PAB
Pesquisa Agropecuária Brasileira

Animal Science/ Original Article

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

Dietary effects of cottonseed and vitamin E on greenhouse gas emissions from cattle feces analyzed in biodigesters

Abstract – The objective of this work was to evaluate the effect of diets with cottonseed and vitamin E on the greenhouse gas (GHG) emissions of cattle feces analyzed in biodigesters. Animal feces were evaluated in 18 laboratory-scale anaerobic digesters through the following treatments: control, feces from cows fed with a control diet; CS, feces from cows fed with a cottonseed diet; and CSVitE, feces from cows fed with a cottonseed and vitamin E diet. The production of biogas and GHG was measured, and no differences were observed for the emissions of biogas, methane (CH_4), and nitrous oxide (N_2O). The partition of the gross energy of the anaerobic digestion process was similar among treatments. On average, 28% of gross energy fed was released as CH_4, 47% during digestion, and 25% as other gases and heat. The addition of cottonseeds to the cattle diet increases the concentration of CH_4 and reduces that of CO_2, but it does not affect the total production of CH_4, CO_2, and N_2O in the biodigesters. The inclusion of vitamin E in the diet has no effect on GHG production in the biodigesters.

Index terms: anaerobic digestion, methane, nitrous oxide.

Efeitos dietéticos de caroço de algodão e vitamina E nas emissões de gases de efeito estufa por fezes de bovinos analisadas em biodigestores

Resumo – O objetivo deste trabalho foi avaliar o efeito de dietas com caroço de algodão e vitamina E nas emissões de gases de efeito estufa (GEE) de fezes de bovinos analisadas em biodigestores. As fezes dos animais foram avaliadas em 18 digestores anaeróbios de laboratório, por meio dos seguintes tratamentos: controle, fezes de vacas alimentadas com dieta controle; CS, fezes de vacas alimentadas com caroço de algodão; e CSVitE, fezes de vacas alimentadas com caroço de algodão e vitamina E. As produções de biogás e GEE foram medidas, e não foram observadas diferenças para as emissões de biogás, metano (CH_4) e óxido nitroso (N_2O). A partição da energia bruta do processo de digestão anaeróbica foi semelhante entre os tratamentos. Em média, 28% da energia bruta alimentada foi liberada como CH_4, 47% na digestão e 25% como outros gases e calor. A adição de caroços de algodão na dieta de bovinos aumenta a concentração de CH_4 e reduz a de CO_2, mas não afeta a produção total de CH_4, CO_2 e N_2O nos biodigestores. A inclusão de vitamina E na dieta não tem efeito na produção de GEE nos biodigestores.

Termos para indexação: digestão anaeróbia, metano, óxido nitroso.

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Received
June 29, 2022

Accepted
October 04, 2022

How to cite
NOGUEIRA, R.G.S.; PERNA JUNIOR, F.; TSEU, R.J.; RODRIGUES, P.H.M. Dietary effects of cottonseed and vitamin E on greenhouse gas emissions from cattle feces analyzed in biodigesters. **Pesquisa Agropecuária Brasileira**, v.58, e03037, 2023. DOI: https://doi.org/10.1590/S1678-3921.pab2023.v58.03037.

Introduction

The manipulation of rumen fermentation by supplementing lipids in cattle diets (Wanapat et al., 2015) is a strategy to reduce methane (CH_4) emissions by ruminants. Lipids are directly related to fatty acids and, therefore, may be increased in diets through the addition of ingredients such as cottonseed (CS), a by-product of the cotton fiber industry extensively used as a source of fiber, protein, or fat in cattle nutrition (Warner et al., 2020), due to its high concentrations of unsaturated fatty acids (Paim et al., 2014). Vitamin E has been used in animal feed to inhibit lipid oxidation (Juárez et al., 2012), relieving the effects caused by free radicals, being beneficial to rumen fermentation as it improves nutrient digestibility (Vázquez-Añón & Jenkins, 2007).

Some studies have investigated the addition of whole cottonseed and vitamin E in cattle diets (Polviset et al., 2015; Ferrinho et al., 2018; Nogueira et al., 2019, 2020). However, there are no known works associating these ingredients with the impact of feces on the environment or potential use in digesters. According to Møller et al. (2014), the effects of changes in cattle's diets on biogas and GHG emissions from feces need to be further studied.

