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Gas Emission from Waste of Cows Fed Monensin and *Acacia mearnsii* Tannins

Research Article

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ABSTRACT

The study aimed to evaluate the effect of combined use of tannins and monensin on biogas production from waste of Nellore cows fed these additives through biodigesters as a way to improve animal waste management. Eight cows were arranged in 2 contemporary 4 × 4 latin squares design and received 8 diets that differed in the level of tannin inclusion (0.00, 0.75, 1.50, and 2.25% DM) and inclusion or not of monensin. Monensin was daily administered to each cow in one square (32 mg/kg DM). Faeces and urine were collected for anaerobic biodigestion. Experimental batch-type biodigesters were arranged in a completely randomised design, in a 2 × 4 factorial arrangement of 8 treatments with 4 replicates. The data were submitted to statistical analysis system. Monensin did not affect total biogas production ($P>0.05$) but reduced CO_2 production by 18.90%. Tannins had a quadratic effect on total solids (TS) biodigestion efficiency, but biodigestion efficiency for volatile solids (VS) and nitrogen linearly reduced ($P<0.05$). Tannins had a quadratic effect on total biogas and CH_4 production (at 2.25% the total biogas and CH_4 production reduced by 36.95 and 36.10%, respectively) and linearly reduced the production of CO_2 ($P<0.05$). Antagonistic interactions between monensin and tannins were observed on TS and VS recovery and VS biodigestion efficiency, where monensin reduced the effect of tannins of reducing the VS or TS biodigestion. Therefore, monensin and tannins may be used to reduce the emission of greenhouse gases from cattle waste when tannins are included above 0.75%. There is a strong evidence that monensin and tannins or their bioactive metabolites may appear in faeces (when used to feed cows) and impair the biodigestion of the waste, but further studies should be carried out to confirm this finding.

KEY WORDS anaerobic biodigestion, biodigestion efficiency, carbon dioxide, feeding additives, methane.

INTRODUCTION

The intensification of animal production and increased size of animal production units are now trends in livestock activity worldwide, representing a considerable pollution hazard through accumulation of high amounts of animal waste (Holm-Nielsen *et al*. 2009). The main emissions within the farms include enteric CH_4 and CH_4 from housing facilities during long-term storage (Rotz, 2017). Although the concentration of CH_4 in the atmosphere is lower than that of CO_2, CH_4 has a heating potential 25 times more than that of CO_2 (IPCC, 2007). The global emission of greenhouse gases (GHG) from manure grew between 1961 and 2010 from 0.57 to 0.99 gigatonnes of carbon dioxide equivalent

($GtCO_2eq$) per year. On average, emissions grew by 1.10% per year (IPCC, 2014), but despite these data, Lynch (2019) concluded that there are still insufficient data available to fully address important questions regarding the climate impacts of agricultural production.

The handling and use of manure on livestock farms contributes to emissions of GHG (Petersen, 2018). Comparing gas emissions from two typical manure handling options at cattle feedlots (composting and static stockpile storage), Bai *et al.* (2020) found that composting inhibits CH_4 emissions but promotes NH_3 and N_2O emissions. Certainly, the efficient treatment of animal waste can support environmental protection in addition to bioenergy management (Achinas *et al.* 2018). Anaerobic digestion is a biological process that can convert organic substrates to biogas (Zhang *et al.* 2016). It is characterised by reactions in which biogas is produced from biodegradable products in the absence of oxygen (Neshat *et al.* 2017). Anaerobic digestion is increasingly used worldwide to generate energy from biogas and brings significant economic and environmental benefits (Scarlat *et al.* 2018) by being an efficient alternative technology that combines biofuel production with waste management (Achinas *et al.* 2017).

Biogas is mainly comprised of CH_4 and CO_2 and minor amounts of other gases, such as nitrogen, hydrogen sulfide, ammonia and water vapour (Neshat *et al.* 2017).

Monensin and tannins, separately, have shown to reduce enteric CH_4 emission from ruminants (Russell and Houlihan, 2003; Aboagye *et al.* 2019; Stewart *et al.* 2019). Monensin's mode of action is by reducing Gram-positive microorganisms, the major producers of methanogenic substrates as the final fermentation products (CO_2, acetate, hydrogen, etc.) (Russell and Houlihan, 2003). There are three major forms by which tannins reduce enteric CH_4 emission. The first is by reducing methanogenic *Archaea*; the second is through reduction of *Archaea* associated rumen protozoa, and the third is through depression of fibre digestion in the rumen (Carrasco *et al.* 2017; Tseu *et al.* 2020).

Methane production from enteric fermentation of ruminants generates feed gross energy losses ranging from 2 to 15% (Johnson and Johnson, 1995; Wanapat *et al.* 2015). Thus, the effect of monensin and tannins on reducing CH_4 production contributes to enhance feed energy efficiency. As seen above, the reduction in CH_4 production by tannins is highly linked to reduction of fibre digestibility, so, according to Patra and Saxena (2011), the reduction in CH_4 production compensates the loss of feed energy only if the tannin content in the diet is low or moderate (usually less than 50 g/kg DM), otherwise the loss of nutrients in faeces may be of high magnitude. In addition to reduce fibre digestion, tannins may also reduce protein digestibility (Tseu *et al.* 2020) by forming complexes with these macromolecules and make them inaccessible to microbial and enzymatic digestion (Nigrant *et al.* 2017). Therefore, the decreased nutrient digestibility is expected to increase fermentable organic matter (referenced in this study as volatile solids) concentration in faeces, which can promote a great anaerobic biodigestion for biogas production including CH_4 (Hristov *et al.* 2013).

Although much is known about the effects of these additives on rumen fermentation, studies reporting their effects on the fermentation of waste from cows (or other kind of ruminants) that have been fed these additives were not found. Hence, the overriding question was whether or not the effect of these additives on the reduction of enteric CH_4 production provides conditions for the emission of GHG from waste.

Given these factors, the hypothesis tested in this study was that the combined use of monensin and tannins to feed cows would increase the production of CH_4 and CO_2 from waste. Therefore, the study aimed to evaluate the effect of combined use of *Acacia mearnsii* tannins and monensin on biogas production from the waste of cows fed these additives by means of anaerobic biodigestion as a way to manage animal waste.

MATERIALS AND METHODS

Ethical issue and place of experimentation

The experiment followed the guidelines established in accordance with the ethical principles of animal experimentation of the commission of ethics in the use of animals of the college of animal science and food engineering of the university of Sao Paulo (USP) under the protocol number CEUA 3080240518. It was carried out at the Animal Nutrition and Production Department of the College of Veterinary Medicine and Animal Science of USP in Brazil.

Treatments and experimental design

The experiment was carried out in two phases, (1) the feeding phase and (2) the anaerobic digestion phase.

In the first phase (the feeding phase), eight Nellore cows, non-pregnant and non-lactating, carrying a rumen cannula and having a mean body weight of 582 kg (±96), were kept in a roofed shed in individual pens with free access to water, feed and sand bedding. They were distributed in two contemporary 4 × 4 Latin squares design (LSD) in a 2 × 4 factorial arrangement and received eight experimental diets which differed in the levels of tannin inclusion (0.00, 0.75, 1.50 and 2.25% of feed DM) and the inclusion or not of monensin (Rumensin® 200, Elanco Animal Health, Brazil), i.e. no monensin was administered in the second square, but tannins at 0.00, 0.75, 1.50 and 2.25%; in addition to receiving tannins (at the same levels) in the first square, the cows also received monensin (300 mg/day, about 32 mg/kg DM).

