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University of Sao Paulo

ABSTRACT

Anaerobic digestion represents an alternative for the treatment of waste, because in addition to allowing the reduction of the polluting potential of waste, it promotes the generation of biogas. The objective of this study was to evaluate the effect of diets with different levels of Acacia mearnsii tannins in two distinct cattle genotype on the greenhouse gas (GHG) emissions of cattle manure. Experimental batch-type digesters placed inside a climatic chamber (30 to 35ºC) were used for anaerobic digestion for 175 days. The digesters were organized in a completely randomized design, in a 2x4 arrangement, with two cattle genotypes (Holstein and Nellore) and four tannins level in the diet (0, 0.5, 1.0 and 1.5% of dietary DM), with four repetitions per treatment, totaling 32 experimental units. The data were subjected to analysis of variance (PROC MIXED), and the level effect was evaluated using orthogonal polynomials at 5% significance. Feeding cattle with Acacia mearnsii extract up to 1.5% of the DM of the diet does not harm the environment, as it does not alter the efficiency of removing nutrients from manure and the emission of GHG. The CH_4 and CO_2 production rates were higher for Holstein cattle, which may reduce Residence Time in the manure digestion process. Therefore, tannins can be used as additives to modify rumen fermentation without affecting the anaerobic digestion process of cattle manure.

Indexterms: *acacia mearsnii* extract, anaerobic digestion, carbon dioxide, cattle manure, greenhouse gas emissions, livestock nutrition, methane, nitrous oxide, rumen fermentation, tannins.

Classification: LCC Code: S631-S631.5

Language: English

Great Britain Journals Press

LJP Copyright ID: 925655
Print ISSN: 2631-8490
Online ISSN: 2631-8504

London Journal of Research in Science: Natural and Formal

Volume 24 | Issue 5 | Compilation 1.0

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ABSTRACT

Anaerobic digestion represents an alternative for the treatment of waste, because in addition to allowing the reduction of the polluting potential of waste, it promotes the generation of biogas. The objective of this study was to evaluate the effect of diets with different levels of Acacia mearnsii tannins in two distinct cattle genotype on the greenhouse gas (GHG) emissions of cattle manure. Experimental batch-type digesters placed inside a climatic chamber (30 to 35ºC) were used for anaerobic digestion for 175 days. The digesters were organized in a completely randomized design, in a 2x4 arrangement, with two cattle genotypes (Holstein and Nellore) and four tannins level in the diet (0, 0.5, 1.0 and 1.5% of dietary DM), with four repetitions per treatment, totaling 32 experimental units. The data were subjected to analysis of variance (PROC MIXED), and the level effect was evaluated using orthogonal polynomials at 5% significance. Feeding cattle with Acacia mearnsii extract up to 1.5% of the DM of the diet does not harm the environment, as it does not alter the efficiency of removing nutrients from manure and the emission of GHG. The CH_4 and CO_2 production rates were higher for Holstein cattle, which may reduce Residence Time in the manure digestion process. Therefore, tannins can be used as additives to modify rumen fermentation without affecting the anaerobic digestion process of cattle manure.

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Author α σ §: Department of Animal Nutrition and Production, College of Veterinary Medicine and Animal Science, University of Sao Paulo, 225 Duque de Caxias Norte Ave, Pirassununga, SP 13635-900, Brazil.

ρ: Department of Animal Science, College of Animal Science and Food Engineering, University of Sao Paulo, 225 Duque de Caxias Norte Ave, Pirassununga, SP 13635-900, Brazil.

Ѡ: Department of Animal Production, Veterinary College, Eduardo Mondlane University, 3453 Julius Nyerere Avenue, Maputo, Mozambique.

I. INTRODUCTION

The livestock sector plays a vital role in climate change, representing 14.5% of human-induced greenhouse gas (GHG) emissions, according to Gerber et al. (2013). The majority of these emissions come from enteric fermentation and cultivated soils. The CH_4 and N_2O emissions are an environmental concern because their global warming potentials are 27.2 and 273 more potent than CO_2, respectively (Pörtner et al., 2023). Cattle manure, when stored, can represent 7 to 27% of the total CH_4 emission by ruminants (Hindrinchsen et al., 2006).

