



Valorization of *Caesalpinia coriaria* Fruit Waste to Enhance the Ruminant Mitigation of Greenhouse Gases Production

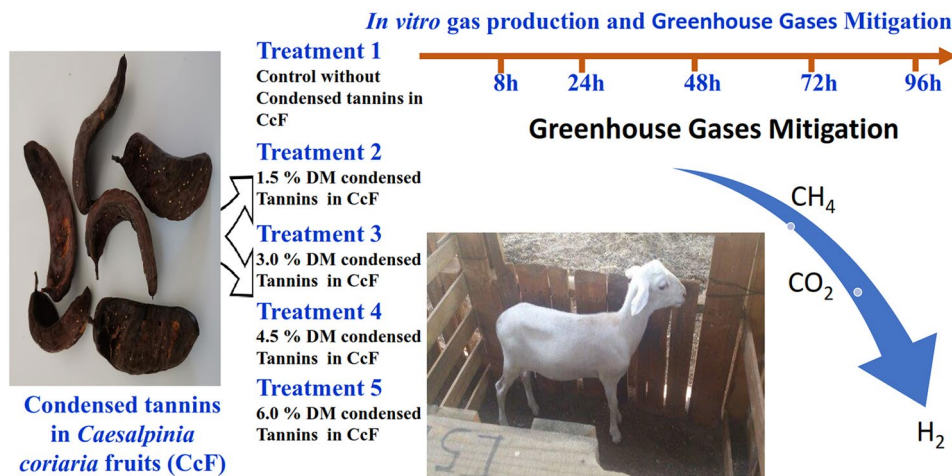
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Abstract

The use of fruits waste from tanniniferous trees represents a new technology that aims to solve or mitigate greenhouse gases emission, and to increase the production of food of animal origin in an ecofriendly manner. This study aims to evaluate the production of methane and carbon dioxide, degradability by in vitro ruminal fermentation in goats diet supplemented with nuts (*Caesalpinia coriaria* Jacq. Willd.) for adoption by livestock farmers. Condensed tannins (CT) of *C. coriaria* inclusion rates were 0 (CT0 or control, no CT), 1.5 (CT1.5), 3.0 (CT3), 4.5 (CT4.5) and 6.0% (CT6) of the total mixed ration. All CT treatments reduced (linear, quadratic and cubic; $P=0.001$) CH_4 , CO_2 and H_2 gases, and had some increasing effect on total biogas production. However, CT3 reduced greenhouse gases and had the highest biogas production. Addition of tannins from cascalote fruit waste (*C. coriaria* Jacq. Willd.) to goats diet at CT3 level reduced methane production, improved fermentation and ruminal degradability in vitro and has potential to be used as ecofriendly feed or feed additive.

Graphic Abstract



Keywords Biogases · Greenhouse gases · *Caesalpinia coriaria* · Tannins · Goats

Statement of Novelty

The use of fruit wastes from tanniniferous trees represents a new technology that aims to solve or mitigate the effects of greenhouse gases, to increase the production of food of animal origin for the population in an ecofriendly manner.

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The impact of *C. coriaria* fruit waste on greenhouse gases emission is not yet established. Thus, there is need for research to screen and evaluate tannin sources and establish optimal concentrations in ruminant diets. The addition of tannins from the *C. coriaria* fruit waste to goats diets may reduce the production of methane, improve fermentation, ruminal degradability and could be good for adoption by local livestock farmers.

Introduction

Increasing human population and affluence will bring increased demand for animal derived protein especially in developing countries and encourage farmers to go into livestock production including ruminants. Currently there is increasing competition for resources such as land, forage and water etc., which has resulted in several clashes especially large ruminant and crop farmers because they need large quantity of resources [1]. To reduce these competitions, farmers are encouraged to go into small ruminant production because of high adaptability to climate changes, harsh environment and emit lower greenhouse gasses compared to large ruminants [2, 3]. From the environmental perspective, ruminants contribute about 5.7 of 7.1 Gt CO₂eq./yr. global livestock greenhouse gases with approximately 44% of these emissions as methane [4]. Specifically, world CO₂-eq (carbon dioxide equivalent) and methane (CH₄) emission of small ruminant is 72–79% and 90–93% lower than cattle respectively while CH₄ emission intensity per kg of final product is lower in small ruminant than cattle [3, 5, 6]. This suggest the environmental friendliness of small ruminant. Despite the low emissions of small ruminant, between 2000 and 2017, sheep and goat population increased globally by 13.40 and 37.62%, respectively [7]. The increase in goat population may cause an increase in overall contributions of small ruminant to ruminant greenhouse gas emission. Therefore, there is need to create a balance by minimizing environmental impacts of small ruminant to ensure sustainable animal-protein production [8]. Animal feed supplementation is part of the global proffered solution to minimize ruminant greenhouse gas emission. Application of natural additives such as of biogenic origin may be suitable for cleaner animal production; and enhance feed digestibility of ruminant residing in tropical areas which is characterized by low quality forage containing low levels of crude protein and fermentable energy.