Kaolin was added as the tannin level decreased from 2.25 to 0.00% to equalise the DM in all treatments.

The tannins, from a commercial extract, were obtained from the bark of *A. mearnsii* (Seta Natur®-Seta Acacia Tannin Extract). The concentration of total phenols (84.40%) was determined by the Folin-Ciocalteau method (Makkar, 2003a), and total tannins (82.30% tannic acid equivalent) were estimated by the difference in total phenol concentration before and after treatment with insoluble polyvinylpolypyrrolidone (Makkar *et al*. 1993). The concentration of condensed tannins (32.30% leucocyanidine equivalent) was determined by the HCl-butanol method (Makkar, 2003a). The feed was offered at 8 a.m. and 4 pm. in the form of total mixed ration with a ratio of 50% of corn silage and 50% of concentrate. The feed consumption was *ad libitum*. The proportions of ingredients and the chemical composition of the diets are shown in Table 1.

The feeding phase was carried out in 4 periods of 24 days each, but the last two days of each period the cows spent together in pasture. The first 16 days were to adapt the animals to the diets, and between days 17 and 21 the collection of faeces for anaerobic digestion phase was performed. The collection of faeces was performed twice a day (8 am. and 4 pm.) by hand. All the faeces corresponding to the same cow per period were mixed and frozen in a single bag until biogas tests were conducted. On day 24 of each period urine was collected every 6 hours (at 6 am., 12 p.m., 6 pm. and 12 am.), obtained either during spontaneous urination or stimulation by vulva massage. All the urine corresponding to the same cow per period was mixed and frozen in a single flask until biogas tests were conducted.

In the second phase (the anaerobic digestion phase), the 32 samples of faeces and 32 samples of urine collected and frozen during the feeding phase, i.e. samples from 4 × 4 latin square design with 8 treatments (tannin inclusion levels of 0.00, 0.75, 1.50 and 2.25% in both squares and 32 mg of monensin per kg DM for each cow only in the first square), were thawed and diluted in water. Firstly, a mixture of faeces and urine (waste) was prepared by using a theoretical ratio of 83:17%, respectively. Then, this mixture was diluted in water, and finally, the inoculum was added, composing a substrate. Hence, the substrate composition was as follows: 40% of waste, 3.30% of inoculum and 56.70% of water. The inoculum was sewage sludge from waste treatment and had 0.16% total solids (TS). Accordingly, the substrates were prepared to ensure an estimation of 6% of TS as per Lucas Junior *et al*. (1993), who found better biogas production in batch-type biodigesters when the TS content of the substrate was less than 8%.

Batch-type biodigesters were used (Figure 1), and 3 kg of substrate were prepared, 2 kg of which were used to fill the biodigesters and 1 kg to perform the characterisation analyses of the substrate (Table 2). The biodigesters were arranged in a completely randomised design (CRD) in a 2 × 4 factorial arrangement of 8 treatments with 4 replicates, totalling 32 experimental units (represented by faeces and urine of the animals which received the different levels of tannins and the inclusion or not of monensin in the diet). After filling, the biodigesters were conditioned in a climate chamber with controlled temperature (33±2 ˚C) by electric resistance heating system and digital temperature recorder to guarantee that the test occurred in mesophilic conditions, ideal for digestion kinetics (Metcalf and Eddy, 2014). The temperature was monitored through a digital thermometer (in ˚C), and the readings and records were made immediately before the biogas reading. The composition of the substrates in the different biodigesters is shown in Table 2.

Quantitative production of biogas through biodigesters

The batch-type biodigesters consisted of three straight cylinders with diameters of 15, 10 and 7.50 cm, with a mean capacity to ferment 2 litres of substrate each (Figure 1). The 15 and 7.50 cm cylinders were inserted one inside the other so that the space between the outer wall of the inner cylinder and the inner wall of the outer cylinder contained a volume of water (water seal), reaching the depth of 60 cm. The cylinder of intermediate diameter (gas meter) had one of the ends sealed to retain a record for biogas discharge while it was capsized in the water seal to provide anaerobic conditions and to store the produced gas.

The reading of biogas production was performed according to the accumulation in the gas meter. It consisted of the height measured by the measuring tape attached to the gas meter according to the vertical displacement. The reading value was multiplied by the internal cross-sectional area of the gas meter. After each reading, the gas meters were emptied by using the biogas discharge register. The correction of the biogas volume for the conditions of 1 atm at 20 ˚C was carried out according to the methodology described by Lucas Junior (1994).

The correction of the biogas volume was performed through the expression resulting from the combination of the laws of Boyle and Gay-Lussac:

$$(V_0P_0) / T_0 = (V_1P_1) / T_1 \quad (1)$$

Where:

V_0: corrected biogas volume, m^3 or L.

P_0: corrected biogas pressure, 10322.27 mm H_2O.

T_0: corrected biogas temperature, 293.15 K.

V_1: gas volume in the gas meter.

P_1: biogas pressure at the time of reading, 10344.11 mm H_2O.

T_1: biogas temperature, in K, at the time of reading.

Table 1 Proportions of ingredients and chemical composition of experimental diets

Ingredients (% dry matter, DM)	Tannin level (% feed DM)			
	0.00	0.75	1.50	2.25
Corn silage	50.00	50.00	50.00	50.00
Dry ground corn grain	32.36	32.36	32.36	32.36
Soybean meal	12.40	12.40	12.40	12.40
White salt	0.50	0.50	0.50	0.50
Mineral mixture[1]	2.00	2.00	2.00	2.00
Tannin extract[2]	0.00	0.91	1.82	2.74
Kaolin	2.74	1.82	0.91	0.00
Chemical composition of the diet for all tannin levels				
Dry matter (%)	60.35			
Crude protein (CP, % DM)	14.43			
Ruminally degradable protein[4] (% CP)	65.30			
Ruminally undegradable protein[4] (% CP)	34.70			
Neutral detergent fibre[3] (% DM)	28.06			
Effective neutral detergent fibre[4] (% DM)	24.47			
Acid detergent fibre[3] (% DM)	15.41			
Non-fibre carbohydrates[3] (% DM)	47.59			
Starch[4] (% DM)	42.58			
Ashes[3] (% DM)	6.73			
Calcium[3] (% DM)	0.69			
Phosphorus[3] (% DM)	0.40			
Ether extract[3] (% DM)	3.19			
Net energy for lactation[4] (Mcal/kg DM)	1.50			

[1] Mineral mixture, quantity per kg of product: calcium: 140 g; Phosphorus: 80 g; Sulfur: 10 g; Sodium: 129 g; Cobalt: 80 mg; Copper: 1400 mg; Fluorine: 800 mg; Iodine: 80 mg; Manganese: 1 g; Selenium: 20 mg and Zinc: 3.50 g.
[2] Extract of *Acacia mearnsii* with 82.30% of total tannins, of which 32.30% of condensed tannins.
[3] Determined through chemical analysis (non-fibre carbohydrates (NFC)=100–(% NDF+% CP+% EE+% Ash)).
[4] Estimated by the spartan dairy ration evaluator/balancer software, version 3.0.3.