Cattle manure is a suitable substrate for developing anaerobic digestion, as it contains carbohydrates, proteins, and fat (Ahring et al., 2001). According to Moller et al. (2014), the effects of changes in livestock diets on biogas and GHG emissions need to be further studied, as many factors can change the characteristics of manure. As an alternative to the use of drugs to modify rumen fermentation and reduce CH_4 emissions, researchers have intensified the study of natural food additives for ruminants, such as tannins (Alves et al., 2017 and Perna Junior et al., 2022). These compounds are classified into hydrolysable (HT) and condensed (CT) tannins, both with effects depending on their plant source, concentration, and other factors such as the animal species, the physiological state, and the composition of the animal diet (Makkar, 2003a). According to Hao et al. (2011), CT in ruminant diets reduces the degradation of rumen N, but there is little research on how these phenolic compounds alter the decomposition of manure. Furthermore, Hegarty (2004), in his review of different genotypes and their impact on the digestive tract of ruminants, states that there are significant differences in digestive function between species, breeds and within breeds. Thus, there is a need to investigate how the use of tannins in the diet of different cattle influences the composition of manure and biogas production by anaerobic digestion.

Therefore, it is expected that the addition of tannins to the cattle diet will increase the N content of the manure and result in higher CH_4 emissions, as it enhances the anaerobic digestion process. The objective of the present study was to evaluate the nutrient removal efficiency and biogas production (CO_2, CH_4 and N_2O) during the anaerobic digestion of manure from two different groups of cattle fed with different levels of tannins from *Acacia mearnsii*.

II. MATERIAL AND METHODS

The trial was conducted in Pirassununga, state of São Paulo, southeastern Brazil (21°59'45"S, 47°25'37" W, and 625 m above sea level). All procedures involving animal care were conducted following the Institutional Animal Care and Use Committee Guidelines (protocol n° 3222290414).

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 non-pregnant and non-lactating cows were used, four Holstein (*Bos taurus*) with a mean live weight of 775 (± 55) kg and four Nellore (*Bos indicus*) with an average live weight of 434 (± 47) kg. The choice of these two genotypes was because they are expressive representatives of the world's dairy production systems (Holstein genotype) and Brazil's large beef cattle sector (Nellore genotype). The cows were housed in individual stalls with a sand bed, feed bunker, drinker, and fans to ensure the animals' thermal comfort. Feed was offered twice daily for ad libitum intake (at least 5% refusal) at 8 am and 4 pm. It contained a mixed ration with a 50:50 roughage to concentrate ratio (DM-basis). The composition of the diet is shown in Table 1. The tannin doses (0, 0.5, 1.0 and 1.5%) were adjusted daily depending on DM intake and manually mixed with the total diet before each feeding. A commercial extract (Natur N, Seta®, Brazil) obtained from Acacia bark (*Acacia mearnsii*) was used as the source of tannins. The concentration of total phenols (84.4%) was determined by the Folin-Ciocalteau method (Makkar, 2003b), and the total tannins (82.3% equivalent in tannic acid) were estimated by the difference of the total phenol concentration before and after treatment with insoluble polyvinylpolypyrrolidone (Makkar et al., 1993). The CT concentration (32.3% equivalent to leucocyanidin) was determined by the HCl-butanol method, according to Makkar, 2003b. To avoid the negative effect of tannins on dry matter intake, low to moderate levels were used in this study, since Grainger et al. (2009) observed reduction in feed intake for dairy cow using only 0.9% of diet DM.

Table 1: Ingredient proportion and chemical composition of the experimental diet.

Item	Diet
Ingredient, % of dry matter (DM)	
Corn silage	50.0
Dry-ground corn grain	32.8
Soybean meal	12.7
Sodium chloride	0.5
Vitamin and mineral premix[a]	2.0
Tannins[b]	*
Caolim[c]	**
Chemical composition, % of DM	
DM (%)	61.1
Ash	6.6
Ether extract	3.2
Crude protein	13.1
Neutral detergent fiber	34.2
Acid detergent fiber	22.2
Nonfiber carbohydrates	43.0
Total digestible nutrients[d]	67.3

For the feeding and collecting feces, the animals were allocated in a duplicated Latin square 4x4 design, in a 2x4 factorial arrangement, with two distinct groups of animals and four levels of inclusion of tannins in the diet. Each experimental period had 22 days, the first 17 days for adaptation to the diet and the last five days for collection of feces, which were collected manually via rectum, at 8-h and 16-h, and frozen at -20° C forming a single sample composed of animals, in each period. Urine samples were obtained from all cows on the 22nd day of each experimental period, every 6 hours, during urination stimulated by massage on the vulva, and then stored at -20ºC in a single bottle, forming a sample composed of 24 hours.

