	0.000119	0.000022	0.010261	0.049197	0.199771
		0.5	0.424343	0.000088	0.000022	0.010056	0.049058	0.212080
		1	0.535373	0.000095	0.000022	0.019571	0.067682	0.266262
	Nano-extract	0.25	0.474953	0.000118	0.000023	0.012518	0.043339	0.236945
		0.5	0.456520	0.000094	0.000023	0.013289	0.044692	0.214252
		1	0.499003	0.000100	0.000023	0.012764	0.049180	0.249136
	Type of extract (TE)		<0.0001	0.6176	0.0001	<0.0001	<0.0001	<0.0001
	Extract dose (ED)		0.0159	<0.0001	0.42	0.3522	0.0179	0.0117
	TE x ED		0.1144	0.3213	0.8006	0.1559	0.154	0.1294
	SEM		0.0606	0.0000	0.0000	0.0017	0.0078	0.0294
CHO40	Without extract	0	0.518660	0.000358	0.000021	0.016653	0.066927	0.259305
	Nano-chitosan	0.25	1.987950	0.000102	0.000023	0.027275	0.191828	0.994322
		0.5	1.751753	0.000087	0.000022	0.020261	0.181899	0.875603
		1	0.987090	0.000096	0.000021	0.014554	0.117480	0.490367
	Crude extract	0.25	0.490357	0.000099	0.000022	0.014531	0.056710	0.243281
		0.5	0.565593	0.000096	0.000022	0.021748	0.073605	0.282499
		1	0.568833	0.000106	0.000022	0.022913	0.070191	0.283519
	Nano-extract	0.25	0.633487	0.000128	0.000024	0.009995	0.043626	0.315965
		0.5	0.506040	0.000102	0.000023	0.009496	0.047849	0.253264
		1	0.536600	0.000102	0.000023	0.013462	0.051036	0.267500
	Type of extract (TE)		<0.0001	0.9724	0.1006	0.0245	<0.0001	<0.0001
	Extract dose (ED)		0.0795	0.9773	0.4834	0.9949	0.2722	0.0767
	TE x ED		0.0528	0.9997	0.9000	0.2120	0.0926	0.0498
	SEM		0.1252	0.0000	0.0000	0.0036	0.0119	0.0625
CHO55	Without extract	0	0.511237	0.000110	0.000022	0.013416	0.065131	0.255970
	Nano-chitosan	0.25	2.098620	0.000111	0.000023	0.026607	0.148727	1.049638
		0.5	1.984410	0.000117	0.000024	0.019357	0.115527	0.993009
		1	2.061550	0.000113	0.000023	0.030681	0.163393	1.029421
	Crude extract	0.25	0.518220	0.000103	0.000022	0.018908	0.061188	0.258343
		0.5	0.453860	0.000107	0.000022	0.016789	0.060041	0.225206
		1	0.498453	0.000118	0.000022	0.008679	0.056971	0.249741
	Nano-extract	0.25	0.529327	0.000101	0.000023	0.003541	0.040361	0.264398
		0.5	0.357510	0.000103	0.000023	0.008319	0.035814	0.178421
		1	0.480083	0.000113	0.000023	0.009852	0.046551	0.257080
	Type of extract (TE)		<0.0001	0.5476	0.001	<0.0001	<0.0001	<0.0001
	Extract dose (ED)		0.146	0.4461	0.9225	0.782	0.3172	0.135
	TE x ED		0.9596	0.8909	0.7381	0.0225	0.5282	0.9349
	SEM		0.0618	0.0000	0.0000	0.0026	0.0106	0.0316
SEM pooled [c]			0.082859	0.000016	0.000000	0.002647	0.010154	0.041316
P value:								
Carbohydrate level (CHO)			0.3595	0.0724	0.0351	0.6038	0.3768	0.3160
Type of extract (TE)			<0.0001	0.9897	0.0003	<0.0001	<0.0001	<0.0001
Extract dose (ED)			0.2146	0.9016	0.3712	0.7197	0.4895	0.2094
CHO x TE			0.0013	0.9960	0.0591	0.0056	0.2169	0.0014
CHO x ED			0.0128	0.9888	0.8161	0.9326	0.0641	0.0105
TE x ED			0.2097	0.9995	0.7507	0.3437	0.3622	0.1862
CHO x ET x ED			0.0139	1.0000	0.9863	0.0165	0.0801	0.0142