Table 2 Composition of substrates of anaerobic batch-type biodigesters supplied with the waste of nellore cows fed monensin (mg/kg DM) and different levels of tannins of *A. mearnsii*

Variable	Monensin (M)		Tannin level (TL, % feed DM)				SEM	P-value		
	0.00	32.00	0.00	0.75	1.50	2.25		M	TL	M × TL
TS (g/kg)	48.20	47.88	48.32	47.77	48.58	47.48	0.68	NS	NS	NS
VS (g/kg)	40.79	40.19	38.48	39.65	41.83	42.01	0.66	NS	0.0464^{L}	NS
N (g/kg TS)	34.11	36.49	34.17	34.74	35.55	36.75	1.00	NS	NS	NS
NDF (g/kg TS)	386.20	423.20	388.00	395.60	392.10	443.20	7.87	NS	0.0105^{L}	NS
pH	6.45	6.52	6.33	6.37	6.51	6.73	0.05	NS	0.0010^{L}	NS

TS: total solids; VS: volatile solids; N: nitrogen and NDF: neutral detergent fibre.
L: linear effect and NS: non-significant.
SEM: standard error of the means.

Considering the average atmospheric pressure of Pirassununga (Sao Paulo–Brazil) equal to 10273.11 mm H_2O and the pressure conferred by the gas meters of 71 mm H_2O, the following expression was obtained to correct the biogas volume:

$$V_0 = (V_1/T_1) \times 293.7703 \quad (2)$$

Biogas sampling was performed whenever the biogas volume was measured.

Samples were collected by using a 60 mL syringe connected to the gas register at the top of the gas meter. Then 50 mL of biogas, for analysis, were injected in collecting flasks (glass flasks of 50 mL of capacity, Frascolex, Sao Paulo, Brazil). The gas meters were then emptied to allow a new accumulation of gas. The test was terminated when the biogas production ceased, i.e. there was no more displacement of the gas meter.

The concentration of CH_4 and CO_2 was determined by gas chromatography (Trace 1300, Thermo Fisher Scien-

tific®, Rodano, Milan, Italy) in controlled temperature (25 ˚C) according to Kaminski *et al.* (2003).

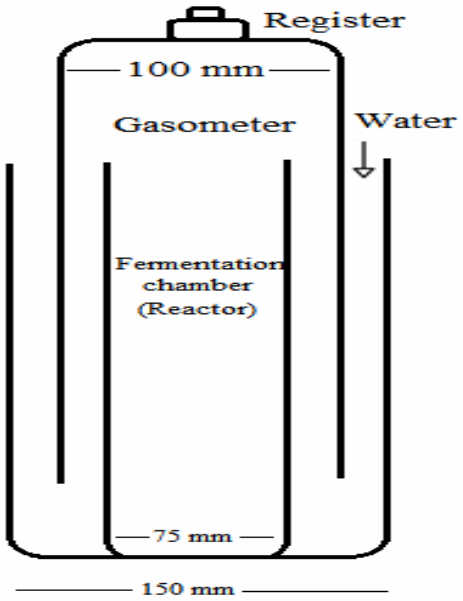

Figure 1 Diagram showing the batch-type biodigester design

The biogas samples were diluted in glass flasks, with a known volume, 16.78 times in atmospheric air. Then, 6 mL were injected into the chromatograph injector (split/splitless), 4 mL of which were used to wash the injection system and 2 mL were used for analysis. One (1) mL was also used for the system with a flame ionisation detector (FID), responsible for the measurement of CO_2 and CH_4.

The chromatograph was calibrated with 3.10% CH_4 and 3.10% CO_2 that was diluted in atmospheric air. One gaseous mixture was used as a reference, one with (50% CH_4 to 50% CO_2) in balance with helium (He) (mol/mol). Helium with a flow rate of 30 mL/min was used as the dragging gas. The volumes of CH_4 and CO_2 produced (m^3 or L) were calculated using the production data and biogas composition of each digester according to the equation:

$$Vol = (Vol_{BIOGAS} \times \% \text{ Gas}) / 100 \quad (3)$$

Where:
Vol: volume (m^3 or L).
Vol_{BIOGAS}: volume of biogas produced (m^3 or L).
% Gas: content of gas of interest in biogas.

The production of CH_4 or CO_2 was calculated by dividing the total production of each gas by the amount of VS added or removed (the difference between VS added in the filling time of the biodigesters and VS eliminated during the fermentation).

The Gompertz model was used to study the biogas production kinetics and its components. The model assumes that the gas production rate is proportional to the microbial activity, but the proportionality decreases with the incubation time which can be interpreted as a loss of efficiency in the fermentation rate (Lavrencic *et al.* 1997).

The mathematical description of the gas production curves allowed the data analysis, the substrate comparison, and the performance of the fermentation. The following equation describes the model used:

$$Y_t = A \exp [-B \exp (-kt)] \quad (4)$$

Where:
Y_t: gas production (L/g VS added) at time t (days).
A: asymptote of the model, indicating the stabilisation value of the production (L/g VS added) in relation to time t.
B: integration constant, with no biological meaning.
kt: growth rate, logarithmic function of the production growth (L/g VS added) per unit of time.

The time (t) at inflection point was determined as follows:

$$t_1 = \ln B / k \quad (5)$$

Where:
t_1: time (days) at inflection point (inflection point is the point at which the production rate is maximum and after which production tends to stabilise).
ln: natural logarithm.
B: integration constant.
k: production constant.

The gas production at inflection point was determined as:

$$y_1 = A / \exp \quad (6)$$

Where:
y_1: gas production at the inflection point.
A: asymptotic gas production.
exp: base of natural logarithm (2.7183).

Nutrient removal

The substrates added (before biodigestion) and recovered residue (after biodigestion) in each biodigester were weighed and multiplied by their DM content in percentage to calculate the DM content in grams. The added or recovered nutrients, expressed in grams, were calculated by multiplying between the added or recovered, and expressed as grams of DM, then were expressed as a percentage and divided by 100 according to the following equation:

Nutrient (g)= (added or eliminated / bio digested nutrient (%) × DM) / 100

The nutrient removal, in percentage, was calculated by using the added and recovered nutrient content and expressed in g/kg of DM according to the following equation:

Removed nutrient (%)= (added nutrient (g)–recovered (g)/added nutrient (g)) × 100

Laboratory analysis

The samples of the substrates before and after anaerobic digestion were collected and dried in an oven with ventilation and constant air renewal at 65 ˚C for 72 hours, according to AOAC (1995).

Then, they were milled with wily-type knives in 1 mm sieves and stored in properly sealed vials. The DM was determined at 105 ˚C for 16 hours in the oven (method 930.15; AOAC, 1995). The mineral matter (MM) was obtained by calcination in a muffle oven at 550 ˚C for 5 hours (AOAC, 1990).

The TS (TS=100-humidity) and VS (VS=TS-MM) contents of the substrates were determined with adaptations to the methodology described in APHA (2005). The total nitrogen (N) content was determined by the micro-Kjeldahl technique (method 920.87; AOAC, 1990). The Neutral detergent fibre (NDF) was determined by the method described by Van Soest *et al*. (1991). The hydrogen ion potential (pH) was measured by portable pH meter (Hanna Instruments®, HI 8424, Italy).