Tannin can be used as additives in mitigating greenhouse gas emission and improving livestock production. These tannins particularly condensed tannin (CT) are commonly distributed in nutritionally important forages, and shrubs, commonly consumed by ruminants [9]. The effectiveness of CT depends on its sources of origin ruminants [9]. In

addition, condensed tannins modify ruminal fermentation by inhibiting ammonia and methane production, partly through their ability to complex protein and fiber in the diet. The effect of tannins on ruminant digestion is associated with the amount of tannin consumed and its chemical composition [10]. Despite the impact of CT, its high level can affect dry matter intake, nutrient digestion, protein utilization, reduce rumen microbial activity and inhibit endogenous digestive enzyme activities [9]. One of such plants with condensed tannin is *Caesalpinia coriaria*, an arboreal legume found in the tropical region of Mexico and it contains various secondary metabolites such as tannins, gallic acid and flavonoids [11]. Furthermore, *C. coriaria* fruit could serve as alternative feed in goat production without affecting health and performance parameters negatively [12] and has ovicidal and larvicidal properties [13] and antioxidant properties [14]. The impact on greenhouse gasses emission is not yet established, however, Manuel-Pablo et al. [12] study gave a glimpse on the potential of *C. coriaria* on methane where its addition in goats diet reduced the concentration of protozoa in rumen fluid. Thus, due to the hydrogen-exchange relationship between protozoa and methanogens, *C. coriaria* shows that it has the potential to reduce methane emission. Therefore, there is need for research to screening and evaluation tannins sources and establish optimal concentrations in ruminant diets.

These in vitro studies provide preliminary information about these resources and their potential production of methane in enteric fermentation. The present study aims to determine methane and carbon dioxide production, degradability and in vitro rumen fermentation in goats diet supplemented with nuts (*C. coriaria* Jacq. Willd.) for adoption by farmers through local livestock associations. Therefore, the addition of tannins from cascalote fruit waste (*C. coriaria* Jacq. Willd.) to goats diet will reduce methane production, improve fermentation and ruminal degradability in vitro.

Materials and Methods

Substrates and Treatments

In vitro fermentation was carried out using a balanced diet based on 50:50 F:C. Treatments include supplementation of 0 (CT0), 1.5 (CT1.5), 3.0 (CT3), 4.5 (CT4.5) and 6.0 (CT6) % of total diet dry matter (DM) of condensed tannins (CT) of *Caesalpinia coriaria* Jacq. Willd. The composition of the diets used as subtracts is shown in Table 1. Mature fruits waste of *C. coriaria* were collected during the harvest season directly from the tree at a height of 1.5 m simulating goats grazing, from there samples were obtained which were

Table 1 Diets ingredients and compositions as well as the composition of cascalote fruit

	Diets				
	CT0	CT1.5	CT3	CT4.5	CT6
Ingredients					
Oat hay	46.79	46.94	47.09	47.24	47.3
Ground corn	27.95	22.38	16.83	11.33	6.8
Soybean meal	12.64	13.24	13.84	14.43	14.9
Molasses	7.17	7.2	7.22	7.24	7.24
Fruit of Cascalote	0	4.58	9.13	13.65	17.6
Sunflower oil	3.17	3.18	3.19	3.2	3.21
Calcium carbonate	1.15	1.15	1.14	1.14	1.14
Urea	0.91	0.91	0.91	0.91	0.91
Vit-mineral mixture ^a	0.22	0.42	0.65	0.86	0.9
Chemical composition (% dry matter basis)					
Organic matter	99.68	99.73	99.7	99.73	99.69
Crude protein	15.23	16.01	14.88	15.49	15.14
Ether extract	5.97	6.85	7.44	2.38	2.42
Total condensed tannins	0	1.5	3.0	4.5	6.0
Chemical composition of cascalote fruit % DM basis					
Organic matter	98.99				
Crude protein	8.14				
Ether extract	2.17				
Acid detergent fiber	10.11				
Neutral detergent fiber	13.20				
Free condensed tannins	22.71				
Protein-bonded condensed tannins	3.17				
Fiber-bonded condensed tannins	7.18				
Total condensed tannins	33.06				