Biogas is a mixture of CH_4 and carbon dioxide (CO_2) with some trace gases (Mata-Alvarez et al., 2014), resulting from the conversion of organic substrates during the biological process of anaerobic digestion (Zhang et al., 2016). Biogas and CH_4 production from organic matter, defined as volatile solids, have been evaluated during the testing of anaerobic digestion under controlled or monitored conditions in laboratory batch on a small scale (Kunz et al., 2022). Nogueira et al. (2020), for instance, observed that the inclusion of cottonseed in cattle's diet improved rumen fermentation and reduced rumen CH_4 production.

However, although different feeding strategies can reduce enteric CH_4 emission from stored manure, or improve ruminal conditions (Benchaar & Hassanat, 2019), the subsequent effects on GHG emissions from feces still remain unclear.

The objective of this work was to evaluate the effect of diets with cottonseed and vitamin E on the GHG emissions of cattle feces analyzed in biodigesters.

Materials and Methods

The study was conducted in the summer of 2017 at Universidade de São Paulo, located in the municipality of Pirassununga, in the state of São Paulo, Brazil (21°59'45"S, 47°25'37"W, at 625 m above sea level). The climate of the season is classified as Aw according to the Köppen-Geiger, with an average temperature of 21.7°C and an average annual rainfall of 1,346 mm. The experiment was approved by the ethics committee on animal use of Universidade de São Paulo (application number 0092013).

Six non-pregnant and non-lactating Holstein dairy cows, with an average body weight of 876±16.1 kg, were arranged in individual pens with free access to water and sand bedding. The cows were fed ad libitum twice a day at 8:00 a.m. and 4:00 p.m with a high-energy ration containing 87% of concentrates, using sugarcane bagasse as roughage. Vitamin E (Lutavit E 50, BASF Australia Limited, Victoria, Australia), with 50% alpha-tocopheryl acetate, was included at an amount of 500 IU per day. Three dietary treatments were tested: control, control diet; CS, control diet with 30% cottonseed replacing ground corn grain; and CSVitE, CS diet supplemented with vitamin E. The ingredients and chemical compositions of the diets are presented in Table 1.

For feeding and feces collection, cows were arranged in a 3x3 Latin-square design with three experimental periods, each lasting 21 days. The first 14 days of each period were used for diet adaptation. Feces were collected during the next 15 to 21 days, twice a day, after the cows were fed. Representative samples – feces of each animal in each period – were pooled together for testing. The substrates (feces and water) were prepared to ensure an estimation of 5% of total solids. The feces were loaded into 18 laboratory-scale digesters, 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).

In a chamber with controlled temperature, the anaerobic digestion test was carried out under mesophilic conditions, ideal for digestion kinetics (Metcalf & Eddy, 2014). The digesters were arranged in a completely randomized design with three feces treatments (control, CS, and CSVitE) and six replicates. The digesters were started up using the feces with no inoculum. Every 15 days, biogas volume was calculated, through the vertical displacement of the gasometer, in centimeters, and the obtained value was standardized for the conditions of 1.0 atm and 20ºC.

Every time biogas volume was measured, biogas samples were collected with a syringe connected to the gas log on top of the gasometer. The concentrations of CH_4, CO_2 and N_2O were determined using the Trace 1300 gas chromatograph (Thermo Fisher Scientific, Milan, Italy), equipped with a flame ionization detector at 280°C, with a 3.5 m column packed with Porapak N matrix (Merck, Darmstadt, Germany), according to Kamiński et al. (2003). The volumes of CH_4, CO_2, and N_2O were calculated by multiplying biogas volume by its respective concentration. Specific gas yield (per gram of volatile solids fed or destroyed) was calculated by dividing the total gas production by the amount of volatile solids fed (before anaerobic digestion) or destroyed (difference between volatile solids fed and eliminated). The test was considered finished when biogas production ceased. The nutrients fed (NF) and eliminated (NE) were then weighed to calculate dry matter (DM) content in grams.

Ingestate and digestate nutrients were obtained using the following equation (Tseu et al., 2021):

Nutrients (g) = (NF or NE – % × DM fed or eliminated – g) × 100

Table 1. Ingredients and chemical composition of dietary treatments for dairy cows.