Statistical analysis

Biogas production was obtained in each biodigester by biogas measurement for about 6 months (175 days). The frequency of biogas measurement was performed following gasometer capacity and speed of gas production. For this reason, production and time for filling were considered as variables over time, not allowing to perform statistical analysis in repeated measurement. In this way, gas production over time was used to run Gompertz model using nonlinear procedures (PROC NLIN) in the SAS software (SAS, 2013). Gompertz model provided not only total gas production, but many other information such as growth rate and inflection point. The data obtained in Gompertz model, as well as other data, were all analysed by using SAS. Before the data were analysed, they were evaluated in relation to the presence of discrepant information (outliers) and normality of the residues by the Shapiro-Wilk test. When the normality premise was not met, the data were transformed. They were next submitted to analysis of variance, which separated, as causes of variation, the monensin effect, tannin level effect, and the interaction between monensin and tannin level (all as fixed effects). The tannin level effect was evaluated by the use of orthogonal polynomials separating the effects in linear, quadratic, and quadratic deviation. A significance level of 5% was adopted. The statistical model used was described according to the equation below:

$$Y_{ijkl} = \mu + M_i + TL_j + M_i \times TL_j + e_{ijkl}$$

Where:

Y_{ijkl}: observation concerning monensin (i) + tannin level (j) + monensin (i) × tannin level (j) + random error associated with each observation (e_{ijkl}).

μ: overall mean.

M_i: effect of monensin (fixed effect).

TL_j: tannin level effect (fixed effect).

$M_i \times TL_j$: interaction between monensin (i) and the tannin level (j) (fixed effect).

e_{ijkl}: random error associated with each observation.

RESULTS AND DISCUSSION

Biodigestion and nutrient removal efficiency

There were no significant differences ($P>0.05$) in the amount of TS, VS or nitrogen (N) on the substrates (i.e. initial TS, VS and N) corresponding to the different treatments (Table 3). A significant interaction was observed between monensin and tannins ($P<0.05$) either in the TS or VS recovery (i.e. the amount of nutrients not used or fermented during biodigestation) and VS removal efficiency (Figures 2, 3 and 4, respectively).

Monensin significantly reduced TS and VS removal efficiency by 29.40 and 29.00% (calculated values), respectively, but no significant effect was observed for N. The different levels of tannins had a quadratic effect on the TS recovery and consequently in removal efficiency, but on VS and N the recovery linearly increased and the removal efficiency linearly reduced. The increase in VS and N recovery was 28.30% and 41.80%, respectively, for the highest level of tannins compared to the control treatment. Monensin increased pH substrate during biodigestion, but although tannins linearly increased the substrate pH before (Table 2), it was quadratic during anaerobic digestion.

Biogas production

The theoretical, non-significant biogas production (in general) was observed about 160 days after biodigesters were filled. Therefore, the biodigestion process was interrupted on day 175. No significant interaction ($P>0.05$) was observed between monensin and tannins on biogas production parameters (Table 4). Monensin did not significantly alter the total biogas or CH_4 production, but it reduced CO_2 production (L) by 18.90%.

Table 3 Biodigestion and removal efficiency of nutrients from anaerobic batch-type biodigesters supplied with the waste of cows fed monensin (mg/kg DM) and different levels of tannins of *A. mearnsii*

Variable	Monensin (M)		Tannin level (TL, % feed DM)				SEM	P-value		
	0.00	32.00	0.00	0.75	1.50	2.25		M	TL	M × TL
Added nutrients										
TS (g)	97.91	95.69	98.75	95.12	96.48	96.84	1.36	NS	NS	NS
VS (g)	81.59	79.67	76.97	79.33	82.98	83.24	1.41	NS	NS	NS
N (g)	3.25	3.43	3.26	3.23	3.45	3.44	0.08	NS	NS	NS
Recovered nutrients										
TS (g)	76.37	80.73	78.09	72.03	78.85	85.22	1.96	NS	0.0639^{Q}	0.0433
VS (g)	57.50	62.70	53.95	55.43	61.77	69.24	1.95	0.0709	0.0003^{L}	0.0346
N (g)	2.18	2.20	1.77	2.17	2.32	2.51	0.07	NS	$< 0.0001^{L}$	NS
pH after biodigestion	7.53	7.61	7.67	7.55	7.52	7.55	0.02	0.0122	0.0157^{Q}	NS
Removal efficiency										
TS (%)	22.14	15.64	20.85	24.38	18.43	11.91	1.64	0.0102	0.0398^{Q}	0.0754
VS (%)	29.80	21.16	29.63	30.03	25.71	16.55	2.02	0.0028	0.0009^{L}	0.0061
N (%)	32.72	35.44	45.14	32.45	32.27	26.46	1.85	NS	0.0003^{L}	NS

TS: total solids; VS: volatile solids; N: nitrogen and NDF: neutral detergent fibre.
L: linear effect; Q: Quadratic effect and NS: non-significant.
SEM: standard error of the means.

Table 4 Gas production (total biogas, CH_4 and CO_2) in batch-type biodigesters with the waste of cows fed monensin (mg/kg DM) and different levels of tannins of *A. mearnsii*

Variable	Monensin (M)		Tannin level (TL, % feed DM)				SEM	P-value		
	0.00	32.00	0.00	0.75	1.50	2.25		M	TL	M × TL
Total biogas (L/175 days)	29.50	25.98	30.80	32.22	28.51	19.42	1.42	NS	0.0213^{Q}	NS
CH_4 (L/175 days)	20.85	18.96	21.98	22.78	20.81	14.05	0.97	NS	0.0159^{Q}	NS
CH_4 (% total gas)	72.77	75.36	73.88	73.10	75.43	73.82	0.68	0.0800	NS	NS
CH_4 / faeces (L/g DM)	0.031	0.028	0.033	0.034	0.031	0.021	0.002	NS	0.0160^{Q}	NS
CH_4/added VS[1]										
A (L/g added VS)	0.28	0.25	0.29	0.30	0.27	0.19	0.01	NS	0.0024^{L}	NS
k (L/g added VS.day)	0.037	0.035	0.043	0.038	0.030	0.032	0.002	NS	0.0902^{L}	NS
t (day)	44.96	37.58	41.18	46.77	50.42	26.72	3.14	NS	0.0259^{Q}	NS
y (L/g added VS)	0.10	0.09	0.11	0.11	0.10	0.07	0.01	NS	0.0024^{L}	NS
CH_4 / removed VS (L/g)	0.84	1.35	1.11	1.30	1.07	0.91	0.10	0.0182	NS	NS
CO_2 (L/175 days)	8.65	7.01	8.82	9.44	6.70	5.36	0.49	0.0482	0.0014^{L}	NS
CO_2 (% total gas)	27.22	24.63	26.10	26.88	24.56	26.16	0.68	0.0797	NS	NS
CO_2 / faeces (L/g DM)	0.013	0.011	0.013	0.014	0.012	0.008	0.001	0.0485	0.0014^{L}	NS
CO_2 / added VS										
A (L/g added VS)	0.13	0.09	0.11	0.14	0.11	0.07	0.01	0.0397	0.0389^{L}	NS
k (L/g added VS.day)	0.045	0.035	0.047	0.045	0.037	0.032	0.003	NS	0.0724^{L}	NS
t (day)	40.10	34.82	32.24	40.51	41.30	35.78	3.21	NS	NS	NS
y (L/g added VS)	0.047	0.032	0.042	0.050	0.040	0.026	0.004	NS	0.0389^{L}	NS
CO_2 / removed VS (L/g)	0.35	0.49	0.43	0.46	0.40	0.38	0.03	0.0591	NS	NS
CH_4:CO_2 (L/L)	2.433	2.861	2.539	2.573	2.732	2.673	0.105	0.0539	NS	NS

A: asymptotic production (L/g added VS); k: production constant (L/g added VS per day); t: time at inflection point (day) and y: production at inflection point (L/g added VS).
L: linear effect; Q: Quadratic effect and NS: non-significant.
SEM: standard error of the means.