Dietary condensed tannins (CT) of *C. coriaria* included at 0 (CT0), 1.5 (CT1.5), 3 (CT3), 4.5 (CT4.5) and 6 (CT6) % of the total mixed ration

^aVit-mineral mixture: Sodium chloride, calcium carbonate, magnesium sulfate, iron sulfate, zinc sulfate, sodium selenium, vitamin A, vitamin D3, vitamin E, vitamin B1, iodine 130 ppm, cereal by-products

dried in the shade and ground in a Willey mill with a 0.5 mm screen for use as substrate in diets [15].

In Vitro Incubations

Four Criollo goats (approximately 20 ± 5 kg live weight) housed in individual cages and fed with the basal diet based on [16] were used as inoculum source (ruminal fluid). The goats were provided with fresh water during and after the inoculum collection phase, afterwards the rumen contents were rinsed with CO₂ and filtered using cheese cloth in a flask with oxygen-free space. The collected rumen fluid was mixed with buffer solution [1:4 v/v] [17] and added to incubation bottles containing 1 g DM pre-weighed substrates and additive solutions. Three incubation runs were performed in three weeks. Bottles containing the samples (five dietary CT levels of *C. coriaria* (0, 1.5, 3.0, 4.5 and 6.0% of CT of diet dry matter) × three different runs) plus three bottles as blanks (rumen fluid only) were incubated for 96 h.

Total Biogas, CH₄, CO₂ and H₂ Production

The substrate containing bottles were replicated three times. Bottles were filled, closed using rubber stoppers, mixed, and incubated at 39 °C in water bath. The volume of biogas production (GP) was estimated up to 96 h (2, 4, 6, 8, 10, 12, 24, 36, 48, 60, 72 y 96 h) using a pressure transducer (Extech Instruments, Waltham, U.S.) as per the methodology of Theodorou et al. [18]. Furthermore, CH₄, CO₂, and H₂ amount were estimated at the same times of incubations by gas detector (AIR QUALITY MONITOR YesAIR, Critical Environment Technologies Canada Inc., Delta, BC, V4G 1M3, Canada). The pH was measured after 72 h using a digital pH meter (Conductronic pH15.0, Puebla, Mexico).

Chemical Composition and Tannins Extractions

Diets and *Caesalpinia coriaria* Jacq. Willd. Chemical Analysis

Samples of the diets used as substrates were analysed for dry matter, ash, nitrogen, and ether extract according to AOAC [15]. Ration's contents for neutral detergent fibre content [NDF, 19], acid detergent fibre (ADF) and lignin [15] analyses were carried out using an ANKOM²⁰⁰ Fibre Analyser Unit (ANKOM Technology Corp., Macedon, NY, USA) with the use of an alpha-amylase and sodium sulphite.

Condensed Tannins Extractions and Determination

The technique of Terrill et al. [20] with modifications made by López et al. [21] was used for tannin extraction. Briefly, to extract the free condensed tannins (Free-CT), 1.0 g of the ground fruit sample of *C. coriaria* was weighed and were placed in 50 mL screw-capped centrifuge tubes and extracted three times with a 20 mL mixture of a 0.1% ascorbic acid solution in 7:3 v/v acetone/water; the tubes were stirred with a Vortex for 3 min and were centrifuged at 18,000×g for 15 min. the free condensed tannin was evaluated by colorimetry method.

Extraction of Protein-Bonded Condensed Tannins (Protein-CT)

Solid residue from process of Free-CT was used. 15 mL of a 10 g/L sodium dodecyl sulfate solution; 2-mercaptoethanol, 50 g/L, and 10 mm tris chloride, adjusted to pH 8 with hydrochloric acid, were placed in a boiling water bath for 45 min; the tubes were removed, cooled for 20 min and centrifuged at 18,000×g for 15 min, taking the supernatant to another 50 mL centrifuge tube. This process was repeated, and the combination of supernatants was centrifuged at 18,000×g for 15 min, to eliminate the non-phenolic residues.

Extraction of Fiber-Bonded Condensed Tannins (Fiber-CT)

The non-phenolic residues from protein-CT process was used. About 500 mg of the solid residues, resulting from the extraction of the protein-bonded condensed tannins [P-CT], were weighed in duplicate, placed in the tubes and 1 mL of sodium dodecyl sulfate and 6 mL of butanol-HCL [butanol—hydrochloric acid] were added, placed in a boiling water bath for 75 min, cooled and filtered on rapid filter paper to separate the non-tannin residues, the readings were taken in a spectrophotometer at 550 nm;

in addition, blanks were run with the butanol-HCl solution. Total condensed tannin content [Total-CT] was the sum of the values obtained from free-CT, protein-CT and fiber-CT.