Ingredient[1]	Dietary treatment[2]		
	Control	CS	CSVitE
Sugarcane bagasse (g kg^{-1} DM)	134	134	134
Cottonseed (g kg^{-1} DM)	-	304	304
Ground corn grain (g kg^{-1} DM)	572	281	281
Citrus pulp (g kg^{-1} DM)	183	183	183
Soybean meal (g kg^{-1} of DM)	817	817	817
Minerals (g kg^{-1} DM)	60	60	60
Limestone (g kg^{-1} DM)	40	40	40
Urea (g kg^{-1} DM)	13.7	2.7	2.7
Vitamin E (mg kg^{-1} DM)	-	-	500
Chemical composition			
Dry matter (g kg^{-1})	891	910	910
Crude protein (g kg^{-1} DM)	158	160	160
Ether extract (g kg^{-1} DM)	26.1	76.9	76.9
Neutral detergent fiber (g kg^{-1} DM)	234	357	357
Acid detergent fiber (g kg^{-1} DM)	171	265	265
Lignin (g kg^{-1} DM)	55.3	136	136
Organic matter (g kg^{-1} DM)	829	845	845
Gross energy (MJ kg^{-1} DM)	17.4	17.9	17.9

(1)DM, dry matter. (2)Control, control diet; CS, control diet with 30% cottonseed replacing ground corn grain; and CSVitE, CS diet supplemented with vitamin E.

In addition, nutrient removal (NR) was determined by the equation:

$$NR (\%) = [(NF - NE)/ NF]\times 100$$

Gross energy release, expressed as CH_4, when expressed as megajoule, was calculated using total CH_4 production in liters and considering the following information about CH_4 molecule: molar volume: 26.22 mol L^{-1}; molar mass: 16.04 g mol^{-1}; heat power: 13.16 kcal g^{-1}.

Other gases and heat released, expressed in megajoule, were determined as follows: gross energy fed minus energy released as CH_4 minus gross energy eliminated in the digestate. The percentage of gross energy released, when expressed as percentage of gross energy fed, was calculated by division between gross energy of CH_4 or other gas and heat or digestate and gross energy fed and multiplied by 100.

Individual feed and feces samples, before and after anaerobic digestion, were collected and composited in representative samples on an equal-weight basis. The samples were dried in a forced-air oven, at 60°C, for 48 hours, and then ground to pass a 1.0 mm Wiley mill screen and analyzed. DM content was determined by method 930.15 of 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). Ether extract was obtained using light petroleum ether in the Soxhlet extraction apparatus, as described in method 920.39 (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 Technology (Ankom Technology, Macedon, NY, USA) and heat-stable α-amylase as in method 973.18 (Cunniff, 1995). The levels of total solid and volatile solids were measured according to American Public Health Association (APHA) (Rice et al., 2012).

The experimental design was completely randomized, with 18 experimental units (digesters) for the three treatments (control, CS, and CSVitE) and six replicates. Data were tested for residual normality using the Shapiro-Wilk test. Statistical analyses were performed with the SAS, version 9.3, software (SAS Institute Inc., Cary, NC, USA) using mixed model, with the fixed effect of treatment. The analyses included descriptive statistics, in which mean values and standard errors of the mean were calculated. The variables were analyzed using the following model:

$$Y_{ijkl} = \mu + T_i + e_{ijk},$$

where Y_{ijkl} is the dependent response variable, μ is the overall mean, T_i is treatment effect, and e_{ij} is the residual error.

Contrast statements, at p≤0.05, were used to evaluate differences between the following treatments: control vs. CS and CSVitE; and CS vs. CSVitE.

Methane yield curve parameters were estimated from the methane yield of individual digesters using the Gompertz model (Kafle & Chen, 2016), specifically through the following three equations:

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

where y1 is methane yield at anaerobic digestion days, A is asymptotic methane yield, B is the interaction constant, k is the yield constant rate, and exp is the base of natural logarithm 2.7183.

$$t_l = \ln B / \exp$$

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

$$y_l = A / K$$

where y_l is methane yield at inflection point, A is asymptotic methane yield, and exp is the base of natural logarithm 2.7183.

Results and Discussion

Feces composition differed in accordance with the diet (Table 2). Cottonseed treatments had a higher concentration of total solids (TS), volatile solids (VS), neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin when compared to the control. This result is indicative that the digesters were not able to destroy the highest content of TS and VS present in the feces from cows fed with the diets with cottonseed, as confirmed by the similar values of TS and VS destroyed. Consequently, there was a higher elimination of TS and VS in the cottonseed treatments.

No significant differences were observed between TS destroyed and VS destroyed, whose mean values were 49.6% and 53.7%, respectively. Volatile solids destroyed are commonly used to measure the performance of processes, being a direct indicator of the metabolic activity of a microorganism community (Møller et al., 2004). The destruction of VS in cattle manure in the anaerobic digestion process is typically in the range of 30–45% (Davidsson et al., 2008). In the present study, regardless of the treatment, the destruction of TS and VS was over 45% and was indicative of a sludge stabilization process and good reactor performance in the batch digesters. As a result, feces from cattle fed with cottonseed, with or without vitamin E, did not affect biogas production.