[a] Basal diet illustrated in Table 1, with different carbohydrate levels of 25 % (CHO25), 40 % (CHO40) and 55 % (CHO55) of the total mixed ration.
[b] *b* = is the asymptotic H_2S production (mL/g DM); *c* = is the rate of H_2S production (mL/h); *Lag* = is the initial delay before H_2S production begins (h).
[c] SEM = standard error of the mean.

production of H_2S in the rumen is associated with the actions of sulfate-reducing Bacteria (SRB) that reduce Sulfur (S) to H_2S [43]. Nano-CS likely favours SRB while nano-extract exhibits inhibitory tendencies. Previous study by Alsubait et al. [44] also reported a decrease in H_2S production due to YSE inclusion. In this study, 0.5 ml of nano-extract in the CHO55 treatment group produced the least H_2S at 48 h of incubation. This shows the interaction effects of carbohydrate level, extract type, and dosage on H_2S production during ruminal fermentation.

4.3. Rumen fermentation profile and CH_4 conversion efficiency

Contrary to the report by Yi et al. [45], both TE and ED significantly affected rumen pH in this study. The crude extract consistently reduced pH irrespective of the dietary CHO levels. However, the CHO × TE interaction had a significant effect on pH. The results showed that at lower dietary carbohydrate levels, the different types of extract reduced rumen inoculum pH, but at higher carbohydrate levels, both nano-chitosan and nano-extract increased rumen pH compared to the negative control. This suggests that rumen pH response is more dependent on the type of extract than on CHO level, as the extracts show varying effects on pH across different carbohydrate levels.

Nano-chitosan at 0.5 mL produced the best SCFA and CH_4 conversion efficiency values, followed by 1 mL of crude extract. Methane values at 24 h of incubation improved from 73.46 % to 76.05 % at CHO15 and CHO55, respectively, with the addition of 0.25 and 1 mL of nano-chitosan. Chitosan, with a pKa of approximately 6.5, is slightly acidic

Table 7
Effect of nanoparticles of *Y. Schidigera* at different doses of each extract (0.0-, 0.25-, 0.5-, and 1.0- ml of extract/g dietary DM) on *in vitro* rumen fermentation profile and CH_4 conversion efficiency of diets with three percent levels of carbohydrate (25 (CHO25), 40 (CHO40, and 55 (CHO55) %) compared with nanoparticles of chitosan (as positive control (PC)) and the crude extract used male bulls as donor animals.