In the second phase (the anaerobic digestion phase), the samples composed of feces and urine collected and frozen during the feeding phase (1) were diluted in water, adopting the total solids (TS) content of 6%. A theoretical manure ratio of 75:25 was used for the mixture of feces and urine, respectively. The substrate composition was 37.5% manure, 10% inoculum, and 52.5% water. Sludge from the manure treatment pond with the following characteristics was used as inoculum: pH = 6.2; TS = 4.61%; VS/TS = 60.30%. The digesters were organized in a completely randomized design, in a 2x4 arrangement, with two cattle genotypes (Holstein and Nellore) and four tannins level in the diet (0, 0.5, 1.0 and 1.5% of DM), with four repetitions per treatment, totaling 32 experimental units. The feces were loaded into batch-type digesters (Figure 1) consisting of a 75 mm reactor, a 100 mm gasometer, and a 150 mm digester made with three PVC pipes, adapted from Sunada et al. (2018). Anaerobic digestion was developed in mesophilic conditions (30 to 35ºC), ideal for digestion kinetics (Metcalf and Eddy, 2014), placing the digesters inside a climatic chamber with an electrical resistance heating system and a digital temperature controller, for 175 days. The treatments and respective characterization of the substrates are shown in Table 2.

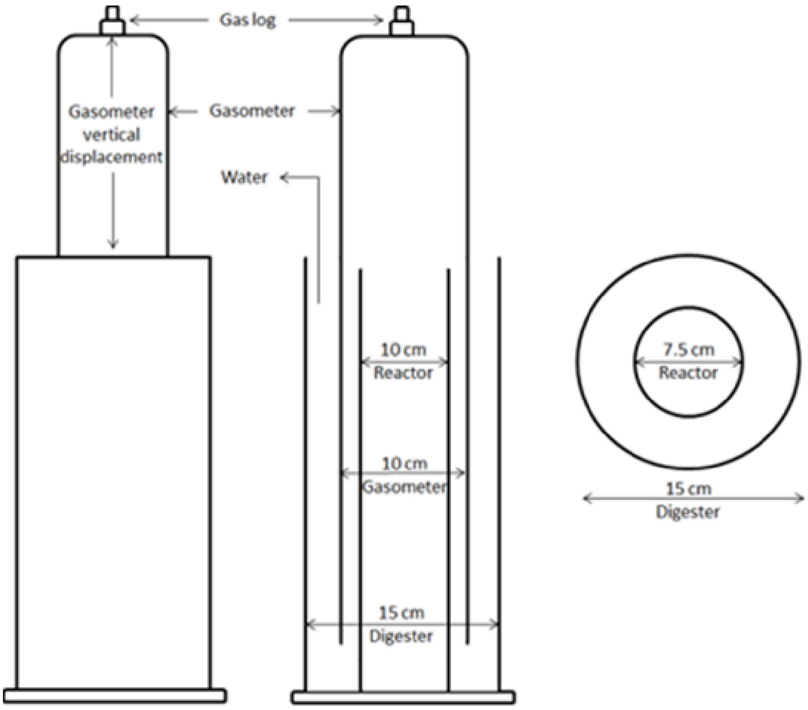

Figure 1: Anaerobic batch-type digester shown in front, side, and top views.

Biogas volume was determined by the displacement of the gasometer and its internal cross-sectional area, and corrected to 1 atm and 20ºC. The frequency of biogas measurement was conducted following gasometer capacity. Every time the biogas volume was measured, biogas samples were collected with a syringe connected to the gas log on top of the gasometer. The CH_4, CO_2 and N_2O concentrations were determined by gas chromatography (Trace 1300, Thermo Fisher Scientific®, Rodano, Milan, Italy), according to Kaminski et al. (2003).

Specific gas yield (per gram of VS fed or destroyed) was calculated by dividing the total gas production (L) by amount of volatile solids fed (before anaerobic digestion), or destroyed (difference between VS fed and eliminated). The test was finished when biogas production ceased. The nutrients fed and eliminated were weighed to calculate the DM content (grams).