Monensin increased the production of CH_4 by 60.70% per gram of VS removed during the biodigestion process, but it did not significantly alter the stabilisation value (A) for CH_4 nor the growth rate (k) or the time to reach the inflection point (t) for CH_4 or CO_2 production. Monensin significantly reduced the stabilisation value for CO_2 production by 33.30% (Table 4 and Figure 5).

The different levels of tannins had a quadratic effect on total biogas and CH_4 production. The highest level of tannins reduced the total biogas and CH_4 production by 36.90 and 36.10%, respectively, when compared to the control treatment. Tannins linearly reduced the stabilisation value (A) as well as the growth rate (K) for CH_4 production, but it showed a quadratic effect on the time to reach the inflection point, and the inclusion levels of 0.75 and 1.50% reached the inflection point significantly later than the control and 2.25% treatments. Tannins linearly reduced CO_2 production as well as the stabilisation value (A) and the growth rate (K). No significant effect of tannins was observed in the production of CH_4 and CO_2 per gram of VS removed (Table 4).

The production ratio of CH_4 and CO_2 (CH_4:CO_2, L/L) was neither significantly affected by monensin (although there was a tendency) nor by the level of inclusion of tannins (Table 4).

The substrate pH before anaerobic biodigestion ranged between 6.33 and 6.73 (Table 2), but after anaerobic biodigestion, it ranged between 7.52 and 7.67 (Table 3). This shows a pH increase during biodigestion process that supports Rabiu *et al.* (2014), Mshandete *et al.* (2006) and Gunaseelan (1995), who stated that the pH of a normal and healthy anaerobic biodigestion system for CH_4 production is generally in the range of 7.00 to 8.50. Hence, the significant effect of monensin on increasing pH (from 7.53 to 7.61) during biodigestion indicates that this ionophore created better pH conditions for CH_4 production compared to the different levels of tannins, as tannins reduced the pH during biodigestion (although it was not of great magnitude in relation to the values of monensin), but it remained within the optimum range cited by the above authors. Perna Junior (2018), who worked with waste of Nellore and Holstein cows fed tannins of *A. mearnsii* up to 1.50% DM basis, also observed linear reduction of biodigestion pH.

The increased concentration of VS and NDF in faeces, and consequently in the substrates (g/kg) (Table 2), may have occurred because tannins reduce nutrient digestibility by forming complexes, which make nutrients inaccessible to microbial and enzymatic digestion in gastrointestinal tract (Patra and Saxena, 2011; Nigrant *et al.* 2017). This effect was also observed by Perna Junior (2018).

The effects of monensin on the reduction of nutrient removal efficiency (Table 3), CO_2 production (Table 4), the increase of CH_4 and CO_2 production per gram of VS removed may be the indication of direct effect of monensin on anaerobic biodigestion. The effects of tannins on increasing nutrient recovery and reducing nutrient removal efficiency as well as the reduction of total biogas, CH_4 and CO_2 production may also be the indication of direct effect of tannins on anaerobic biodigestion. The hypothesis for this study was that both additives would improve anaerobic biodigestion by increasing the concentration of nutrients in faeces (due to the effect of reducing enteric digestion of organic matter mainly caused by tannins). Therefore, the reduction of the performance of biodigestion may suggest that significant concentrations of these additives or their bioactive metabolites might have been present in faeces. Hao *et al.* (2011), adding 25 g/kg of *A. mearnsii* condensed tannins (i.e. 2.50% of inclusion) to cattle diets, found increased agronomic value of the manure and compost as fertiliser, but found no increase in the production of CH_4 and CO_2. Perna Junior (2018) found no differences in CH_4 and CO_2 production; the only difference he observed was a linear increase in the concentration of CO_2, an effect not observed in the present study. There is some evidence that most bioactive metabolites of monensin are eliminated via faeces in ruminants. Davison (1984), investigating whether or not monensin was absorbed, metabolised and eliminated through the bile of calves and other animal species, found that most of the consumed monensin was recovered in faeces but had a minimal recovery in urine and tissues. Determining the excretion pattern and tissue distribution of [^{14}C] monensin in cattle, Herberg *et al.* (1978) recovered almost 95% of active monensin metabolites in faeces. Hydrolysable tannins may be degraded and metabolised by rumen microorganisms (Mcsweeney *et al.* 2001), but condensed tannins are not degraded in the rumen. In addition, the complexes (tannin-protein or tannin-fibre) formed in gastrointestinal tract may not be reversible and hence eliminated in faeces (Makkar, 2003b). Therefore, the high recovery rates of monensin and tannins in faeces may suggest that these additives (mainly tannins) negatively affect the fermentation of faeces, at least when fresh (Hamilton *et al.* 2010). It is still not clear how long monensin and condensed tannins or their bioactive metabolites remain active to hinder anaerobic biodigestion or rumen fermentation. Using a rumen simulation technique (RUSITEC), Makkar *et al.* (1995) exposed rumen microbes to small amounts of quebracho (*Schinopsis* spp.) tannins for 8 days to induce enzymes capable of degrading condensed tannins, but there was no degradation.

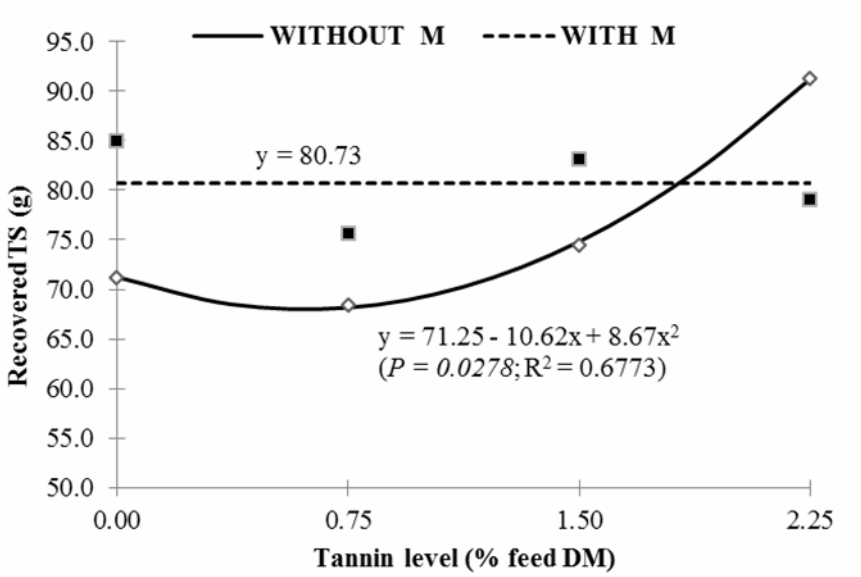

Figure 2 Graph showing the interaction between monensin (M) and tannins on the amount of TS recovered after anaerobic biodigestion. The square points in bold represent the means observed in the different tannin levels only in the biodigesters whose substrates were also treated with M. In these biodigesters, the joint effect of M and tannins was not significant; therefore, it was chosen to present the general mean observed (dashed line). The empty square points show the means observed in the different tannin levels in biodigesters whose substrates were only treated with tannins (quadratic effect). The continuous line passing over the empty squares shows the estimated means for the biodigesters whose substrates received M and tannins if they had not received M (quadratic effect). Then, it may be observed that when monensin was administered jointly with tannins, the effect of tannins was not observed. This suggests that monensin blocked the effect of tannins by antagonistic interaction

