



Effect of replacement of sugarcane by oilseed press cakes in greenhouse gases and volatile fatty acids production *in vitro*

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ABSTRACT. This study aimed to evaluate the production of methane, carbon dioxide, and volatile fatty acids and changes in ruminal pH *in vitro* with oilseed press cakes inclusion, such as, cottonseed, sunflower, castor bean, moringa and jatropha at four different levels (0, 30, 50 and 70%) in replacement to the sugarcane in ruminant feeding using semi-automated *in vitro* technique. The byproduct that produced less CO₂ was cottonseed cake (p = 0.0059). The cakes that produced the least amount of CH₄ were moringa at 70% (p < 0.05) and cottonseed at 70% levels (p < 0.0001). The cakes that had the highest increases in VFAs were cottonseed and castor (p < 0.0001). Additionally, greater pH was moringa at 70% and cottonseed at 50% levels (p < 0.0001). The greater acetate concentration was 70% cottonseed cake, propionate concentration with 30% cottonseed and butyrate concentration with 50% moringa in sugarcane replace. At the 70% level, the moringa cake displayed the highest decreases in methane production and reduction in energy loss. At the 50% substitution level, the cottonseed cake is the most suitable replacement for sugarcane in order to reduce the production of greenhouse gases.

Keywords: acetate, butyrate, N-NH₃, propionate, VFAs.

Efeito da substituição de cana-de-açúcar por tortas oleaginosas sobre a produção *in vitro* de gases do efeito estufa e ácidos graxos voláteis

RESUMO. Objetivou-se avaliar a produção de metano, dióxido de carbono, ácidos graxos voláteis e mudanças no pH ruminal *in vitro* com tortas de oleaginosas: algodão, girassol, mamona, moringa e pinhão manso incluídas em quatro níveis diferentes (0, 30, 50 e 70%) em substituição à cana-de-açúcar na dieta de ruminantes, utilizando-se a técnica semiautomática *in vitro*. O subproduto que produziu menos CO₂ foi o algodão (p = 0,0059). As tortas de oleaginosas que produziram menos CH₄ foram moringa (p < 0,05) e algodão (p < 0,0001) a 70% de inclusão. As tortas que aumentaram a produção de AGVs foram algodão e mamona (p < 0,0001). Além disso, o pH aumentou nos níveis de inclusão a 70% de moringa e 50% de algodão (p < 0,0001). A maior concentração de acetato ocorreu na torta de algodão a 70%, já o propionato na torta de algodão à 30% e a maior concentração de butirato na torta de moringa à 50% em substituição à cana-de-açúcar. Em nível de 70%, a torta de moringa apresentou maior redução no metano e na energia alimentar. Em nível de substituição de 50%, a torta de algodão foi a substituição mais adequada para a cana-de-açúcar, a fim de reduzir a produção de gases com efeito de estufa.

Palavras-chave: acetato, butirato, NH₃, propionato, AGVs.

Introduction

Research involving the production of methane (CH₄) and carbon dioxide (CO₂) has globally increased due to changes in the ozone layer. Brazil has the world's second largest cattle herd and has signed the Kyoto Protocol; therefore, the country monitors the amount of greenhouse gases produced by the national herd. Methane is the second main greenhouse gas, contributing to approximately 15% of all gases to global warming (Hristov et al., 2013; Muller & Aubert, 2014).

The gases and volatile fatty acids (VFAs) production derived from rumen fermentation depends on the feed characteristics, the intake and digestibility of the feed by animal (Moreira et al., 2014; Oliveira et al., 2015). Thus, there is the possibility that gas mitigation could be accomplished by modifying the rumen fermentation through changes of diet and thus manipulating the microbiota of the rumen with feed additives or components naturally present in oilseed press cake (Morais et al., 2015; Silva et al., 2016).