The nutrients ingested and digested were calculated according to the following equation (Nogueira et al., 2023):

$$Nutrients\ (g) = \frac{nutrient\ fed\ or\ eliminated\ (\%) \times DM\ fed\ or\ eliminated\ (g)}{100}$$

Nutrient removals were calculated according to this equation:

$$Nutrient\ removal,\ \% = 100 - \frac{nutrient\ digestate\ (g)}{nutrient\ ingestate\ (g)} \times 100$$

$$Nutrient\ removal(\%) = \frac{nutrient\ fed\ (g) - nutrient\ eliminated}{nutrient\ fed\ (g)} \times 100$$

Individual feed and feces samples were collected before and after anaerobic digestion. The samples were dried in a forced-air oven, at 60°C for 48 hours, ground to 1.0 mm and analyzed. DM content was determined by method 930.15 of the Association of Official Analytical Chemists (AOAC) (Cunniff, 1995) in the forced-air oven at 105°C for 2 hours, followed by cold weighing. Nitrogen content was obtained by the micro Kjeldahl method, being multiplied by 6.25 to calculate crude protein (Cunniff, 1995). Neutral detergent fiber, acid detergent fiber, and lignin were determined by the methods described in the literature (Van Soest et al., 1991), using the Filter Bag and heat-stable α-amylase as in method 973.18 (Cunniff, 1995). The levels of total solid and volatile solids were measured according to the American Public Health Association (APHA) (Rice et al., 2012). The hydrogen ion potential (pH) was measured by a portable pH meter (Hanna Instruments®, HI 8424, Italy).

Table 2: Characteristics of the substrates and removal efficiency of nutrients in anaerobic batch-type digesters supplied with manure of Nellore and Holstein cattle fed *Acacia mearnsii* tannins

Variables	Genotype (G)		Tannin Level (TL)					Probability		
	Holstein	Nellore	0%	0.5%	1.0%	1.5%	SEM	G	TL	G*TL
Substrates (g kg^{-1})										
TS	45.5	49.3	49.3	47.6	47.5	45.2	-	-	-	-
VS	36.8	39.4	38.6	37.7	38.5	37.7	-	-	-	-
N (TS)	40.1	37.7	35.7	37.7	40.4	41.9	-	-	-	-
NDF (TS)	558.2	474.2	465.8	496.1	525.0	577.8	-	-	-	-
ADF (TS)	368.2	346.3	301.4	356.8	373.5	397.2	-	-	-	-
Eliminated nutrients										
TS (g)	71.40	79.75	78.65	77.91	76.91	75.03	2.07	0.003	ns	ns
VS (g)	51.55	56.72	54.85	54.92	53.97	55.81	1.73	0.005	ns	ns
N (g)	2.29	2.48	2.24	2.33	2.42	2.74	0.06	0.003	0.005^{L}	ns
NDF (g)	27.72	30.59	28.15	29.39	29.96	31.29	1.22	ns	ns	ns
ADF (g)	24.74	26.95	24.19	27.10	28.64	29.47	0.90	0.074	0.031^{L}	ns
pH	7.16	7.27	7.28	7.24	7.20	7.15	0.02	0.006	0.014^{L}	ns
Nutrient removal efficiency										
TS (%)	21.62	19.05	20.19	18.14	19.02	18.90	0.95	ns	ns	ns
VS (%)	29.99	27.07	28.90	27.20	29.86	25.85	1.06	ns	ns	ns
N (%)	38.22	30.82	36.50	36.61	35.47	29.42	1.72	0.029	ns	ns
NDF (%)	44.44	32.08	37.94	36.32	39.36	38.56	2.73	0.028	ns	ns
ADF (%)	32.63	20.64	20.95	20.90	22.28	21.75	2.67	0.003	ns	ns

*SEM: Standard error of the mean; G*TL: Interaction between Genotype and Tannin Level; TS: Total solids; SV: Volatile solids; N: Nitrogen; NDF: Neutral detergent Fiber; ADF: Acid detergent fiber.*