The microorganisms present in the control degraded more protein and organic matter than those in CS and CSVitE (Table 2). These results suggest that the microorganisms in the control had to hydrolyze higher quantities of crude protein and organic matter to obtain nutrients, as reported by Coelho et al. (2022). By contrast, the microorganisms in the CS and CSVitE had to hydrolyze higher quantities of protein from the NDF provided by the cottonseeds included for nutrients. This means that both treatments showed a similar destruction of VS and TS, in addition to CH_4 yield. Although the CS and CSVitE have a higher amount of TS available for the anaerobic digestion, the nutrients

Table 2. Characteristics of feces and nutrients destroyed in batch anaerobic digesters loaded with feces from cows fed with different diets.

Variable(1)	Treatment(2)			SEM(3)	Probability(4)	
	Control	CS	CSVitE		C1	C2
TS (g kg^{-1})	18.7	22.5	22.5	0.50	*	ns
VS (g kg^{-1})	16.7	20.4	21.0	0.50	*	ns
OM (g kg^{-1} TS)	806	819	824	5.14	ns	ns
CP (g kg^{-1} TS)	127	133	133	2.26	ns	ns
EE (g kg^{-1} TS)	15.6	16.5	14.4	0.61	ns	ns
NDF (g kg^{-1} TS)	399	453	472	16.3	*	ns
ADF (g kg^{-1} TS)	309	379	412	17.1	*	ns
Lignin (g kg^{-1} TS)	99.3	187	137	14.9	*	ns
$TS_{eliminated}$ (g kg^{-1})	10.0	10.9	11.9	0.23	*	ns
$VS_{eliminated}$ (g kg^{-1})	7.92	9.06	9.81	0.21	*	ns
$TS_{destroyed}$ (%)	47.7	52.6	48.6	1.34	ns	ns
$VS_{destroyed}$ (%)	52.5	55.5	53.1	1.43	ns	ns
$OM_{destroyed}$ (%)	58.7	53.2	49.8	1.37	*	ns
$CP_{destroyed}$ (%)	54.4	44.6	38.0	2.50	*	ns
$NDF_{destroyed}$ (%)	39.6	41.1	40.6	2.61	ns	ns
$ADF_{destroyed}$ (%)	44.3	41.7	41.6	2.89	ns	ns
$Lignin_{destroyed}$ (%)	43.0	39.2	26.5	6.04	ns	ns
$EE_{destroyed}$ (%)	41.6	38.0	27.6	3.19	ns	ns

(1)TS, total solids; VS, volatile solids; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; and ADF, acid detergent fiber. (2)Control, control diet; CS, control diet with 30 % cottonseed replacing ground corn grain; and CSVitE, CS diet supplemented with vitamin E. (3)SEM, standard error of mean. *Significant at 5% probability. nsNonsignificant. (4)Contrast statements were used to evaluate differences between the following treatments: C1, control vs. CS and CSVitE; and C2, CS vs. CSVitE.

consisted of fibrous contents, i.e., of NDF, ADF, and lignin. Orrico Junior et al. (2012) concluded that a higher concentration of fiber in the diet may favor a lower reduction in TS and VS, whereas Kafle & Chen (2016) observed a strong negative relationship, with a coefficient of determination (R^2) of 0.85, between lignin concentration in manure and CH_4 emissions. Therefore, although there was an increase in lignin content in the feces in the cottonseed treatments, there was no reduction in CH_4 production.

The crude protein content of a diet is the major factor determining the percentage of excreted N (Külling et al., 2002). In the present study, a similar crude protein content was obtained for the diet and substracts used to load the digesters (Table 2). Since the crude protein content in the substracts is a N source for the process of N_2O production, a similar crude protein resulted in similar N_2O emissions. The same authors reported similar N losses and N_2O emissions for dairy manure under anaerobic conditions.

Regarding biogas, methane concentration was higher in the cottonseed treatments when compared to the control (Table 3). However, the diets with cottonseeds had a lower CO_2 concentration, CO_2 yield per gram of VS fed, and CO_2 yield per gram of VS destroyed, with no significant differences for the N_2O concentration in the biogas, total yield, yield, and Gompertz parameters. Mathot et al. (2012) only found alterations in feces characteristics but not in GHG emissions, as observed here.