The byproducts originating from biodiesel production have been presented as potential ingredients for ruminant diets to replace conventional ingredients as in the case of sugarcane, contributing to the methane mitigate and improve the microbiological fermentation of the rumen and consequently the performance of animals. Thus, studies aimed at characterizing the ruminal metabolism of these byproducts (e.g., by measuring gas production *in vitro*) are needed to identify potential oilseed press cakes that could be used efficiently in the diet of ruminants (Gonzaga Neto et al., 2015; Medeiros et al., 2015; Cerutti et al., 2016).

These tests must determine whether an adequate supply of these feeds is available and the feed ingredients do not harm animal health, development and production. Thus, this study aims to evaluate the production of CH₄, CO₂, VFA and pH from several oilseed press cakes: cottonseed (*Gossypium hirsutum* L.), sunflower (*Helianthus annuus* L.), castor bean (*Ricinus communis*), moringa (*Moringa oleifera* L.) and Jatropha (*Jatropha curcas* L.) from the production of biodiesel when replacing sugarcane.

Material and methods

This research was conducted at the Experimental Station of Coronel Pacheco, MG (owned by Embrapa Gado de Leite – CNPGL), Minas Gerais State, Brazil. This study was carried out in strict accordance with the recommendations in the Guide for the National Council for Animal Experiments Control (Concea). The protocol was approved by the Committee on the Ethics of Animal Experiments of the Federal University of Piau , Piau  State, Brazil (Permit Number: 16-2014).

Feeds and substrates

In this experiment, the following oilseed press cakes were used: cottonseed (*Gossypium hirsutum* L.), sunflower (*Helianthus annuus* L.), castor bean (*Ricinus communis*), moringa (*Moringa oleifera* L.), jatropha (*Jatropha curcas* L.) and sugarcane from the experimental field of Embrapa in Coronel Pacheco, Minas Gerais State. Approximately 300 g sample of each byproduct, derived from the processing and/or extraction of vegetable oil to produce fuel for the biodiesel industry, was collected. Subsequently, samples were transported to the laboratory, packaged and stored at 5°C prior to analysis.

In the laboratory, the samples belonging to each oilseed press cake were mixed and homogenized to form a single sample. From this sample, a portion (100 g) was used for chemical analysis, and the

remainder of the material was used to formulate the test diets. Each diet was formulated to evaluate various levels of cakes, with ratios of 100/0, 70/30, 50/50 and 30/70% (sugarcane/cakes), as fed basis.

Chemical analyses

The substrates consisting of forage materials and the oilseed press cakes were pre-dried in forced air ovens at 55°C for 72 hours and then ground in a Willey mill equipped with a 1.0 mm sieve to determine dry matter (DM) (Method 967.03 - Association of Official Analytical Chemists [AOAC], 1990), ash (Method 942.05 -AOAC, 1990), crude protein (CP) (Method 981.10 - AOAC, 1990), and ether extract (EE) (Method 920.29 - AOAC, 1990). To determine the NDF and ADF contents, the methodology of Van Soest, Robertson, and Lewis (1991) was used with the modifications that were proposed in the Ankon device manual (Ankon Technology Corporation, Macedon, New York, US). Acid detergent lignin (ADL) was determined according to method 973.18 (Association of Official Analytical Chemists [AOAC], 2002), in which the ADF residue was treated with 72% sulfuric acid. The proportion of ingredients and chemical composition are shown in Table 1. The total carbohydrate (TC) values were determined using the equation described by Sniffen, O'Connor, Van Soest, Fox, and Russel (1992): TC = 100 - (% CP + % EE + % Ash).

Table 1. Chemical composition (g kg⁻¹ DM) of sugarcane and oilseed press cakes.

Ingredients	DM	CP	NDF	ADF	ADL	EE	ASH	IVDMD	TC	NFC
Sugarcane	270.4	22.5	518.2	362.2	40.4	11.9	48.5	554.6	917.1	646.7
Cottonseed	922.9	549.9	303.6	207.7	32.1	40.3	68.3	595.6	341.5	37.9
Sunflower	914.5	329.4	439.7	384.0	120.4	162.0	41.3	463.1	467.3	85.1
Castor bean	912.6	420.2	423.3	383.4	154.4	43.8	42.3	497.1	493.6	61.6
Moringa	901.2	577.6	202.7	80.5	10.3	84.8	49.8	791.3	287.8	27.6
Jatropha	920.7	356.9	391.4	334.5	43.4	110.6	79.5	571.3	453.0	74.1

Abbreviations: DM, Dry Matter; CP, Crude Protein; NDF and ADF, Neutral and Acid Detergent Fiber; ADL, Acid Detergent Lignin; EE, Ether Extract; ASH, Ashes; IVDMD, *In Vitro* Dry Matter Degradability; TC, Total Carbohydrates; NFC, Non Fiber Carbohydrates.