Preparation of Standards

Free condensed tannins from *C. coriaria*. were purified with Hagerman's technique [22]. One gram of *C. coriaria* with 10 mL of an 80: water 20 (v/v) ethanol mixture, were added and left to stand for 12 h at 4 °C and filtered through a Pyrex crucible with a porous glass bottom (40–60 μ) with a vacuum pump. After extraction, the filtrate containing the condensed tannins was lyophilized. The determination of the curve was made with the absorbance values, given by the concentration of *C. coriaria* CT, as leucocyanidin equivalents, considering that the color production of condensed tannins is of 460 absorbance units.

Calculations

To calculate gas production (GP) kinetic parameters, GP results (mL/g dry matter) were fit as per NLIN option of SAS [23], following France et al. [24] equation:

$$A = b \times [1 - e^{-ct-L}] \quad (1)$$

where A = volume of GP at time t; b = asymptotic GP (mL/g dry matter); c = rate of GP (/h), and L (h) = discrete lag time prior to GP.

Statistical Analyses

Collected data were analysed using the linear model procedure in R software. The model fitted to the data was.

$$Y_{ijk} = \mu + D_i + R_j + (D \times R)_{ij} + \varepsilon_{ijk} \quad (2)$$

where Y_{ijk} = observation which was one of the following: total gas, methane (CH₄), carbondioxide (CO₂), and hydrogen (H₂) expressed in mL/g dry matter, μ is the overall mean, D_i is the fixed effect of ith treatment, R_j is the fixed effect of hour, $(D \times R)_{ij}$ is the interaction between treatment and diet, and ε_{ijk} is the random residual error.

For the in vitro gas fractions of CH₄, CO₂, and H₂ (ml/g dry matter), only the effect of treatment was evaluated. All results were expressed as least square mean ± SEM (standard error of mean). The differences were declared significant at $P \leq 0.05$.

Fig.1 Rumen total biogas production (mL/g dry matter) of the five total mixed rations in the presence of condensed tannins (CT) of *C. coriaria* at 0 (CT0), 1.5 (CT1.5), 3.0 (CT3), 4.5 (CT4.5) and 6.0 (CT6) % of the total mixed ration. SEM (Pooled)=2.784; $P < 0.001$

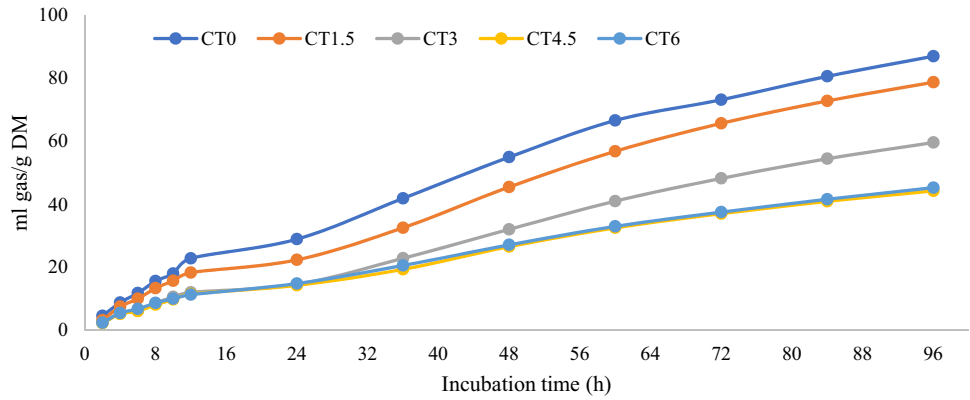


Fig.2 Rumen methane production (mL CH₄/g dry matter) of the five total mixed rations in the presence of condensed tannins (CT) of *C. coriaria* at 0 (CT0), 1.5 (CT1.5), 3.0 (CT3), 4.5 (CT4.5) and 6.0 (CT6) % of the total mixed ration. SEM (Pooled)=3.346; $P < 0.001$