Data were statistically analyzed using the SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Before the actual analysis, the data were analyzed for the presence of disparate information ("outliers") and normality of residuals (Shapiro-Wilk). Individual observation was considered an outlier when standard deviations to mean were more than +3 or less than −3. When the normality assumption was not accepted, the logarithmic transformation or the square root was required. The following statistical model was used:

$$Y_{ijkl} = \mu + G_i + L_i + G_i * L_i + P_k + A_l(G_i) + e_{ijkl}$$

Where Yijkl is the observation, μ is the general mean, Gi is the genotype effect (fixed effect), Lj is the tannin level effect (fixed effect), Gi* Lj is the interaction effect of genotype and tannin level, Pk is the period effect (random effect), Al(Gi) is the animal within genotype effect (random effect) eijkl is the residual error.

The data were subjected to analysis of variance (PROC MIXED), and the level effect was evaluated by the use of orthogonal polynomials, separating the effects in linear, quadratic and deviation from the quadratic. The 0.05 significance level was adopted.

Methane yield curve parameters were estimated from each digester yield using the Gompertz model (Kafle and Chen, 2016) according to the equation:

$$y_1 = A \exp exp\,[-\ B \exp exp\,(-\ k)\,]$$

Where y_1 is the methane yield at anaerobic digestion days, A is the asymptotic methane yield, B is the interaction constant, k is the yield constant rate, and exp is the base of natural logarithmic (2.7183).

$$t_1 = \frac{\ln ln\, B}{K}$$

Where t_1 is the point of inflection, ln is the logarithmic, B is the interaction constant, and k is the yield constant rate.

$$y_1 = \frac{A}{exp}$$

Where y_1 is the methane yield at inflection point, A is the asymptotic methane yield, and exp is the base of natural logarithmic (2.7183).

III. RESULTS AND DISCUSSION

The use of anaerobic digesters under controlled temperature conditions was a strategy of the present experiment to promote the maximum activity of the different microbial groups that convert the complex organic substrate into biogas through the anaerobic food chain, as the vast majority of anaerobic microorganisms they develop better at temperatures ranging between 20ºC and 40ºC (Gavala et al., 2003). According to Dohányos and Zábranská (2001), the efficiency of removal of organic matter (represented by the VS) is generally between 25-50% in reactors operated at mesophilic temperatures, in agreement with the values of approximately 30% found in the present experiment.

The pH, after the digestion process, was 1.54% higher for the Nellore than for the Holstein. Additionally, with increasing levels of tannins in the diet, there was a linear reduction in the pH of the degraded manure (Table 2). The different levels of tannins in the diet did not cause significant differences in nutrient removal efficiency. However, Holstein manure showed higher removal efficiency for N, NDF and ADF compared to Nellore (Table 2).

Hegarty (2004), in his review of the different genotypes and their impact on the digestive tract function of ruminants, states that there are significant differences in digestive function between species, breeds, and within breeds. Additionally, it is known that there are differences between zebu and taurine cattle regarding nutrient use and performance (Frisch and Vercoe, 1977). In the present experiment, it was observed that Nellore cattle (zebu) possibly had a better use of nutrients in the diet and, consequently, the generation of manure with fewer nutrients, which justifies having a worse efficiency in removing nutrients in the anaerobic digestion process, when compared to Holstein (taurine) manure (Table 2).

Cumulative CH_4 production was estimated by the Gompertz curve (Figure 2). The higher R^2 indicates that the Gompertz model was able to explain the variability in the response data. The Gompertz curve is an excellent modeling tool for predicting biogas yield. Kafle and Chen (2016) reported that Gompertz model was the better model to predict CH_4 yield compared to than others models. Holstein manure showed a higher growth rate (k) for CH_4 production ($P<0.05$) than Nellore cattle. Additionally, they had a shorter time to reach the inflection point (t). The inflection point (t) for CO_2 production was also lower ($P<0.05$). The proportion of CO_2 in percentage was linearly reduced ($P<0.05$) with increasing levels of tannins in the diet (Table 3).