In vitro digestibility

In vitro digestibility of the dry matter (DDM) was performed according to the methodology of Tilley and Terry (1963). Three fistulated steers were used for this *in vitro* test. The ruminal contents were collected in the morning, transferred to preheated thermo-flasks (39°C) and transported immediately to the laboratory. In the laboratory, the rumen fluid was filtered using three layer of gauze. A mineral buffer solution was later added to the rumen fluid in a water bath maintained at 39°C with continuous CO₂ injection. The samples were incubated and

rotated for 72 hours. Peptides were added to the incubators within 48 hours to act as an intermediate compound for the microorganisms.

***In vitro* incubations**

The substrates used for the *in vitro* incubations were cakes of biodiesel chain, which was used to replace the sugarcane in ratios of 100/0, 70/30, 50/50 and 30/70% (sugarcane/cakes). The feed ingredients were dried at 55°C for 24 hours and then ground to paste through a 1 mm screen. Each *in vitro* incubation was conducted according to the methods reported by Meale, Chaves, Baah, and Mcallister (2012). The entire incubation procedure was repeated twice (*i.e.*, two incubation runs x three replicates per treatment, resulting in a total of six replicate vials per treatment).

The inoculum for the *in vitro* incubation was obtained from the three ruminally fistulated cows that were grazing on beard grass supplemented with 2 kg of concentrate (22 g kg⁻¹ CP and 12.6 g kg⁻¹ NDF in DM). Rumen fluid was collected two hours before morning milking from four distinct sites in the rumen, filtered through four layers of cheesecloth, combined in equal portions from each animal and immediately transported in a pre-warmed Thermos® flask to the laboratory. Inocula were prepared by mixing the rumen fluid with a mineral buffer and 0.5 mL of cysteine sulfide solution (Vitti et al., 1999) in a ratio of 1:5. The inoculum (30 mL) was then transferred into pre-loaded, pre-warmed (39°C) vials under a stream of O₂-free N gas. The vials were sealed and placed on an orbital shaker rack set at 120 oscillations per minute in an incubator set at 39°C.

The net gas production of each vial was measured at 6:00, 12:00, 24:00 and 48:00 hour of incubation with a water displacement apparatus (Fedora & Hruday, 1983). At 6:00 and 12:00 hour prior to the gas measurement, the headspace gas was sampled from each vial with a 20 mL syringe and immediately transferred into a 5.9 mL evacuated Exetainer® (Labco Ltd., High Wycombe, Buckinghamshire, UK), which was analyzed to determine the CH₄ concentration using gas chromatography. CH₄ and CO₂ concentration was expressed as mL g⁻¹ of incubated DM. After the gas was sampled for CH₄ and CO₂ was measured at 48 hour of incubation, the fermentation vials were opened, and the pH of the ruminal fluid was measured using a pH meter (Orion Model 260A,

Fisher Scientific, Toronto, ON, Canada). The ANKOM® bags containing the residues were then removed from the bottles, rinsed thoroughly with distilled water and dried at 55°C for 48 hour to a constant weight to estimate the IVDMD.

A subsample (1.6 mL) of the ruminal fluid from each vial was transferred to a 2 mL micro-centrifuge tube and centrifuged at 14,000 x g for 10 min at 4°C (Spectrafuse 16M, National Labnet Co., Edison, NJ, USA) to precipitate the particulate matter and proteins. The supernatant was frozen at -20°C until the analysis of the VFAs concentrations. The 0 hour samples were also analyzed for ammonia-N and VFA to calculate net ammonia-N and net total VFAs production (Holtshausen et al., 2009).