Carbohydrate level (% of diet CHO)[a]	Type of extract	Extract dose (ml/g DM)	Rumen fermentation profile[b]				CH_4 conversion efficiency[c]		
			pH	DMD%	SCFA mmol/g DM	ME, MJ/kg DM 24 h	CH4: ME (g/MJ)	CH4:OM (ml/g)	CH4: SCFA at 24h (mmol/mmol)
CHO25	Without extract	0	6.76	89.58	8.12	8.37	4.50	9.13	28.86
	Nano-chitosan	0.25	6.70	85.29	13.47	11.12	1.49	4.02	7.66
		0.5	6.65	61.27	13.30	11.03	1.57	4.25	8.13
		1	6.65	62.83	14.98	11.90	1.55	4.43	7.66
	Crude extract	0.25	6.31	81.57	10.17	9.43	6.33	14.45	36.47
		0.5	6.03	81.65	10.45	9.57	3.82	8.82	21.79
		1	5.61	75.09	12.15	10.44	3.87	9.80	20.70
	Nano-extract	0.25	6.07	69.26	8.93	8.79	6.89	14.68	42.15
		0.5	6.38	86.93	9.12	8.89	8.63	18.55	52.39
		1	6.32	84.72	8.95	8.80	8.91	18.96	54.58
	Type of extract (TE)		<0.0001	0.0904	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Extract dose (ED)		0.1728	0.6726	0.0686	0.07	0.8848	0.87	0.8628
	TE x ED		0.0108	0.0222	0.4753	0.4753	0.0063	0.0191	0.0026
	SEM		0.0779	4.4623	0.4359	0.2242	0.4712	1.1723	2.5689
CHO40	Without extract	0	6.33	83.72	10.66	9.63	5.33	12.38	30.07
	Nano-chitosan	0.25	6.60	75.95	14.37	11.53	1.53	4.26	7.66
		0.5	6.76	70.01	15.85	12.30	9.58	31.09	11.12
		1	6.92	64.59	17.13	12.96	7.61	26.18	34.55
	Crude extract	0.25	5.96	83.75	10.92	9.76	6.37	15.05	35.52
		0.5	5.90	80.89	12.30	10.48	6.75	17.15	35.73
		1	5.61	80.29	13.36	11.02	5.47	14.63	28.10
	Nano-extract	0.25	6.38	69.04	8.34	8.44	8.68	17.65	55.75
		0.5	6.45	81.68	9.22	8.89	8.14	17.54	48.83
		1	6.45	76.86	9.54	9.06	7.68	16.83	45.41
	Type of extract (TE)		<0.0001	0.0889	<0.0001	<0.0001	0.6009	0.8394	<0.0001
	Extract dose (ED)		0.5178	0.8325	0.0192	0.0185	0.5626	0.4952	0.6658
	TE x ED		0.0015	0.4099	0.8677	0.8727	0.5821	0.6427	0.1155
	SEM		0.0616	3.9494	0.6519	0.3341	1.7365	5.6373	5.1863
CHO55	Without extract	0	6.44	88.01	10.05	9.31	6.91	15.54	39.88
	Nano-chitosan	0.25	6.67	55.37	14.12	11.39	2.18	6.01	10.96
		0.5	6.67	60.60	14.31	11.49	1.91	5.31	9.55
		1	6.51	73.01	14.65	11.67	2.57	7.23	12.74
	Crude extract	0.25	5.98	85.61	10.88	9.73	9.41	22.21	52.30
		0.5	5.85	81.23	11.03	9.81	5.77	13.70	32.03
		1	5.59	79.40	11.24	9.91	5.29	12.68	29.06
	Nano-extract	0.25	6.70	80.72	8.04	8.27	5.88	11.74	37.65
		0.5	6.66	78.73	7.83	8.16	5.52	10.84	36.35
		1	6.46	79.49	9.22	8.88	7.47	16.05	44.76
	Type of extract (TE)		<0.0001	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Extract dose (ED)		<0.0001	0.5736	0.0819	0.0811	0.0115	0.0201	0.0142
	TE x ED		0.1618	0.1752	0.6096	0.6059	0.0003	0.0007	0.0005
	SEM		0.0410	3.1488	0.3124	0.1606	0.3960	1.0123	2.2568
SEM pooled [d]			0.060	3.815	0.467	0.240	0.862	2.601	3.282
P value:									
Carbohydrate level (CHO)			0.3205	0.7812	0.0002	0.0004	0.1114	0.1029	0.1232
Type of extract (TE)			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.1476	<0.0001
Extract dose (ED)			0.0006	0.9113	0.0003	0.0003	0.9294	0.7531	0.3467
CHO x TE			<0.0001	0.4035	0.2111	0.2131	0.0678	0.0995	0.0052
CHO x ED			0.0830	0.7059	0.3251	0.3227	0.4203	0.4356	0.6176
TE x ED			<0.0001	0.0762	0.8210	0.8202	0.1557	0.3499	0.0012
CHO x ET x ED			0.0616	0.0491	0.8119	0.8101	0.6584	0.7739	0.0223