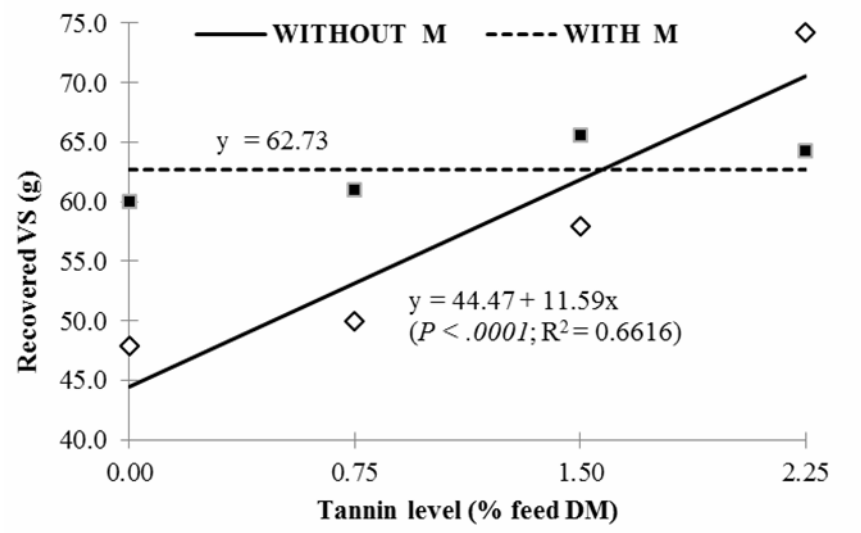

Figure 3 Graph depicting the interaction between monensin (M) and tannins on the amount of VS recovered after anaerobic biodigestion. The square points in bold represent the means observed in the different tannin levels only in the biodigesters whose substrates were also treated with M. In these biodigesters, the joint effect of M and tannins was not significant, then it was chosen to present the general mean observed (dashed line). The empty square points show the means observed in the different tannin levels in biodigesters whose substrates were only treated with tannins (quadratic effect). The continuous line shows the estimated means for the biodigesters whose substrates received M and tannins if they had not received M (linear effect). Therefore, it may also be observed that when monensin was administered with tannins, the effect of tannins was not observed, suggesting inhibition of tannin effect by monensin through antagonistic interaction

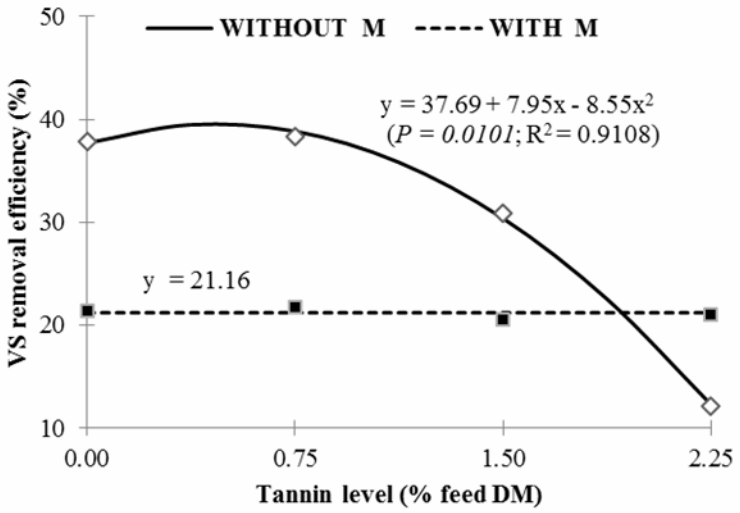

Figure 4 Graph depicting the interaction between monensin (M) and tannins on VS removal efficiency. The square points in bold represent the means observed in the different tannin levels only in the biodigesters whose substrates were also treated with M. In these biodigesters, the joint effect of M and tannins was not significant, then it was preferred to present the general mean observed (dashed line). The empty square points show the means observed in the different tannin levels in biodigesters whose substrates were only treated with tannins (quadratic effect). The continuous line passing over the empty squares shows the estimated means for the biodigesters whose substrates received M and tannins if they had not received M (quadratic effect). As a consequence of the effect depicted in the former graph, it may be observed that it was necessary to increase the concentration of tannins to cause-effect. Hence, it appears to be obvious that when monensin was administered along with tannins, the effect of tannins was not observed. This also suggests that monensin inhibited the effect of tannins by antagonistic interaction

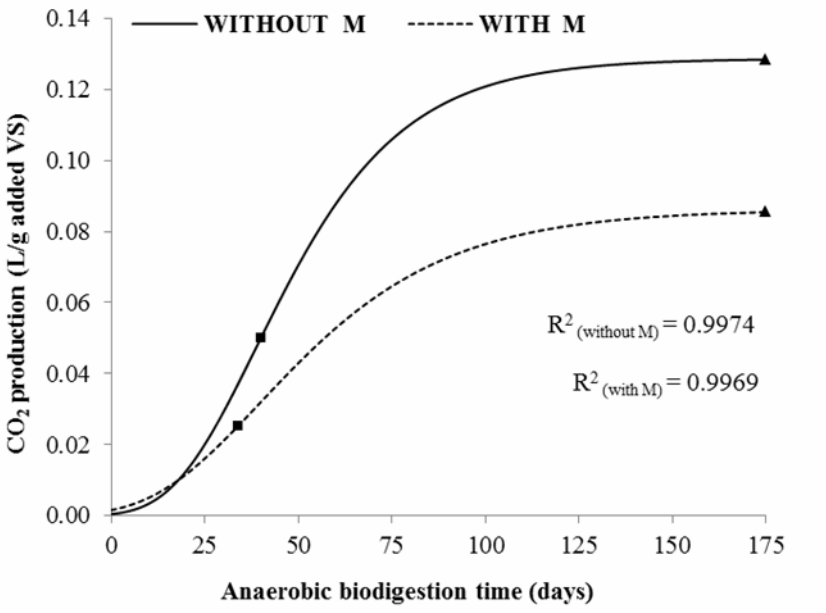

Figure 5 Graph adjusted by the Gompertz model depicting the time (in days) and cumulative CO_2 production (M: monensin; ■ inflection point and ▲stabilisation value)

Makkar (2003b) reported degradation of purified quebracho and *A. nilotica* condensed tannins within 7 days, but this occurred under aerobic conditions in artificial fermenters. It is difficult to believe that even if they are present in faeces, monensin and tannins or their bioactive metabolites, can remain active up to 175 days (the time duration of biodigestion in the present study) and thereby continually impair the kinetics of biodigestion and reduce the performance of the fermentation. This suggests that there must be some process whereby if biodigestion is disturbed, by the presence of bioactive substances or other factors, future biodigestion performance is also impaired.

The reduction of nutrient utilisation by microorganisms is a characteristic of tannins (Tseu *et al*. 2020). The increased nutrient recovery observed in the present study was the consequence of the reduction of the removal efficiency of TS, VS, and N (Table 3), i.e. the reduced capacity to biodigest nutrients caused by tannins, even though, the VS removal efficiency, which ranged between 16.55 to 30.03%, was only below the range stipulated by Dohányos and Zábranská (2001) (25-50%) when monensin and up to 2.25% of tannins were included in the diet.

Although monensin has significantly reduced the nutrient (TS and VS) removal efficiency (Table 3), the interactions observed between this additive and tannins (Figures 2, 3 and 4) suggest that monensin has the potential to reduce the effect of tannins by an antagonistic interaction. It may be understood from Figures 2, 3 and 4 that if monensin and tannins had not been administered simultaneously, the effect of tannins in reducing the VS or TS biodigestion would have been of higher magnitude.