Statistical analyses

The experimental design used was completely randomized in a 5 x 4 factorial arrangement (cakes and substitution levels). Four experimental observations were removed from the initial four periods, and an additional period was added to the experiment to provide 4 replications per treatment. Effects were considered significant at a p value of ≤ 0.05. The results of increasing levels were interpreted through regression models using Proc Reg of Statistical Analysis System (SAS, 2004). Interaction effects were assessed using Tukey's test between the cakes within each level of substitution and levels substitution within each oilseed press cake.

Results

From the results of the chemical composition shown in Table 1, the sugarcane contained 22.5 g kg CP⁻¹ and 11.9 g kg EE⁻¹. The moringa and cottonseed cakes showed the highest CP and *in vitro* dry matter digestibility. For levels of neutral detergent fiber, acid detergent fiber and ash were highest in the castor bean and sunflower cake. Jatropha cake had greater amounts of lipids.

The production of methane (CH₄) was dependent of the substitution of sugarcane with oilseed press cakes (Table 2). All cakes increased the production of CH₄ compared to sugarcane, and moringa and cottonseed cake produced the lowest amount of CH₄ (p < 0.05). Other cakes led to relatively high CH₄ production; however, 70% castor bean cake substitution (p < 0.0001) produced the highest amount of CH₄ among all cakes tested at all levels.

Table 2. Mean values, probability (p value) and regression equations of the effects on production of CH₄ (mL g⁻¹) and CO₂ (mL g⁻¹) of substitution of sugarcane by oilseed press cakes.

Cakes	Levels of substitution				Regression Equations	R ²	p value
	0%	30%	50%	70%			
Methane production (CH ₄ ; mL g ⁻¹)							
Cottonseed	2.28	4.27 ^b	4.39 ^b	5.63 ^b	$\hat{Y} = 2.4616 + 0.449x$	0.6881	< 0.0001
Sunflower	2.28	7.65 ^a	5.95 ^{ab}	8.02 ^b	$\hat{Y} = 3.2892 + 0.0718x$	0.5220	0.0004
Castor bean ¹	2.28	5.52 ^{ab}	6.54 ^a	11.58 ^a	$\hat{Y} = 2.4668 + 0.0385x + 0.0012x^2$	0.8727	< 0.0001
Moringa	2.28	5.15 ^{ab}	3.48 ^b	1.96 ^c	$\hat{Y} = 3.4618$	0.0061	> 0.05
Jatropha ²	2.28	6.76 ^a	5.87 ^{ab}	7.13 ^b	$\hat{Y} = 2.4785 + 0.1552x - 0.0013x^2$	0.7121	< 0.0001
Carbon Dioxide (CO ₂ ; mL g ⁻¹)							
Cottonseed	35.43	33.14 ^b	25.30 ^c	26.53 ^d	$\hat{Y} = 35.6909 - 0.1490x$	0.2968	0.0059
Sunflower ³	35.43	33.49 ^b	27.66 ^c	59.84 ^a	$\hat{Y} = 36.9540 - 0.8055x + 0.0156x^2$	0.6579	< 0.0001
Castor bean ⁴	35.43	30.25 ^b	35.42 ^b	44.63 ^b	$\hat{Y} = 35.3254 - 0.3710x + 0.0072x^2$	0.4509	0.0018
Moringa ⁵	35.43	53.23 ^a	54.81 ^a	30.81 ^c	$\hat{Y} = 35.3254 - 0.3710x + 0.0072x^2$	0.8303	< 0.0001
Jatropha	35.43	36.69 ^b	27.57 ^c	27.33 ^d	$\hat{Y} = 36.9554 - 0.1387x$	0.3599	0.0019

Means in the same column with different letters differ statistically by the Tukey test at 5% probability. ¹Pmin. = 16.0%; ²Pmax. = 59.7%; ³Pmax. = 25.8%; ⁴Pmax. = 25.7%; ⁵Pmax. = 33.9%.