[a] Basal diet illustrated in Table 1, with different carbohydrate levels of 25 % (CHO25), 40 % (CHO40) and 55 % (CHO55) of the total mixed ration.
[b] pH = ruminal pH; DMD = dry matter degradability; SCFA = short-chain fatty acids; ME = the metabolizable energy.
[c] CH_4:SCFA = methane:short-chain fatty acids ratio; CH_4:ME = methane:metabolizable energy ratio; CH_4:OM = methane:organic matter ratio.
[d] SEM = standard error of the mean.

and can influence rumen pH, affecting microbial fermentation and methane production. Studies have shown that chitosan inclusion in ruminant diets increases ruminal propionic acid levels and decreases acetic acid concentrations [19,46]. These changes in short-chain fatty acid profiles suggest that chitosan can enhance fermentation efficiency and potentially reduce methane emissions. Araújo et al. [47] also observed that higher nutrient intake improves digestibility and alters SCFA concentrations, supporting the idea that chitosan's impact on methane conversion efficiency is influenced by its acidity and dietary context. The combined effects of extract type, dosage, and carbohydrate levels are critical in determining methane conversion efficiency, as they interact to influence ruminal fermentation and methane production. Additionally, Cardozo et al. [48] suggested that rumen VFA production due to saponins depends on diet, rumen pH, and dosage.

5. Conclusions

Incorporating nano-chitosan into cattle diets enhances rumen fermentation and increases short-chain fatty acid production while reducing methane emissions. However, it also leads to higher carbon monoxide and hydrogen sulfide production. Among the carbohydrate levels tested, a 40 % carbohydrate diet resulted in the highest gas production after 48 h. Both nano-extract and crude extract reduced hydrogen sulfide production and improved dry matter digestibility. Chitosan and yucca extract show potential for improving rumen fermentation and reducing methane production but using chitosan as a carrier for yucca extract requires careful balancing of extract type, dosage, and carbohydrate levels in the diet.

Funding

Not applicable.

Ethics approval

The ruminal contents of cattle were taken from the slaughterhouse of Toluca, Estado de Mexico, Mexico.

Consent for publication

Not applicable.

Data availability statement

The data presented in this study are available on request from the corresponding author.

Code availability

Not applicable.

Institutional review board statement

Not applicable.

CRediT authorship contribution statement

Conceptualization, A.Z.M.S.; methodology, E.O.B.C., M.M.M.Y.E., O. A.D. and R.D.T.; software, E.O.B.C. and R.D.T.; validation, M.M.M.Y.E. and A.Z.M.S.; formal analysis, E.O.B.C., O.A.D. and R.D.T.; investigation, E.O.B.C., O.A.D., R.D.T. and M.M.M.Y.E.; resources and data curation, E.O.B.C. and A.Z.M.S.; writing—original draft preparation, E. O.B.C., A.M., O.A.D. and R.D.T.X.X.; writing—review and editing, M.M. M.Y.E., M.L. and A.Z.M.S.; visualization, A.Z.M.S.; supervision, M.M.M. Y.E., M.L. and A.Z.M.S.; project administration, A.Z.M.S. and M.L.; funding acquisition, A.Z.M.S. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgement

Authors would like to thank BIORGANIX MEXICANA S.A. DE C.V, Coahuila, Mexico for providing INSILATO AL[@] as a probiotic cocktail during the experiments.