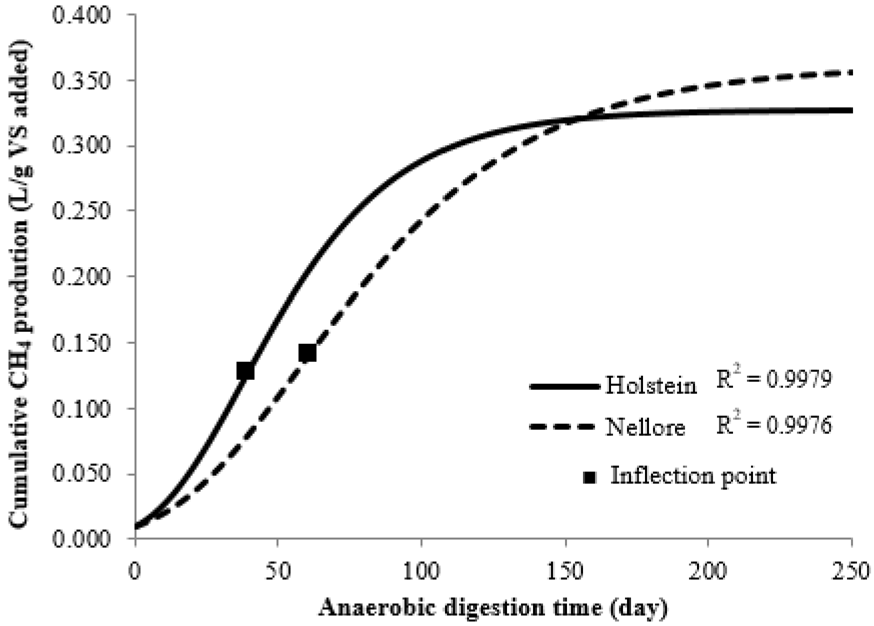

Figure 2: Cumulative CH_4 production, adjusted by the Gompertz model in anaerobic batch-type digesters supplied with manure of Nellore and Holstein cattle fed *Acacia mearnsii* tannins. The inflection point represents the day when the maximum point of CH_4 production occurred.

Tannins are known for the formation of complexes with dietary nutrients, especially with proteins, which can result in reduced nutrient digestibility and consequent increase in their excretion in feces, as can be seen in the present study, with an increase in the amounts of N, NDF, and ADF in the manure used to supply the anaerobic digesters (Table 2). According to Hristov et al (2013), the decreased nutrient digestibility is expected to increase fermentable organic matter concentration in feces (volatile solids), which can promote a tremendous anaerobic digestion for biogas production, including CH_4. However, the doses of tannins used in this study were not enough to modify the efficiency of nutrient removal.

Investigation of CH_4 production potential is a prerequisite to better predict CH_4 emission by anaerobic digestion or during storage of feces under anaerobic conditions. Moller et al. (2004) reported emissions of 0.4 L of CH_4 per gram of VS from bovine manure maintained in treatment systems with anaerobic lagoons, being close to 0.34 L/g of VS added, on average, found in the present study. However, it should be considered that the CH_4 yield of manure from different sources can be highly variable and is affected by several factors, including species, breed, animal growth stage, food, quantity and type of bedding material, as well as the degradation processes during pre-storage (Angelidaki and Ahring, 2000).

Unlike our hypothesis, tannins could not promote changes in CH_4 production. Hao et al. (2011), using 25g/kg of *A. mearnsii* CT for confined cattle, also found no increase in GHG emissions (CO_2, CH_4 and N_2O) when the manure was composted. However, Tseu et al. (2021), using the same tannin extract of this experiment (0, 0.75, 1.50, and 2.25% DM), had a quadratic effect on total biogas and CH_4 production when tannins are included above 0.75%, in addition, they suggest that tannin bioactive metabolites may appear in feces (when used to feed cows) and impair the digestion of the manure. A fact that may have contributed to the lack of change in CH_4 production in the present experiment may be due to the levels of inclusion of tannins in the diet of the animals, since these levels are considered low and were intentionally used for not cause the effect of reducing food consumption by animals due to the known astringent impact of these compounds (Grainger et al., 2009). The substrates of the different genetic groups evaluated showed that the growth rate of CH_4 and CO_2 production were higher for Holstein manure (Table 3), causing the time needed to reach the inflection point of the curve, or that is, the maximum production potential would occur 22 (Figure 2) and 25 days before the Nellore manure, respectively. This fact is of great relevance, as it can contribute to the reduction of the Residence Time (RT), which indicates the time in which the liquid fraction of manure remains in the digester in contact with the biomass (Metcalf & Eddy, 2014), being the time necessary to achieve a certain degree of waste treatment dependent on the microbial metabolism rate.