All oilseed cakes increased the production of CH₄. With moringa and cottonseed cake the increase was lower (p < 0.05) than with the other cakes. The highest increase in CH₄ production was observed with castor bean cake that had a minimum level of 16% when producing 1.54 mL g DM⁻¹ CH₄, and 59% of jatropha cake was the maximum point when producing 7.11 mL g DM⁻¹ CH₄. The production of carbon dioxide (CO₂) was dependent on cakes level used to replace sugarcane. In Table 2, we note that the most effective cakes substituted for sugarcane to reduce CO₂ production were cottonseed (p = 0.0059) and jatropha (p = 0.0019).

Sunflower, castor bean and moringa cake the greater level of addition (70%) produced the greater the amount of CO₂. Already for moringa, 30 and 50% levels in replacing sugar cane had the greater

CO₂ production; the sunflower cake at 70% level produced the most amount of CO₂ (p < 0.0001). Analyzing the equations of the four levels of each replacement byproduct, the oilseed press cakes of cottonseed and jatropha have decreasing linear equations where as sunflower cake, castor bean and moringa have quadratic equations. In summary, 25.8% sunflower cake replacing sugar cane produced 26.65 mL g DM⁻¹ CO₂, castor bean at 25.7% sugar cane substitution produced 30.54 mL g DM⁻¹ CO₂ and moringa cake 33.9% of sugar cane substitution produced 50.27 mL g DM⁻¹ CO₂.

The oilseed press cakes of moringa, among the five tested cakes, showed decreased acetate production; even at a high substitution level of 70%, the acetate production levels were lower than with sugarcane (p < 0.0001) (Table 3).

Table 3. Mean values, probability (p value) and regression equations illustrating of the effects on production of volatile fatty acids (VFAs) of substitution of sugarcane by oilseed press cakes.

Cakes	Levels of substitution				Regression Equations	R ²	p value
	0%	30%	50%	70%			
Concentration of Acetate (μmol mL ⁻¹)							
Cottonseed	24.49	30.48 ^a	32.52 ^a	34.58 ^a	$\hat{Y} = 25.1481 + 0.1433$	0.9102	< 0.0001
Sunflower ¹	24.49	31.52 ^a	31.39 ^{ab}	31.96 ^{ab}	$\hat{Y} = 24.6605 + 0.2828x - 0.0026x^2$	0.6318	< 0.0001
Castor bean	24.49	29.40 ^a	32.46 ^a	33.20 ^a	$\hat{Y} = 25.0448 + 0.1292x$	0.8621	< 0.0001
Moringa ²	24.49	20.20 ^b	21.17 ^c	23.25 ^c	$\hat{Y} = 24.4211 - 0.2167x + 0.0020x^2$	0.7048	< 0.0001
Jatropha ³	24.49	28.94 ^a	29.50 ^b	29.50 ^b	$\hat{Y} = 24.5433 + 0.1918x - 0.0018x^2$	0.8738	< 0.0001
Concentration of Propionate (μmol mL ⁻¹)							
Cottonseed ⁴	21.80	25.22 ^a	20.48 ^a	17.82 ^a	$\hat{Y} = 22.0853 + 0.1625x - 0.0033x^2$	0.7254	< 0.0001
Sunflower	21.80	23.95 ^a	16.68 ^b	17.49 ^a	$\hat{Y} = 23.0711 - 0.0823x$	0.3263	0.0070
Castor bean ⁵	21.80	25.17 ^a	21.02 ^a	18.14 ^a	$\hat{Y} = 22.0368 + 0.1696x - 0.0033x^2$	0.7054	< 0.0001
Moringa ⁶	21.80	18.62 ^b	17.15 ^b	17.74 ^a	$\hat{Y} = 21.8604 - 0.1581x + 0.0014x^2$	0.6333	< 0.0001
Jatropha ⁷	21.80	23.20 ^a	18.77 ^a	16.37 ^b	$\hat{Y} = 22.0349 + 1.2162x - 0.0023x^2$	0.7246	< 0.0001
Concentration of Butyrate (μmol mL ⁻¹)							
Cottonseed	10.30	10.18 ^b	10.58 ^b	9.45 ^a	$\hat{Y} = 10.4578$	0.1199	> 0.05
Sunflower	10.30	9.95 ^b	7.97 ^c	8.68 ^a	$\hat{Y} = 10.3363 - 0.02956x$	0.2760	0.0084
Castor bean	10.30	10.31 ^b	10.38 ^b	9.70 ^a	$\hat{Y} = 10.4328$	0.0738	> 0.05
Moringa ⁸	10.30	12.43 ^a	11.77 ^a	7.49 ^a	$\hat{Y} = 10.2311 + 0.1744x + 0.0036x^2$	0.8528	< 0.0001
Jatropha ⁹	10.30	10.27 ^b	10.38 ^b	8.66 ^a	$\hat{Y} = 10.2315 + 0.0357x - 0.0008x^2$	0.4395	0.0023
Acetate: propionate ratio							
Cottonseed	1.12	1.20a	1.58b	1.94a	$\hat{Y} = 0.284x + 0.75$	0.942	< 0.0001
Sunflower	1.12	1.31a	1.88a	1.82b	$\hat{Y} = 0.267x + 0.865$	0.842	< 0.0001
Castor bean	1.12	1.16a	1.54b	1.83b	$\hat{Y} = 0.251x + 0.785$	0.926	< 0.0001
Moringa	1.12	1.08b	1.23c	1.31c	$\hat{Y} = 0.072x + 1.005$	0.787	< 0.0001
Jatropha	1.12	1.24a	1.57b	1.80b	$\hat{Y} = 0.237x + 0.84$	0.972	< 0.0001