References

[1] IPCC, Climate change 2013, in: The Physical Science Basis, Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, WMO/UNEP, Cambridge, 2013.
[2] P.J. Gerber, H. Steinfeld, B. Henderson, A. Mottet, C. Opio, J. Dijkman, A. Falcucci, G. Tempio, Tackling Climate Change through Livestock: a Global Assessment of Emissions and Mitigation Opportunities, Food and agriculture Organization of the United Nations (FAO), 2013.
[3] J.J. Carrique-Mas, J. Rushton, Integrated interventions to tackle antimicrobial usage in animal production systems: the ViParc project in vietnam, Front. Microbiol. 8 (2017), https://doi.org/10.3389/fmicb.2017.01062.
[4] D. Alviano, C. Alviano, Plant extracts: search for new alternatives to treat microbial diseases, Curr. Pharmaceut. Biotechnol. 10 (2009) 106–121.
[5] N. Puvača, V. Tufarelli, I. Giannenas, Essential oils in broiler chicken production, immunity and meat quality: review of *Thymus vulgaris, Origanum vulgare*, and *Rosmarinus officinalis*, Agriculture 12 (2022), https://doi.org/10.3390/agriculture12060874.
[6] G.M. Molina-Salinas, M.C. Ramos-Guerra, J. Vargas-Villarreal, B.D. Mata-Cárdenas, P. Becerril-Montes, S. Said-Fernández, Bactericidal activity of organic extracts from *Flourensia cernua* DC against strains of *Mycobacterium tuberculosis*, Arch. Med. Res. 37 (2006) 45–49, https://doi.org/10.1016/j.arcmed.2005.04.010.
[7] Y. Rosas-Piñón, A. Mejía, G. Díaz-Ruiz, M.I. Aguilar, S. Sánchez-Nieto, J.F. Rivero-Cruz, Ethnobotanical survey and antibacterial activity of plants used in the Altiplane region of Mexico for the treatment of oral cavity infections, J. Ethnopharmacol. 141 (2012) 860–865, https://doi.org/10.1016/j.jep.2012.03.020.
[8] P.R. Cheeke, Actual and potential applications of *Yucca schidigera* and *Quillaja saponaria* saponins in human and animal nutrition, in: Saponins in Food, Feedstuffs and Medicinal Plants, Springer, 2000, pp. 241–254.
[9] D. Headon, Glycofractions of the Yucca plant and their role in ammonia control, in: T.P. Lyon (Ed.), Biotechnology in the Feed Industry, Alltech Technical Publication, Nicholasville, KY, 1991, pp. 95–108.
[10] S. Patel, Yucca: a medicinally significant genus with manifold therapeutic attributes, Natural Prod. Biopros. 2 (2012) 231–234, https://doi.org/10.1007/s13659-012-0090-4.
[11] S.R. Zeid, M.S. Moharam, A.A. Nour, H.M. El-Zalat, S.M.A. Sallam, In vitro responses of *Yucca Schidigera* extract on ruminal methane production, fermentation characteristics and degradability, Egypt. J. Nutrition Feeds 22 (2) (2019) 321–328.
[12] U.Y. Anele, X. Crumel, L. Olagunju, D.P. Compart, Effects of *Yucca schidigera* based feed additive on *in vitro* dry matter digestibility, efficiency of microbial production, and greenhouse gas emissions of four dairy diets, Dairy 3 (2022) 326–332, https://doi.org/10.3390/dairy3020025.
[13] A. Müller, S.K. Das, P. Kögerler, H. Bögge, M. Schmidtmann, A.X. Trautwein, V. Schünemann, E. Krickemeyer, W. Preetz, A New Type of supramolecular compound: molybdenum-oxide-based composites consisting of magnetic nanocapsules with encapsulated keggin-ion electron reservoirs cross-linked to a two-dimensional network, Angew. Chem. Int. Ed. 39 (2000) 3413–3417, https://doi.org/10.1002/1521-3773(20001002)39:19.
[14] Y. Liang, X. Zhao, P.X. Ma, B. Guo, Y. Du, X. Han, pH-responsive injectable hydrogels with mucosal adhesiveness based on chitosan-grafted-dihydrocaffeic acid and oxidized pullulan for localized drug delivery, J. Colloid Interface Sci. 536 (2019) 224–234, https://doi.org/10.1016/j.jcis.2018.10.056.
[15] M.A. Elgadir, M.S. Uddin, S. Ferdosh, A. Adam, A.J.K. Chowdhury, M.Z.I. Sarker, Impact of chitosan composites and chitosan nanoparticle composites on various drug delivery systems: a review, J. Food Drug Anal. 23 (2015) 619–629, https://doi.org/10.1016/j.jfda.2014.10.008.
[16] I.A. Siddiqui, D.J. Bharali, M. Nihal, V.M. Adhami, N. Khan, J.C. Chamcheu, M. I. Khan, S. Shabana, S.A. Mousa, H. Mukhtar, Excellent anti-proliferative and pro-apoptotic effects of (−)-epigallocatechin-3-gallate encapsulated in chitosan nanoparticles on human melanoma cell growth both *in vitro* and *in vivo*, Nanomed.