Table 3: Production of biogas, CH_4, CO_2 and N_2O in anaerobic batch-type digesters supplied with manure of Nellore and Holstein cattle fed *Acacia mearnsii* tannins

Variables	Genotype (G)		Tannin Level (TL)					Probability		
	Holstein	Nellore	0%	0.5%	1.0%	1.5%	SEM	G	N	G*TL
Biogas (L)	36.27	36.42	34.77	37.26	36.89	36.46	0.65	ns	ns	ns
CH_4	25.22	26.11	25.03	26.20	26.40	25.09	0.45	ns	ns	ns
CH_4 (%)	71.45	73.93	73.48	71.99	72.42	70.58	0.36	ns	ns	ns
CH_4/VS digested (L/g)	1.11	1.26	1.24	1.07	1.18	1.23	0.051	ns	ns	ns
CH_4/VS added										
A (L/g)	0.393	0.432	0.419	0.445	0.385	0.403	0.02	ns	ns	ns
t (day)	39.28	61.35	51.02	47.87	44.51	57.86	5.49	0.039	ns	ns
y (L/g)	0.145	0.162	0.154	0.163	0.142	0.145	0.006	ns	ns	ns
CO_2, L	11.00	10.87	9.99	11.05	11.58	11.11	0.21	ns	ns	ns
CO_2, %	28.54	28.15	27.09	28.06	29.28	28.92	0.21	ns	0.003^L	ns
CO_2/VS digested (L/g)	0.479	0.523	0.488	0.442	0.518	0.557	0.020	ns	ns	ns
CO_2/VS added										
A (L/g)	0.161	0.168	0.150	0.178	0.159	0.169	0.008	ns	ns	ns
t (day)	36.82	64.45	53.99	52.47	47.96	48.12	5.76	0.038	ns	ns
y (L/g)	0.059	0.061	0.055	0.065	0.059	0.062	0.003	ns	ns	ns
N_2O (mL)	8.02	8.95	8.868	6.948	9.336	8.784	0.38	ns	ns	ns
N_2O (%)	0.023	0.028	0.026	0.022	0.026	0.025	0.0013	0.064	ns	ns
N_2O/VS digested (mL/g)	0.362	0.496	0.461	0.391	0.428	0.437	0.030	ns	ns	ns
N_2O/VS added										
A (mL/g)	0.122	0.115	0.083	0.122	0.131	0.138	0.009	ns	ns	ns
t (day)	40.29	57.31	43.76	47.35	47.63	56.47	5.24	ns	ns	0.075
y (mL/g)	0.046	0.042	0.03	0.046	0.048	0.050	0.003	ns	ns	ns

*SEM: Standard error of the mean; G*TL: Interaction between Genotype and Tannin Level; A: Asymptotic production (L/g VS added); k: production constant (L/g of VS added per day); t: time at inflection point (day); y: production at the inflection point (L/g of VS added).*

As expected, in the present study the production of N_2O was not interfered by the type of substrate or by the tannin levels, probably because it occurs under strict experimental anaerobic conditions and due to the high C/N ratio. According to Bernet et al. (1996), when the C/N ratio is above 18 (characteristic of most animal manure) denitrification is complete and the formation of N_2O does not occur.

IV. CONCLUSIONS

Anaerobic digestion represents an alternative for waste treatment, as it not only reduces the polluting potential of waste, but also promotes the generation of biogas. The use of *Acacia mearnsii* extract up to 1.5% of the DM of the cattle diet did not promote changes in the nutrient removal efficiency, or in the production of biogas and its compounds (CH_4, CO_2 and N_2O). The production rates of CH_4 and CO_2 were influenced by the composition of the waste of the different groups of cattle evaluated, being faster for Holstein, which could be a positive factor for the reduction of the Residence Time. As there was no increase in GHG production, this is good for environmental issues. On the other hand, from the point of view of energy generation, it would be irrelevant. Therefore, this extract can be used as a food additive to modify rumen fermentation without modifying the anaerobic digestion process of cattle manure. Furthermore, we recommended more studies on livestock nutrition and anaerobic digestion of waste as an analytical tool to manage the sustainability of livestock production and the environment.

ACKNOWLEDGMENTS

To Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for financial support (grant number 201705301-0).

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