To calculate the CH_4 emissions of the manure of dairy cows, the Intergovernmental Panel on Climate Change (IPCC) (Dong et al., 2006) suggests a default value of 0.240±36 L g^{-1} of VS as the maximum amount of CH_4 producing capacity of manure. In the present work, the specific CH_4 productivity, measured in terms of VS destroyed, was, on average, 0.303 L g^{-1} of VS destroyed, which is indicative of the complete degradation of the organic components of the feces. The ultimate CH_4 yield, obtained in terms of VS fed (Dong et al., 2006), was 54.0% of the theoretical CH_4 yield, confirming the reduction of 53.7% in VS. The ultimate CH_4 yield will always be lower than the theoretical yield because a fraction of the feces is used to synthesize bacterial mass; therefore, the fraction of the organic material lost in the effluent and lignin-containing compounds will only be degraded to a limited degree (Franco et al., 2007).

The cumulative CH_4 yield was estimated by the Gompertz curve (Figure 1), which explains most of the variability in the response data, confirmed by the higher R^2 values obtained. A CH_4 yield difference of 3.4% was observed between the measured and predicted values, which is in alignment with Kafle & Chen (2016), who concluded that the Gompertz model was the one that better predicted CH_4 yield. Inflection point was reached, on average, on day 38 when cumulative CH_4 represented 38% of total CH_4 yield, which increased up to day 38, after which it decreased until day 220, when yield ceased.

The cottonseed treatments had higher gross energy fed and released in the digestate when compared to

Table 3. Biogas and greenhouse gas emissions of batch anaerobic digesters loaded with feces from dairy cows fed with different diets.

Variable[(1)]	Treatment[(2)]			SEM[(3)]	Probability[(4)]	
	Control	CS	CSVitE		C1	C2
$Biogas_{total}$ (L)	7.593	8.256	8.373	0.403	ns	ns
CH_4						
$CH_{4concentration}$ (%)	74.39	79.69	80.05	0.842	*	ns
CH_{4total} (L)	5.637	6.573	6.701	0.320	ns	ns
CH_4 per VS_{fed} (L g^{-1})	0.168	0.166	0.159	0.007	ns	ns
CH_4 per $VS_{-destroyed}$ (L g^{-1})	0.320	0.290	0.300	0.014	ns	ns
t_I (day)	35.03	43.37	36.93	2.374	ns	ns
y_I (L g^{-1})	0.067	0.058	0.062	0.002	ns	ns
CO_2						
$CO_{2concentration}$ (%)	25.46	20.21	19.32	0.858	*	ns
CO_{2total} (L)	1.956	1.667	1.662	0.118	ns	ns
CO_2 per VS_{fed} (L g^{-1})	0.058	0.041	0.038	0.003	*	ns
CO_2 per $VS_{destroyed}$ (L g^{-1})	0.112	0.074	0.072	0.007	*	ns
t_I (day)	35.27	47.63	36.14	3.432	ns	ns
y_I (L g^{-1})	0.019	0.015	0.014	0.001	ns	ns
N_2O						
$N_2O_{concentration}$ (%)	0.113	0.093	0.866	0.008	ns	ns
N_2O_{total} (mL)	8.803	7.707	7.314	0.864	ns	ns
N_2O per VS_{fed} (mL g^{-1})	0.261	0.189	0.174	0.022	ns	ns
N_2O per $VS_{destroyed}$ (mL g^{-1})	0.495	0.349	0.322	0.004	ns	ns
t_I (day)	35.28	47.81	34.80	3.459	ns	ns
y_I (mL g^{-1})	0.081	0.070	0.071	0.007	ns	ns

[(1)]TS, total solids; VS, volatile solids; t_I, inflection point; and y_I, yield rate at inflection point. [(2)]Control, control diet; CS, control diet with 30 % cottonseed replacing ground corn grain; and CSVitE, CS diet supplemented with vitamin E. [(3)]SEM, standard error of the mean. [(4)]Contrast statements were used to evaluate differences between the following treatments: C1, control vs. CS and CSVitE; and C2, CS vs. CSVitE. *Significant at 5% probability. [ns]Nonsignificant.

the control (Table 4). However, no differences were observed for gross energy released when expressed as a percentage of gross energy fed. On average, 47% of the gross energy fed was released during digestion, 25% as other gas and heat, and 28% as CH_4 that can be used for electricity or heat generation.