Nanotechnol. Biol. Med. 10 (2014) 1619–1626, https://doi.org/10.1016/j.nano.2014.05.007.
[17] R.C. Goy, D.d. Britto, O.B.G. Assis, A review of the antimicrobial activity of chitosan, Polímeros 19 (2009).
[18] I. Goiri, A. Garcia-Rodriguez, L.M. Oregui, Effect of chitosans on *in vitro* rumen digestion and fermentation of maize silage, Anim. Feed Sci. Technol. 148 (2009) 276–287, https://doi.org/10.1016/j.anifeedsci.2008.04.007.
[19] A.O.C. Dias, R.H.T.B. Goes, J.R. Gandra, C.S. Takiya, A.F. Branco, A.G. Jacaúna, R. T. Oliveira, C.J.S. Souza, M.S.M. Vaz, Increasing doses of chitosan to grazing beef steers: nutrient intake and digestibility, ruminal fermentation, and nitrogen utilization, Anim. Feed Sci. Technol. 225 (2017) 73–80.
[20] N.R. Sudarshan, D.G. Hoover, D. Knorr, Antibacterial action of chitosan, Food Biotechnol. 6 (1992) 257–272, https://doi.org/10.1080/08905439209549838.
[21] A.K. Patra, J. Saxena, The effect and mode of action of saponins on the microbial populations and fermentation in the rumen and ruminant production, Nutr. Res. Rev. 22 (2009) 204–219, https://doi.org/10.1017/S0954422409990163.
[22] A. Zúñiga-Serrano, H.B. Barrios-García, R.C. Anderson, M.E. Hume, M. Ruiz-Albarrán, Y. Bautista-Martínez, N.A. Sánchez-Guerra, J. Vázquez-Villanueva, F. Infante-Rodríguez, J. Salinas-Chavira, Antimicrobial and digestive effects of *Yucca schidigera* Extracts related to production and environment implications of ruminant and non-ruminant animals: a Review, Agriculture 12 (2022) 1198, https://doi.org/10.3390/agriculture12081198.
[23] J.C. Ribeiro, W.L.C. Ribeiro, A.L.F. Camurça-Vasconcelos, I.T.F. Macedo, J.M. L. Santos, H.C.B. Paula, J.V. Araújo Filho, R.D. Magalhães, C.M.L. Bevilaqua, Efficacy of free and nanoencapsulated *Eucalyptus citriodora* essential oils on sheep gastrointestinal nematodes and toxicity for mice, Vet. Parasitol. 204 (2014) 243–248, https://doi.org/10.1016/j.vetpar.2014.05.026.
[24] H.K. Goering, P.J. Van Soest, Forage Fiber Analyses (Apparatus, Reagents, Procedures, and Some Applications), US Agricultural Research Service, 1970.
[25] E.R. Alvarado-Ramírez, A. Maggiolino, M.M.M.Y. Elghandour, M.A. Rivas-Jacobo, G. Ballesteros-Rodea, P.D. Palo, A.Z.M. Salem, Impact of Co-Ensiling of maize with *Moringa oleifera* on the production of greenhouse gases and the characteristics of fermentation in ruminants, Animals 13 (2023) 764, https://doi.org/10.3390/ani13040764.
[26] O.V. Vazquez-Mendoza, V. Andrade-Yucailla, M.M.M.Y. Elghandour, D. A. Masaquiza-Moposita, J.A. Cayetano-De-Jesús, E.R. Alvarado-Ramírez, M. J. Adegbeye, M. Barros-Rodríguez, A.Z.M. Salem, Effect of dietary guanidinoacetic acid levels on the mitigation of greenhouse gas production and the rumen fermentation profile of alfalfa-based diets, Animals 13 (11) (2023) 1719, https://doi.org/10.3390/ani13111719.