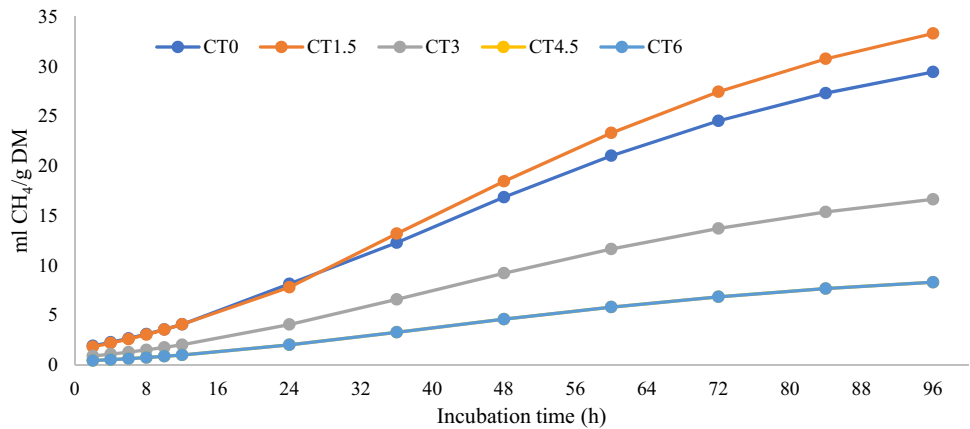


Fig.3 Rumen CO₂ production (mL CO₂/g dry matter) of the five total mixed rations in the presence of condensed tannins (CT) of *C. coriaria* at 0 (CT0), 1.5 (CT1.5), 3.0 (CT3), 4.5 (CT4.5) and 6.0 (CT6) % of the total mixed ration. SEM (Pooled)=1.518; $P < 0.001$

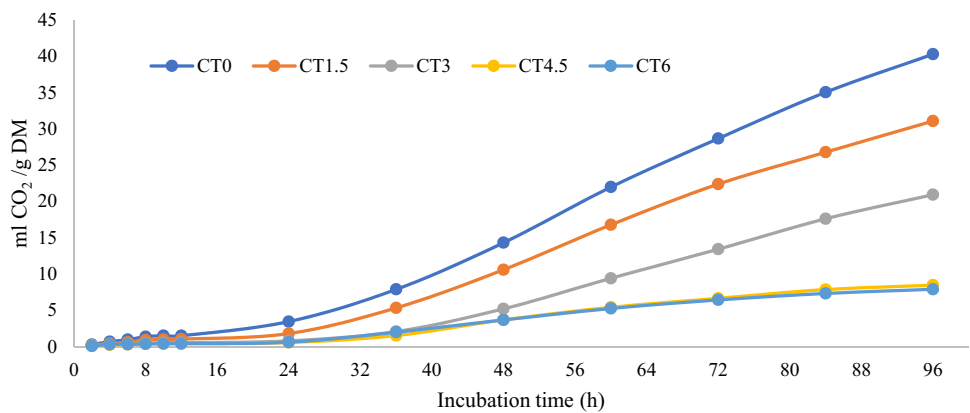
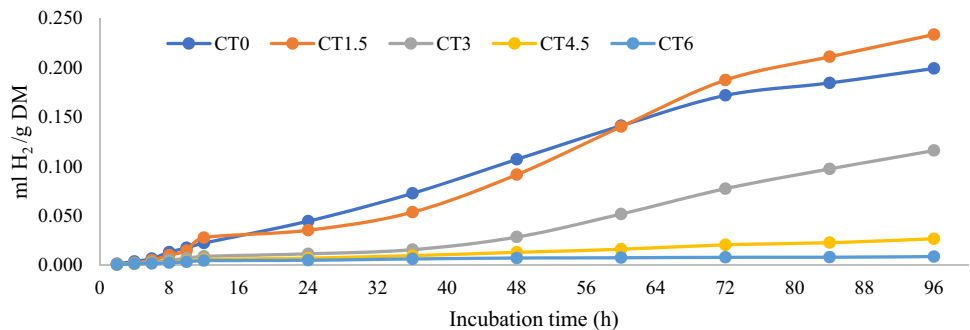


Fig.4 Rumen H₂ production (mL H₂/g dry matter) of the five total mixed rations in the presence of condensed tannins (CT) of *C. coriaria* at 0 (CT0), 1.5 (CT1.5), 3.0 (CT3), 4.5 (CT4.5) and 6.0 (CT6) % of the total mixed ration. SEM (Pooled)=0.018; $P < 0.001$