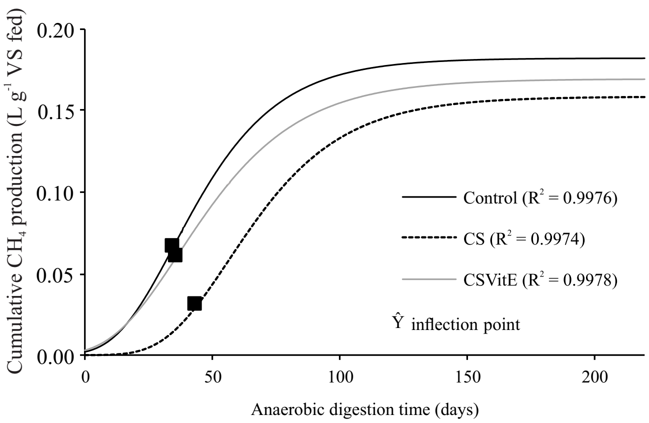

Figure 1. Cumulative CH_4 production in relation to anaerobic digestion time observed and adjusted by the Gompertz curve. The inflection point represents the day when the maximum point of CH_4 production occurred. Control, control diet; CS, control diet with 30% cottonseed replacing ground corn grain; and CSVitE, CS diet supplemented with vitamin E.

Table 4. Gross energy released and eliminated by batch anaerobic digesters loaded with feces from dairy cows fed with different diets.

Variable	Treatment(1)			SEM(2)	Probability(3)	
	Control	CS	CSVitE		C1	C2
Gross energy fed (MJ)	0.64	0.80	0.80	0.02	*	ns
Methane released (MJ)	0.18	0.22	0.22	0.01	ns	ns
Percentage of gross energy fed (%)	28.9	27.1	27.9	1.23	ns	ns
Digestate released (MJ)	0.31	0.35	0.38	0.01	*	ns
Percentage of gross energy fed (%)	48.1	44.3	48.2	1.59	ns	ns
Others gases and heat released (MJ)	0.15	0.23	0.19	0.01	ns	ns
Percentage of gross energy fed (%)	22.9	28.4	23.8	2.07	ns	ns

(1)Control, control diet; CS, control diet with 30% cottonseed replacing ground corn grain; and CSVitE, CS diet supplemented with vitamin E. (2)SEM, standard error of the mean. (3)Contrast statements were used to evaluate differences between the following treatments: C1, control vs. CS and CSVitE; and C2, CS vs. CSVitE. Statistical significance was declared at p≤0.05. *Significant at 5% probability. nsNonsignificant.

Conclusions

1. The addition of cottonseeds to cattle diets increases the concentration of CH_4 and reduces that of CO_2, but does not affect the total yield of CH_4, CO_2 and N_2O in biodigesters.

2. The inclusion of vitamin E in cattle diets has no effect on greenhouse gas production in the biodigesters.

Acknowledgment

To Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), for financial support (grant number 201311983-5).

References

BENCHAAR, C.; HASSANAT, F. Methane emissions of stored manure from dairy cows fed conventional or brown midrib corn silage. **Journal of Dairy Science**, v.102, p.10632-10638, 2019. DOI: https://doi.org/10.3168/jds.2019-16822.

COELHO, L. de M.; BRITO, L. de F.; MESSANA, J.D.; CARDOSO, A. da S.; CARVALHO, G.M.; TORRES, R. de N.S.; CARLOS, R.S.; MALHEIROS, E.B.; CRUZ, M.C.P. da; BERCHIELLI, T.T. Effects of rumen undegradable protein sources on nitrous oxide, methane and ammonia emission from the manure of feedlot-finished cattle. **Scientific Reports**, v.12, art.9166, 2022. DOI: https://doi.org/10.1038/s41598-022-13100-9.

CUNNIFF, P. (Ed). **Official Methods of Analysis of AOAC International**. 16th ed. Arlington: AOAC International, 1995. Official Methods 920.39; 930.15 and 973.18.

DAVIDSSON, A.; LOVSTEDT, C.; LA COUR JANSEN, J.; GRUVBERGER, C.; ASPEGREN, H. Co-digestion of grease trap sludge and sewage sludge. **Waste Management**, v.28, p.986-992, 2008. DOI: https://doi.org/10.1016/j.wasman.2007.03.024.

DONG, H.; MANGINO, J.; MCALLISTER, T.A.; HATFIELD, J.L.; JOHNSON, D.E.; LASSEY, K.R.; LIMA, M.A. de; ROMANOVSKAYA, A. Emissions from Livestock and Manure Management. In: EGGLESTON, S.; BUENDIA, K.M.; MIWA, K.; NGARA, T.; TANABE, K. (Ed). **2006 IPCC Guidelines for National Greenhouse Gas Inventories**: v.4: Agriculture, forestry and other land use. Kanagawa: iGES, 2006. p.1-87.