[27] M.M.M.Y. Elghandour, E.B. Figueroa Pacheco, A. Khusro, D.N. Tirado-González, M. Lackner, J.L. Ponce-Covarrubias, P. De Palo, A. Maggiolino, A.Z.M. Salem, Deciphering the role of *Moringa oleifera* seeds and probiotic bacteria on mitigation of biogas production from ruminants, Amb. Express 14 (2024) 86, https://doi.org/10.1186/s13568-024-01744-x.
[28] M.K. Theodorou, B.A. Williams, M.S. Dhanoa, A.B. McAllan, J. France, A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds, Anim. Feed Sci. Technol. 48 (1994) 185–197, https://doi.org/10.1016/0377-8401(94)90171-6.
[29] AOAC, in: A.O.A. Chem, M.D. Gathersburg (Eds.), Official Methods of Analysis, eighteenth ed. ed., 1997.
[30] P.J. Van Soest, J.B. Robertson, B.A. Lewis, Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition, J. Dairy Sci. 74 (1991) 3583–3597, https://doi.org/10.3168/jds.S0022-0302(91)78551-2.
[31] I.I. Sas, SAS, Cary, NC, 2018.
[32] J. France, J. Dijkstra, M.S. Dhanoa, S. Lopez, A. Bannink, Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed *in vitro*:derivation of models and other mathematical considerations, Br. J. Nutr. 83 (2000) 143–150, https://doi.org/10.1017/S0007114500000180.
[33] K.H. Menke, L. Raab, A. Salewski, H. Steingass, D. Fritz, W. Schneider, The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor *in vitro*, J. Agric. Sci. 93 (1979) 217–222, https://doi.org/10.1017/S0021859600086305.
[34] G. Getachew, H.P.S. Makkar, K. Becker, Tropical browses: contents of phenolic compounds, *in vitro* gas production and stoichiometric relationship between short chain fatty acid and *in vitro* gas production, J. Agric. Sci. 139 (2002) 341–352, https://doi.org/10.1017/S0021859602002393.
[35] A. Belanche, E. Pinloche, D. Preskett, C.J. Newbold, Effects and mode of action of chitosan and ivy fruit saponins on the microbiome, fermentation and methanogenesis in the rumen simulation technique, FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 92 (2016) fiv160, https://doi.org/10.1093/femsec/fiv160.
[36] T.A. Del Valle, P.G.d. Paiva, E. Ferreira de Jesus, G.F.d. Almeida, F. Zanferari, A.G. B.V.B. Costa, I.C.S. Bueno, F.P. Rennó, Dietary chitosan improves nitrogen use and feed conversion in diets for mid-lactation dairy cows, Livest. Sci. 201 (2017) 22–29, https://doi.org/10.1016/j.livsci.2017.04.003.
[37] F. Zanferari, T.H.A. Vendramini, M.F. Rentas, R. Gardinal, G.D. Calomeni, L. G. Mesquita, C.S. Takiya, F.P. Rennó, Effects of chitosan and whole raw soybeans on ruminal fermentation and bacterial populations, and milk fatty acid profile in dairy cows, J. Dairy Sci. 101 (2018) 10939–10952, https://doi.org/10.3168/jds.2018-14675.