¹Means in the same column with different letters differ statistically by the Tukey test at 5% probability. ¹Pmax. = 54.0%; ²Pmax. = 37.0%; ³Pmax. = 53.0%; ⁴Pmax. = 24.0%; ⁵Pmax. = 25.0%; ⁶Pmax. = 56.0%; ⁷Pmax. = 16.0%; ⁸Pmax. = 29.0%; ⁹Pmax. = 22.0%.

The greater acetate concentration was presented by cottonseed and castor bean cake ($p < 0.0001$) (Table 3). However, the sunflower cake showed a constant production of acetate regardless of the substitution levels. Observing the equations presented in the acetate production, the oilseed press cakes of cottonseed and castor bean showed increasing linear equations, while the other cakes showed quadratic equations. The maximum points occurred for 54% substitution with sunflower cake (producing 32.35 mmol mL⁻¹ acetate), 53% substitution with jatropa cake (producing 29.65 mmol mL⁻¹ acetate) and 37% substitution with moringa cake (producing 20.37 mmol mL⁻¹ acetate).

Propionate concentration indicated that VFA production decreased for all cakes, where as the 30% substitution level with any of the cakes, except for moringa, increased the propionate production. At substitution levels of 50 and 70%, the cakes showed a decreased production of propionate ($p < 0.0001$), and castor bean cake produced more propionate than the other cakes at the three levels tested ($p < 0.05$). Observing the equations, the sunflower cake had an increasing linear equation, the other cakes showed quadratic equations. The maximum points occurred with 24% cottonseed cake substitution leading to the production of 24.08 mmol mL⁻¹ VFA, 25% castor bean cake substitution leading to the production of 24.21 mmol mL⁻¹ VFA, 56% moringa cake substitution yielding 21.85 mmol mL⁻¹ VFA and the 16% jatropa cake leading to the production of 22.64 mmol mL⁻¹ VFA.

The production of butyrate from substitutions with the cottonseed, castor bean and jatropa cakes were stable, except for the butyrate production from 70% oilseed press cake substitutions, for which there was a decrease in the butyrate production ($p < 0.0001$).