Results

Total Biogas, Methane, Carbon Dioxide and Hydrogen Production

Figures show trends for in vitro rumen total biogas (Fig. 1), methane (Fig. 2), CO₂ (Fig. 3) and H₂ (Fig. 4) on graded level of *C. coriaria* CT. Overall total gas production showed that the control had the highest production while CT4.5 produced the lowest gas. Among the greenhouse gases, CT1.5 had highest methane and hydrogen production, whereas CT0 produced the most CO₂ while CT6 had the lowest greenhouse gas. Table 2 shows the influence of treatment ($P=0.001$), time ($P=0.001$) and treatment x time ($P=0.001$) interaction on various gas production parameters. Across all treatments (CT0, CT1.5, CT3, CT4.5 and CT6.0), there was a linear ($P=0.001$) decrease in total biogas production. Furthermore, greenhouse gases (CH₄ and CO₂ and H₂) linearly ($P=0.001$) decreased across treatments with exception to CT1.5 which increased.

Results of Table 2 showed that total biogas, CH₄, CO₂ and H₂ production linearly ($P=0.001$) increased at 8 h, 24 h, 48 h, 72 h, and 96 h into the experiment. Suggesting that at each measuring hour all gases were increasingly produced. Treatment x hours interaction showed that 8 h and 96 h production of (total biogas, CH₄, CO₂ and H₂) were the lowest and highest ($P=0.001$), respectively for each treatment, and gases production increased linearly ($P=0.001$).

Fractions of Biogas, Methane, Carbon Dioxide and Hydrogen Production

Treatments of CT0, CT1.5, CT3, CT4.5 and CT6 had (linear, quadratic and cubic; $P=0.001$) effect on asymptomatic gas production of total biogases, CH₄, CO₂ and H₂. Total biogas production increased ($P=0.001$) except in CT4.5 and CT6 which decreased while the rate of gas production decreased linearly ($P=0.001$) with increasing dosage of CT. The CO₂ decreased ($P=0.001$) in a dose-dependent manner of CT inclusion. The CH₄ and H₂ gasses decreased linearly ($P=0.001$) with increasing concentration of CT, but, CT1.5 had the highest asymptomatic CH₄ and H₂ production (Table 3).

Discussion

Ruminant production continues to come under intense scrutiny due to its contribution to greenhouse gasses –CH₄ and nitrous oxide especially. Therefore, it is imperative to find enviro-friendly additives that can be added to animal diets

Table 2 Rumen biogases of methane, carbon dioxide and hydrogen (ml/g dry matter) production of diets included different levels of condensed tannins concentrations from fruit of the cascalote (*Caesalpinia coriaria* (Jacq.) Willd.

Treatment	Hour	Total biogas	CH ₄	CO ₂	H ₂	
CT0	8	15.63	2.97	1.36	0.01	
	24	28.95	6.77	3.45	0.04	
	48	54.98	14.44	14.32	0.11	
	72	73.20	24.34	28.67	0.17	
	96	87.03	29.36	40.30	0.20	
	SEM ^a	13.301	5.020	7.461	0.042	
	P value:					
	Linear	0.000	0.001	0.001	0.001	
	Quadratic	0.206	0.948	0.136	0.743	
	Cubic	0.774	0.815	0.329	0.684	
CT1.5	8	13.37	1.60	0.90	0.01	
	24	22.38	3.37	1.79	0.04	
	48	45.50	9.88	10.59	0.09	
	72	65.71	28.99	22.40	0.19	
	96	78.77	40.11	31.08	0.23	
	SEM ^a	12.40	7.62	5.91	0.043	
	P value:					
	Linear	0.001	0.001	0.001	0.001	
	Quadratic	0.163	0.036	0.001	0.470	
	Cubic	0.218	0.260	0.002	0.212	
CT3	8	8.61	0.94	0.47	0.00	
	24	14.63	1.53	0.79	0.01	
	48	32.06	3.78	5.20	0.03	
	72	48.23	13.11	13.43	0.08	
	96	59.64	22.78	20.94	0.12	
	SEM ^a	9.701	4.212	4.001	0.0211	
	P value:					
	Linear	0.001	0.001	0.001	0.001	
	Quadratic	0.904	0.003	0.019	0.011	
	Cubic	0.184	0.847	0.422	0.494	
CT4.5	8	8.13	0.57	0.37	0.00	
	24	14.29	1.30	0.57	0.01	
	48	26.57	2.66	3.69	0.01	
	72	37.04	3.89	6.65	0.02	
	96	44.28	5.26	8.47	0.03	
	SEM ^a	6.811	0.801	1.610	0.004	
	P value:					
	Linear	0.001	0.001	0.001	0.001	
	Quadratic	0.050	0.805	0.551	0.894	
	Cubic	0.092	0.951	0.005	0.875	
CT6	8	8.71	0.59	0.37	0.00	
	24	14.85	1.11	0.61	0.00	
	48	27.14	2.32	3.67	0.01	
	72	37.52	3.29	6.43	0.01	