The reduction of the total CO_2 production (L) by monensin was accompanied by the reduction of the stabilisation value of the production (A) (Table 4 and Figure 5), but it did not affect the production growth rate (k) and the time to reach inflection point, i.e. the point at which the production rate is maximum and after which production tends to stabilise. This may have been due to the fact that monensin reduced microbial capacity to remove nutrients; therefore, it was not possible to reach the production potential. According to IPCC (2006), the specific productivity of fermentation products is measured in terms of removed VS. Besides the reduction of CH_4 production, the tannins also reduced the production growth rate and the stabilisation value. This may have been the reason why the average production of CH_4 (0.25 L) per gram of added VS was below the production found by Møller *et al.* (2004) (0.40 L) and Perna Junior (2018) (0.34 L). The lack of effect of monensin and the different levels of inclusion of tannins on the ratio of CH_4 and CO_2 production shows that although these additives may reduce the production of these two gases, the reduction is directly proportional. Monensin and tannins are feed additives used to reduce the emission of enteric CH_4 (Finlay *et al*. 1994; Guan *et al*. 2006; Odongo *et al*. 2007; Patra and Saxena, 2011; Carrasco *et al*. 2017; Nawab *et al*. 2020), this effect is beneficial in two aspects, the first is in increasing the feed energy efficiency and the second is in protecting the environment by reducing CH_4 emission into the atmosphere. Several researchers, such as Carrasco *et al*. (2017) and Tseu *et al*. (2020), have reported that the reduction in the production of enteric CH_4 (through the use of tannins, for example) is usually at the expense of reducing the enteric digestion of nutrients, leading to the faecal excretion of large amounts of nutrients such as VS. This fact led to believe that the use of these additives in the ruminant feeding is seen as beneficial in the context of animal feeding, but due to the higher concentration of VS in faeces, it may be providing better conditions for greater emission of gas, including CH_4, from the waste (an effect poorly studied by many researchers). Many studies, such as Orhorhoro *et al.* (2017), point out that the production of gas through anaerobic biodigestion increases with the increase in the concentration or amount of VS in the substrate. Therefore, the overriding question to conduct this research was whether or not the effect of these additives on the reduction of enteric CH_4 production provides conditions for the emission of GHG from waste. Nonetheless, the results revealed that the use of monensin and tannins in cattle feeding presents no risk of increasing emissions of GHG from waste (although tannins have increased faecal VS) and it is believed that they or their bioactive metabolites might have been present in faeces and hindered the biodigestion. If so, monensin or monensin's bioactive metabolites reduced Gram-positive microorganisms, the major producers of methanogenic substrates as the final fermentation products (CO_2, acetate, hydrogen, etc.) (Russell and Houlihan, 2003) and the tannins or tannin bioactive metabolites may have reduced gas, including CH_4, production by reducing methanogenic microorganisms and directly through depression of fibre biodigestion (Carrasco *et al*. 2017; Tseu *et al*. 2020). Therefore, the combined or isolate use of these additives in cattle feeding can be a sustainable way to continue ruminant production with less environmental pollution hazard. Then, the hypothesis that the combined or isolate use of monensin and tannins in cow feeding increases the production of CH_4 and CO_2 from waste (tested in the present study) might not be accepted if tannins are included at a rate above 0.75%.

CONCLUSION

Monensin (32 mg/kg DM) did not affect total biogas and CH_4 production, but reduced CO_2 production by 18.90%. Tannins had the potential to reduce total biogas and CH_4 production when the inclusion level in the diet was more

than 0.75% of feed DM, but for CO_2 the potential to reduce was linear. Monensin reduced the tannin effect (of reducing the volatile solids or total solids biodigestion) by antagonistic interaction. Monensin and tannins may be used in cattle feeding with no risk of increasing greenhouse gas (e.g. CH_4 and CO_2) production from the waste. According to the results observed in the present study there is a strong evidence that monensin and tannins or their bioactive metabolites may appear in faeces and impair the biodigestion of the waste. Therefore, further studies should be carried out to confirm (by observing effects or by identifying) the presence of these additives or their bioactive metabolites in the faeces.

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REFERENCES

Aboagye I.A., Oba M., Koenig K.M., Zhao G.Y. and Beauchemin K.A. (2019). Use of gallic acid and hydrolyzable tannins to reduce methane emission and nitrogen excretion in beef cattle fed a diet containing alfalfa silage. *J. Anim. Sci*. **97,** 2230-2244.

Achinas S., Achinas V. and Euverink G.J.W. (2017). A technological overview of biogas production from biowaste. *Engineering.* **3,** 299-307.

Achinas S., Li Y., Achinas V. and Euverink G.J.W. (2018). Influence of sheep manure addition on biogas potential and methanogenic communities during cow dung digestion under mesophilic conditions. *Sust. Environ. Res*. **28,** 240-246.

American Public Health Association (APHA). (2005). Standard Methods for the Examination of Water and Wastewater. American Public Health Association. Washington, DC, USA.

AOAC. (1995). Official Methods of Analysis. 12th Ed. Association of Official Analytical Chemists, Arlington, Washington, DC., USA.

AOAC. (1990). Official Methods of Analysis. Vol. I. 15th Ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Bai M., Flesch T., Trouvé R., Coates T., Butterly C., Bhatta B., Hill J. and Chen D. (2020). Gas emissions during cattle manure composting and stockpiling. *J. Environ. Qual.* **49,** 228-235.

Carrasco J.M.D., Cabral C., Redondo L.M., Viso N.D.P., Colombatto D., Farber M.D. and Miyakawa M.E.F. (2017). Impact of chestnut and quebracho tannins on rumen microbiota of bovines. *BioMed Res. Int.* **2017,** 1-11.

Davison K.L. (1984). Monensin absorption and metabolism in calves and chickens. *J. Agric. Food Chem*. **32,** 1273-1277.

Dohányos M. and Zábranská J. (2001). Anaerobic digestion. Pp. 278-314 in Sludge into biosolids. Spinosa L. and Vesilind P.A. Ed., IWA Publishing, London, United Kingdom.

Finlay B.J., Esteban G., Clarke K.J., Williams A.G., Embley T.M. and Hirt R.P. (1994). Some rumen ciliates have endosymbiotic methanogens. *FEMS Microbiol. Lett.* **117,** 157-162.

Guan H., Wittenberg K.M., Ominski K.H. and Krause D.O. (2006). Efficacy of ionophores in cattle diets for mitigation of enteric methane. *J. Anim. Sci.* **84,** 1896-1906.

Gunaseelan V.N. (1995). Effect of inoculum/substrate ratio and pretreatments on methane yield from Parthenium. *Biom. Bioen.* **8,** 39-44.

Hamilton S.W., Depeters E.J., Mcgarvey J.A., Lathrop J. and Mitloehner F.M. (2010). Greenhouse gas, animal performance, and bacterial population structure responses to dietary monensin fed to dairy cows. Atmospheric pollutants and trace gases. *J. Environ. Qual*. **39,** 106-114.

Hao X., Benke M.B., Li C., Larney F.J., Beauchemin K.A. and Mcallister T.A. (2011). Nitrogen transformations and greenhouse gas emissions during composting of manure from cattle fed diets containing corn dried distillers' grains with solubles and condensed tannins. *Anim. Feed Sci. Technol.* **166,** 539-549.

Herberg R., Manthey J., Richardson L., Cooley C. and Donoho A. (1978). Excretion and tissue distribution of [^{14C}] monensin in cattle. *J. Agric. Food Chem.* **26,** 1087-1090.

Holm-Nielsen J.B., Al Seadi T. and Oleskowicz-popiel P. (2009). The future of anaerobic digestion and biogas utilization. *Biores. Technol.* **100,** 5478-5484.