[38] M. Besharati, I. Giannenas, V. Palangi, T. Ayasan, F. Noorian, A. Maggliolino, J. M. Lorenzo, Chitosan/calcium-alginate encapsulated flaxseed oil on dairy cattle diet: *In vitro* fermentation and fatty acid biohydrogenation, Animals 12 (11) (2022) 1400.
[39] X. Sun, L. Cheng, A. Jonker, S. Munidasa, D. Pacheco, A Review: Plant Carbohydrate Types—The Potential Impact on Ruminant Methane Emissions, vol. 9, 2022, https://doi.org/10.3389/fvets.2022.880115.
[40] Y.Q. Guo, J.X. Liu, Y. Lu, W.Y. Zhu, S.E. Denman, C.S. McSweeney, Effect of tea saponin on methanogenesis, microbial community structure and expression of mcrA gene, in cultures of rumen micro-organisms, Lett. Appl. Microbiol. 47 (2008) 421–426, https://doi.org/10.1111/j.1472-765X.2008.02459.x.
[41] K. Haarstad, O. Bergersen, R. Sørheim, Occurrence of carbon monoxide during organic waste degradation, Air Waste Manag. Assoc. 56 (2006) 575–580.
[42] S.M. Techtmann, A.S. Colman, F.T. Robb, That which does not kill us only makes us stronger: the role of carbon monoxide in thermophilic microbial consortia, Environ. Microbiol. 11 (2009) 1027–1037.
[43] S. Wang, K. Giller, M. Kreuzer, S.E. Ulbrich, U. Braun, A. Schwarm, Contribution of ruminal fungi, archaea, protozoa, and bacteria to the methane suppression caused by oilseed supplemented diets, Front. Microbiol. 8 (2017) 1864.
[44] I.S. Alsubait, I.A. Alhidary, A.A. Al-Haidary, Effects of different levels of yucca supplementation on growth rates, metabolic profiles, fecal odor emissions, and carcass traits of growing lambs, Animals 13 (2023) 755, https://doi.org/10.3390/ani13040755.
[45] X. Yi, B. Wu, J. Ma, X. Cui, Z. Deng, S. Hu, W. Li, R. A, X. Li, Q. Meng, et al., Effects of dietary capsaicin and *Yucca schidigera* extracts as feed additives on rumen fermentation and microflora of beef cattle fed with a moderate-energy diet, Fermentation 9 (2023), https://doi.org/10.3390/fermentation9010030.
[46] P.G. de Paiva, E.F. de Jesus, T.A. Del Valle, G.F. de Almeida, A.G.B.V.B. Costa, C.E. C. Consentini, F. Zanferari, C.S. Takiya, I.C. da Silva Bueno, F.P. Rennó, Effects of chitosan on ruminal fermentation, nutrient digestibility, and milk yield and composition of dairy cows, Anim. Prod. Sci. 57 (2017) 301–307.
[47] A. Araújo, B. Venturelli, M. Santos, R. Gardinal, N. Cônsolo, G. Calomeni, J. Freitas, R. Barletta, J. Gandra, P. Paiva, Chitosan affects total nutrient digestion and ruminal fermentation in Nellore steers, Anim. Feed Sci. Technol. 206 (2015) 114–118.
[48] P.W. Cardozo, S. Calsamiglia, A. Ferret, C. Kamel, Screening for the effects of natural plant extracts at different pH on *in vitro* rumen microbial fermentation of a high-concentrate diet for beef cattle1, J. Anim. Sci. 83 (2005) 2572–2579, https://doi.org/10.2527/2005.83112572x.