FERRINHO, A.M.; NASSU, R.T.; ALDAI, N.; BRAVO-LAMAS, L.; FURLAN, M.L.N.; TODA, B.M.; UTEMBERGUE, B.L.; REZENDE, R.G.; MUELLER, L.F.; FURLAN, J.J.M.; ZANATA, M.; BALDI, F.; PEREIRA, A.S.C. Whole cottonseed, vitamin E and finishing period affect the fatty acid profile and sensory traits of meat products from Nellore cattle. **Meat Science**, v.138, p.15-22, 2018. DOI: https://doi.org/10.1016/j.meatsci.2017.12.002.

FRANCO, A.; MOSQUERA-CORRAL, A.; CAMPOS, J.L.; ROCA, E. Learning to operate anaerobic bioreactors. In:

MÉNDEZ-VILAS, A. (Ed.). **Communicating Current Research and Educational Topics and Trends in Applied Microbiology**. Santiago de Compostela: Formatex, 2007. p.618-627.

JUÁREZ, M.; DUGAN, M.E.R.; ALDAI, N.; BASARAB, J.A.; BARON, V.S.; MCALLISTERT, T.A.; AALHUS, J.L. Beef quality attributes as affected by increasing the intramuscular levels of vitamin E and omega-3 fatty acids. **Meat Science**, v.90, p.764-769, 2012. DOI: https://doi.org/10.1016/j.meatsci.2011.11.010.

KAFLE, G.K.; CHEN, L. Comparison on batch anaerobic digestion of five different livestock manures and prediction of biochemical methane potential (BMP) using different statistical models. **Waste Management**, v.48, p.492-502, 2016. DOI: https://doi.org/10.1016/j.wasman.2015.10.021.

KAMIŃSKI, M.; KARTANOWICS, R.; JASTRZEBSKI, D.; KAMIŃSKI, M.M. Determination of carbon monoxide, methane and carbon dioxide in refinery hydrogen gases and air by gas chromatography. **Journal of Chromatography A**, v.989, p.277-283, 2003. DOI: https://doi.org/10.1016/S0021-9673(03)00032-3.

KÜLLING, D.R.; DOHME, F.; MENZI, H.; SUTTER, F.; LISCHER, P.; KREUZER, M. Methane emissions of differently fed dairy cows and corresponding methane and nitrogen emissions from their manure during storage. **Environmental Monitoring and Assessment**, v.79, p.129-150, 2002. DOI: https://doi.org/10.1023/A:1020248700255.

KUNZ, A.; STEINMETZ, R.L.R.; AMARAL, A.C. do. (Ed.). **Fundamentals of anaerobic digestion, biogas purification, use and treatment of digestate**. Concórdia: Sbera: Embrapa Suínos e Aves, 2022. 201p.

MATA-ALVAREZ, J.; DOSTA, J.; ROMERO-GÜIZA, M.S.; FONOLL, X.; PECES, M.; ASTALS, S. A critical review on anaerobic co-digestion achievements between 2010 and 2013. **Renewable and Sustainable Energy Reviews**, v.36, p.412-427, 2014. DOI: https://doi.org/10.1016/j.rser.2014.04.039.

MATHOT, M.; DECRUYENAERE, V.; STILMANT, D.; LAMBERT, R. Effect of cattle diet and manure storage conditions on carbon dioxide, methane and nitrous oxide emissions from tie-stall barns and stored solid manure. **Agriculture, Ecosystems & Environment**, v.148, p.134-144, 2012. DOI: https://doi.org/10.1016/j.agee.2011.11.012.

METCALF, L.; EDDY, H. **Wastewater Engineering**: treatment and resource recovery. 5th ed. New York: McGraw-Hill Education, 2014.

MØLLER, H.B.; MOSET, V.; BRASK, M.; WEISBJERG, R.; LUND, P. Feces composition and manure derived methane yield from dairy cows: influence of diet with focus on fat supplement and roughage type. **Atmosphere Environment**, v.94, p.36-43, 2014. DOI: https://doi.org/10.1016/j.atmosenv.2014.05.009.

MØLLER, H.B.; SOMMER, S.G.; AHRING, B.K. Methane productivity of manure, straw and solid fractions of manure. **Biomass and Bioenergy**, v.26, p.485-495, 2004. DOI: https://doi.org/10.1016/j.biombioe.2003.08.008.

NOGUEIRA, R.G.S.; PERNA JUNIOR, F.; PEREIRA, A.S.C.; CASSIANO, E.C.O.; CARVALHO, R.F.; RODRIGUES, P.H.M. Methane mitigation and ruminal fermentation changes in cows fed cottonseed and vitamin E. **Scientia Agricola**, v.77, e20180247, 2020. DOI: https://doi.org/10.1590/1678-992X-2018-0247.