Hristov A.N., Oh J., Lee C., Meinen R., Montes F., Ott T., Firkins J., Rotz A., Dell C., Adesogan A., Yang W., Tricarico J., Kebreab E., Waghorn G., Dijkstra J. and Oosting S. (2013). Mitigation of Greenhouse Gas Emissions in Livestock Production-A review of technical options for non-CO_2 emissions. FAO Animal Production and Health Paper No. 177. FAO, Rome, Italy.

IPCC. (2007). Climate change 2007: The Physical Science Basis. In Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom.

IPCC. (2014). Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom.

IPCC. (2006). IPCC Guidelines for National Greenhouse Gas Inventories, v. 4 Agriculture, Forestry and Land Use. Institute for Global Environmental Strategies, Hayama, Japan.

Johnson K.A. and Johnson D.E. (1995). Methane emissions from cattle. *J. Anim. Sci.* **73,** 2483-2492.

Kaminski M., Kartanowics R., Jastrzebski D. and Kaminski M.M. (2003). Determination of carbon monoxide, methane and car-

bon dioxide in refinery hydrogen gases and air by gas chromatography. *J. Chromatogr.* **989,** 277-283.

Lavrencic A., Stefanon B. and Susmel P. (1997). An evaluation of the Gompertz model in degradability studies of forage chemical components. *J. Anim. Sci.* **64,** 423-431.

Lucas Junior J. (1994). Some considerations on the use of swine manure as substrate for three anaerobic biodigester systems. MS Thesis. Universidade Estadual Paulista, Jaboticabal, Brazil.

Lucas Junior J., Ortolani A., Benincasa M. and Ymada R.Y. (1993). Avaliação do uso de inóculo no desempenho de biodigestores abastecidos com dejeto de frangos de corte com cama de maravalha. Pp. 915-930 in Congr. Bras. Engen. Agríc., Ilhéus, Brazil.

Lynch J. (2019). Availability of disaggregated greenhouse gas emissions from beef cattle production: A systematic review. *Environ. Impact Assess. Rev*. **76,** 69-78.

Makkar H.P.S. (2003a). Quantification of Tannins in Tree and Shrub Foliage: A Laboratory Manual. Springer, Viena, Australia.

Makkar H.P.S. (2003b). Effects and fate of tannins in ruminant animals, adaptation to tannins and strategies to overcome detrimental effects of feeding tannin rich feeds. *Small Rumin. Res.* **49,** 241-256.

Makkar H.P.S., Blümmel M., Borowy N.K. and Becker K. (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *J. Sci. Food Agric.* **61,** 161-165.

Makkar H.P.S., Borowy N., Becker K. and Degen R. (1995). Some problems in fiber determination in tannin-rich forages (*Acacia saligna*) and their implications in *in vivo* studies. *Anim. Feed Sci. Technol.* **55,** 67-76.

Mcsweeney C.S., Palmer B., Mcneil D.M. and Krause D.O. (2001). Microbial interactions with tannins: Nutritional consequences for ruminants. *Anim. Feed Sci. Technol.* **91,** 83-93.

Metcalf L. and Eddy H. (2014). Wastewater Engineering: Treatment and Resource Recovery. McGraw-Hill, New York.

Møller H.B., Sommer S.G. and Ahring B.K. (2004). Methane productivity of manure, straw and solid fractions of manure. *Biom. Bioener*. **26,** 485-495.

Mshandete A., Bjornosson L., Kivaisi A.K., Rubindamayugi M.S.T. and Mattiasson B. (2006). Effect of particle size on biogas yield from sisal fibre waste. *Renew. Energy.* **31,** 2385-2392.

Nawab A., Li G., An L., Nawab Y., Zhao Y., Xiao M., Tang S. and Sun C. (2020). The potential effect of dietary tannins on enteric methane emission and ruminant production, as an alternative to antibiotic feed additives-a review. *Ann. Anim. Sci.* **20,** 355-388.

Neshat S.A., Mohammadi M., Najafpour G.D. and Lahijani P. (2017). Anaerobic co-digestion of animal manures and lignocellulosic residues as a potent approach for sustainable biogas production. *Renew. Sust. Energy Rev.* **79,** 308-322.

Nigrant R.W.S., Zain M., Epormen and Suryani H. (2017). Effects of doses and different sources of tanninis on *in vitro* ruminal methane, volatile fat acids production and on bacterial and protozoa populations. *Asian J. Anim. Sci.* **11,** 47-53.

Odongo N.E., Bagg R., Vassie G., Dick P., Or-Rashid M.M., Hook S.E., Gray J.T., Kebreab E., France J. and Mcbride B.W. (2007). Long-term effects of feeding monensin on methane production in lactating dairy cows. *J. Dairy Sci.* **90,** 1781-1788.

Orhorhoro E.K., Ebunilo P.E. and Sadjere G.E. (2017). Experimental determination of effect of total solid (TS) and volatile solid (VS) on biogas yield. *American J. Mod. Energy.* **3,** 131-135

Patra A.K. and Saxena J. (2011). Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *J. Sci. Food Agric.* **91,** 24-37.

Perna Junior F. (2018). Tannins as a feed additive to mitigate methane emissions in ruminants. Universidade de São Paulo, Pirassununga, Brazil.

Petersen S.O. (2018). Symposium review: Greenhouse gas emissions from liquid dairy manure: Prediction and mitigation. *J. Dairy Sci.* **101,** 6642-6654.

Rabiu A., Yaakub H., Liang J.B. and Samsudin A.A. (2014). Increasing biogas production of rumen fluid using cattle manure collected at different time as a substrate. *J. Agric. Vet. Sci.* **7,** 44-47.

Rotz C.A. (2017). Symposium review: Modeling greenhouse gas emissions from dairy farms. *J. Dairy Sci.* **101,** 6675-6690.

Russell J.B. and Houlihan A.J. (2003). Ionophore resistance of ruminal bacteria and its potential impact on human health. *FEMS Microbiol. Rev.* **27,** 65-74.

SAS Institute. (2013). SAS®/STAT Software, Release 9.3. SAS Institute, Inc., Cary, NC. USA.

Scarlat N., Fahl F., Dallemand J., Monforti F. and Matola V. (2018). A spatial analysis of biogas potential from manure in Europe. *Renew. Sust. Energy Rev*. **94,** 915-930.

Stewart E.K., Beauchemin K.A., Dai X., MacAdam J.W., Christensen R.G. and Villalba J.J. (2019). Effect of tannin-containing hays on enteric methane emissions and nitrogen partitioning in beef cattle. *J. Anim. Sci.* **97,** 3286-3299.

Tseu R.J., Perna Junior F., Carvalho R.F., Sene G.A., Tropaldi C.B., Peres A.H. and Rodrigues P.H.M. (2020). Effect of tannins and monensin on feeding behaviour, feed intake, digestive parameters and microbial efficiency of nellore cows. *Italian J. Anim. Sci*. **19,** 262-273.

Van Soest P.J., Robertson J.B. and Lewis B.A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysscharides in relation to animal nutrition. *J. Dairy Sci.* **74,** 3583-3597.

Wanapat M., Cherdthong A., Phesatcha K. and Kang S. (2015). Dietary sources and their effects on animal production and environmental sustainability. *Anim. Nutr*. **1,** 196-103.

Zhang Q., Hu J. and Lee D. (2016). Biogas from anaerobic digestion processes: Research updates. *Renew. Energy*. **98,** 108-119.