Table 2 (continued)

Treatment	Hour	Total biogas	CH ₄	CO ₂	H ₂
	96	45.28	4.42	7.90	0.01
	SEM ^a	6.810	0.721	1.513	0.001
	P value:				
	Linear	0.001	0.001	0.003	0.002
	Quad-ratic	0.011	0.728	0.001	0.089
	Cubic	0.188	0.834	0.001	0.231
SEM (Pooled) ^a		2.111	0.303	0.113	0.015
	P value				
Treat.					
	Linear	0.001	0.001	0.001	0.001
	Quadratic	0.404	0.905	0.369	0.001
	Cubic	0.771	0.131	0.186	0.095
Hour					
	Linear	0.001	0.001	0.001	0.001
	Quadratic	0.307	0.251	0.098	0.771
	Cubic	0.702	0.601	0.230	0.553
Treat × Hour		0.001	0.001	0.001	0.001

Dietary condensed tannins (CT) of *C. coriaria* included at 0 (CT0), 1.5 (CT1.5), 3 (CT3), 4.5 (CT4.5) and 6 (CT6) % of the total mixed ration

^aStandard error of mean

reduce greenhouse gasses produced without compromising the rumen fermentation ability, digestibility, and production performance. Phytogetic feed additives rich in plant secondary metabolites has the ability to aid digestion or to reduce digestion depending on its effect. Furthermore, in the tropical area, which is characterized by poor quality forages,

in vitro assessment helps to give potential assessment of feed additives in the rumen ecosystem whether it promotes rumen health or not. Successful evaluation of these trees will go a long way to show if it can be integrated with rangelands, or as additives in livestock diets.

Biogas Production

Plant additives rich in secondary metabolites can help to improve animals’ nutrient digestion and availability because their metabolites can enhance rumen activity [25]. Inclusion of condensed tannin at moderate level has the tendency to improve amino acid supply to the intestine or protect the amino acid from being deaminated/degraded [26]. At high concentration however, they can decrease rumen microbial activity and inhibit endogenous digestive enzyme activities [9]. However, CT showed that there was no inhibition of digestive activity as the biogases continued to increase steadily. This suggests that irrespective of concentration, *C. coriaria* CT has the potential to have a favourable impact on digestion for a prolong period of time and improve rumen microbial activity. It suggests that if fed to ruminant, it could be effective in providing stable rumen environment to favor continuous activity of microbial digestion. The gasses produced among the CT concentrations reduced at dose-dependent rate. This may be attributed to the negative effect of higher *C. coriaria* CT concentration. This suggest that at higher concentration, CT impeded the microbials activity or function or perhaps reduced the enzymatic activity of the microbes which affected the degradation function compared to the control. It is well-known that gas production volume commensurate with the rate of feed degradation; therefore,

Table 3 Rumen biogas fractions^a of methane, carbon dioxide and hydrogen (ml/g dry matter) production of diets included different levels of condensed tannins (CT) concentrations from fruit of the cascalote (*Caesalpinia coriaria* (Jacq.) Willd.

	Total biogas			CH ₄			CO ₂			H ₂		
	<i>b</i>	<i>c</i>	<i>Lag</i>	<i>b</i>	<i>c</i>	<i>Lag</i>	<i>b</i>	<i>c</i>	<i>Lag</i>	<i>b</i>	<i>c</i>	<i>Lag</i>
CT0	65.0	0.08	3.85	35.0	0.03	3.05	42.5	0.03	6.10	0.24	0.04	10.76
CT1.5	65.0	0.06	3.10	40.0	0.01	3.00	32.5	0.01	5.22	0.34	0.03	10.26
CT3	67.5	0.03	3.18	20.0	0.02	3.08	20.0	0.05	4.11	0.27	0.02	15.51
CT4.5	50.0	0.01	2.85	10.0	0.05	3.02	10.0	0.08	4.90	0.05	0.03	7.41
CT6	52.5	0.02	2.10	10.0	0.07	3.09	10.0	0.04	5.81	0.01	0.12	10.58
SEM (Pool) ^b	2.41	0.011	0.91	7.40	0.001	0.640	3.03	0.005	0.341	0.040	0.021	2.980
P value												
Treat.												
	Linear	0.001	0.026	0.314	0.001	0.158	0.001	0.158	0.108	0.001	0.001	0.001
	Quadratic	0.001	0.518	0.180	0.001	0.232	0.001	0.232	0.331	0.001	0.001	0.001
	Cubic	0.001	0.052	0.294	0.001	0.479	0.001	0.001	0.479	0.580	0.001	0.001