NOGUEIRA, R.G.S.; PERNA JUNIOR, F.; PEREIRA, A.S.C.; RODRIGUES, P.H.M. Nutrient digestibility and changes in feeding behavior of cattle fed cottonseed and vitamin E. **Scientia Agricola**, v.76, p.112-122, 2019. DOI: https://doi.org/10.1590/1678-992X-2017-0334.

ORRICO JUNIOR, M.A.P.; ORRICO, A.C.A.; LUCAS JUNIOR, J. de; SAMPAIO, A.A.M.; FERNANDES, A.R.M.; OLIVEIRA, E.A. de. Biodigestão anaeróbia dos dejetos da bovinocultura de corte: influência do período, do genótipo e da dieta. **Revista Brasileira de Zootecnia**, v.41, p.1533-1538, 2012. DOI: https://doi.org/10.1590/S1516-35982012000600030.

PAIM, T. do P.; VIANA, P.; BRANDÃO, E.; AMADOR, S.; BARBOSA, T.; CARDOSO, C.; DANTAS, A.M.M.; SOUZA, J.R. de; MCMANUS, C.; ABDALLA, A.L.; LOUVANDINI, H. Carcass traits and fatty acid profile of meat from lambs fed different cottonseed by-products. **Small Ruminant Research**, v.116, p.71-77, 2014. DOI: https://doi.org/10.1016/j.smallrumres.2013.11.004.

POLVISET, W.; SCHONEWILLE, J.T.; EVERTS, H.; WACHIRAPAKORN, YUANGKLANG, C.; CLAEYS, E.; DE SMET, S. Effect of whole cottonseed *v. sunflower* seed on the fatty acid profile of subcutaneous fat, *longissimus dorsi* and blood of Thai Native and Holstein bulls. **Animal**, v.9, p.2072-2080, 2015. DOI: https://doi.org/10.1017/S1751731115001627.

RICE, E.W.; BAIRD, R.B.; EATON, A.D.; CLESCERI, L.E. (Ed). **Standard methods for the examination of water and wastewater**. 22nd ed. Washington: APHA, 2012.

SUNADA, N. da S.; ORRICO, A.C.A.; ORRICO JUNIOR, M.A.P.; LUCAS JUNIOR, J. de; LOPES, W.R.T.; SCHWINGEL, A.W. Anaerobic co-digestion of animal manure at different waste cooking oil concentrations. **Ciência Rural**, v.48, e20170517, 2018. DOI: https://doi.org/10.1590/0103-8478cr20170517.

TSEU, R.J.; PERNA JUNIOR, F.; CARVALHO, R.F.; SENE, G.A.; PERES, A.H.; TROPALDI, C.B.; DOS ANJOS, F.; RODRIGUES, P.H.M. Gas emission from waste of cows fed monensin and *Acacia mearnsii* Tannins. **Iranian Journal of Applied Animal Science**, v.11, p.443-455, 2021.

VAN SOEST, P.J.; ROBERTSON, J.B.; LEWIS, B.A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. **Journal of Dairy Science**, v.74, p.3583-3597, 1991. DOI: https://doi.org/10.3168/jds.S0022-0302(91)78551-2.

VÁZQUEZ-AÑÓN, M.; JENKINS, T. Effects of feeding oxidized fat with or without dietary antioxidants on nutrient digestibility, microbial nitrogen, and fatty acid metabolism. **Journal of Dairy Science**, v.90, p.4361-4367, 2007. DOI: https://doi.org/10.3168/jds.2006-858.

WANAPAT, M.; CHERDTHONG, A.; PHESATCHA, K.; KANG, S. Dietary sources and their effects on animal production and environmental sustainability. **Animal Nutrition**, v.1, p.96-103, 2015. DOI: https://doi.org/10.1016/j.aninu.2015.07.004.

WARNER, A.L.; BECK, P.A.; FOOTE, A.P.; PIERCE, K.N.; ROBISON, C.A.; HUBBELL, D.S.; WILSON, B.K. Effects of utilizing cotton byproducts in a finishing diet on beef cattle performance, carcass traits, fecal characteristics, and plasma metabolites. **Journal of Animal Science**, v.98, p.1-9, 2020. DOI: https://doi.org/10.1093/jas/skaa038.

ZHANG, Q.; HU, J.; LEE, D.-J. Biogas from anaerobic digestion processes: Research updates. **Renewable Energy**, v.98, p.108-119, 2016. DOI: https://doi.org/10.1016/j.renene.2016.02.029.