Dietary condensed tannins (CT) of *C. coriaria* included at 0 (CT0), 1.5 (CT1.5), 3 (CT3), 4.5 (CT4.5) and 6 (CT6) % of the total mixed ration

^a*b* is the asymptotic gas production (mL/g dry matter); *c* is the rate of gas production (/h); *Lag* is the initial delay before gas production begins (h)

^bStandard error of mean

lower gases production with increasing CT suggest that it created unhealthy condition either by nutrient binding or microbial growth inhibition through toxicity (10, 27). Plant containing condensed tannins are effective in reducing the total in vitro gas [27]. Among the fractional rate of gas production, some CT concentration improved digestion. Higher fractional and rate of GP suggest higher digestion and the growth of the microorganisms, and enzymatic activity [28]. Therefore, CT improved fractional GP until CT3.0 before it started decreasing despite the linear decrease in rate of gas production. This indicate that at moderate level, *C. coriaria* CT has the potential to improve feed digestion without compromising microbial activities. Lower GP at higher level of CT suggest that CT can have negative effect on feed digestion or on rumen environment if consumed at higher quantity.

Methane, Carbon Dioxide and Hydrogen Production

Biogenic methane and CO₂ are enteric fermentation byproducts and important greenhouse gasses because they contribute to global warming. The gases also increase loss of net feed energy from the animal [29, 30]. Furthermore, the H₂ synergizes with rumen CO₂ to form CH₄ by methanogens. Therefore, a reduction in H₂ level or creating an alternative sink for H₂ might reduce methane emissions [31]. Although, there was a progressive increase in cumulative gasses production, application of *C. coriaria* CT decreased the CH₄, CO₂ and H₂ gas production with increasing level of CT. The decrease may be attributed to the reduction in digestion which is indicated through lower gas production with increasing CT inclusion. However, the fraction of these gases showed that CH₄, CO₂ and H₂ decreased linearly with increasing CT inclusion. The reason for the decrease in CH₄ might be attributed to the decrease in H₂ or CO₂ production which are important gases for methane production. It is reported that some feed additives are capable of competing and metabolizing H₂ for other uses thereby preventing methanogens from using them for methane [32]. As such, it could be that the CT created an alternative form of H₂ sink/utilization which reduced availability or prevented methanogen from utilizing H₂. The reduction in lag time for CH₄ production by all CT treatments indicate that methane production was delayed compared to the CT0 which had the highest lag time, suggesting that the methanogens or methanogenesis processes was delayed by CT inclusion. Lower proportional CH₄ production are evidences that the additives were effective in decreasing CH₄ emission through decline in ruminal methanogenic and gram-positive hydrogen producing bacteria and consequently CH₄ emission [33, 34]. These effects may be attributed to the antimethanogenic activity associated with condensed tannins [27, 35]. The reduction in CH₄ emission could potentially increase body weight gain and

milk production in ruminant [36]. It is surprising that despite linear decrease in H₂ production, there was linear increase in the lag time of H₂ at lower CT concentrations while at higher concentrations the lag time reduced. The reason for this is not known. In tropical areas characterized with low quality forage diet, which supports more greenhouse gas emission, inclusion of *C. coriaria* CT in the concentrate diet or planting it on grazing field in a silvopastoral system could help to reduce greenhouse gas emission in small ruminant. Apart from the its potential as antimethanogenic, the nitrogen fixing ability of this plant suggest that it could be planted on the field and help fix nitrogen in soil. The tree like nature of this plant indicates that it can provide shade for animals in silvopastoral system to help reduce heat stress in grazing animals.

Conclusions

Inclusion of CT from *C. coriaria* at different doses had varying effect on gas production, and from the environmental point of view, more dietary CT inclusion associated with more decreased of greenhouse gas emitted. But nutritionally, increasing CT level improves gas production, to a certain extent before decreasing biogas production. Thus, animal nutrition is not only about the reduction of greenhouse gas emission, but also about efficient feed digestion. From a balanced perspective, inclusion of CT3, appears to be environmentally friendly and nutritionally efficient.

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Compliance with Ethical Standards

Conflict of interest The authors declare that there to be no competing interests.

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