The sunflower cake showed the lowest butyrate production levels. Adding moringa cake did not increase the production of butyrate at 30 and 50% substitution levels; however, the butyrate production decreased at the 70% level. According to the regression equations, the sunflower cake showed a decreasing linear equation and the moringa and jatropa cakes showed quadratic equations. The minimum point occurred with the substitution of moringa cake at a level of 29% leading to the production of 13.36 mmol mL⁻¹ butyrate and minimalmaximum point to jatropa cake at a level of 22% leading to the production of 10.62 mmol mL⁻¹ butyrate.

Regarding the ratio of acetate to propionate, all cakes and levels displayed an increase in this ratio

($p < 0.0001$). Comparing the cakes within the levels of substitution to sugar cane, moringa had the lowest proportions acetate: propionate while cottonseed and sunflower showed the greatest relationship. This increase in the ratio indicated that with increasing levels of substitution of sugarcane by cakes, an increased production of acetate occurred compared to propionate, except for 0 to 30% for which the opposite occurred.

Changes in pH (Table 4) were dependent on the levels of replacement of sugarcane with the cakes. With the addition of the cakes, no increase in the pH was noted for all levels. The highest pH values at the 30% substitution level occurred with the moringa cake, while at 50% substitution level, the highest pH was induced the sunflower cake. The highest pH values were observed in the castor bean and jatropa cakes at the 70% substitution level ($p < 0.0001$). From the equations, all cakes showed increasing linear equations for pH.

Table 4. Mean values, probability (p value) and regression equations of the pH when substituting sugarcane by oilseed press cakes.

Cakes	Levels of substitution				Regression Equations	R ²	p value
	0	30%	50%	70%			
Cottonseed	4.62	4.97 ^a	5.17 ^b	5.48 ^c	$\hat{Y} = 4.6127 + 0.0120x$	0.8426	< 0.0001
Sunflower	4.62	5.02 ^a	5.61 ^a	5.56 ^a	$\hat{Y} = 4.6496 + 0.0148x$	0.6026	< 0.0001
Castor bean	4.62	5.01 ^a	5.44 ^a	5.73 ^a	$\hat{Y} = 4.5977 + 0.0162x$	0.8912	< 0.0001
Moringa	4.62	5.10 ^a	5.38 ^a	5.32 ^b	$\hat{Y} = 4.7103 + 0.0107x$	0.6858	< 0.0001
Jatropa	4.62	5.02 ^a	5.35 ^a	5.71 ^a	$\hat{Y} = 4.5981 + 0.0154x$	0.8997	< 0.0001

Means in the identical column with different letters differ statistically by the Tukey test at 5% probability.

Discussion

The low protein values of the sugarcane (22.5 g kg⁻¹), compared to the cakes, support the need to utilize alternative sources that can correct this deficiency. Feed supplied to ruminants with a protein deficiency can cause a reduction in the degradability by the rumen microorganisms. Reducing the digestibility of feed decreases the utilization of nutrients by the animal, causes damage in various systems of the ruminant (digestive, reproductive, immune) and reduces animal production. Thus, the use of cottonseed (549.9 g kg⁻¹), sunflower (329.4 g kg⁻¹), castor bean (420.2 g kg⁻¹) moringa (577.6 g kg⁻¹) and jatropa cakes (356.9 g kg⁻¹) can correct this deficiency and improve the digestibility of the diet for the animals.

Depending on the type of feed supplied to a ruminant, fermentation and the production of gases (CO₂, CH₄ and VFAs) respond differently. With the addition of the cottonseed and moringa cakes, no reduction in CO₂ production was observed. This lack of change was because of the incubation was

rich in proteins. Substrates high in protein may to reduce the production of CO₂ (Khazaal, Dentinho, Ribeiro, & Ørskov, 1995; Manatbay, Cheng, Mao, & Zhu, 2014). Therefore, CO₂ production decreases with increases in the level of cakes, even in moringa that had the highest production among all cakes.

However, the castor bean and sunflower cakes had a greater CO₂ production with increasing levels of substitution. According to Mizubuti et al. (2011), the production of acetate yields two moles of CO₂ per mole of glucose produced. By monitoring acetate production (Table 3), we noted that castor bean and sunflower cakes showed increased production of acetate. CO₂ production occurs during the formation of acetate and butyrate, along with H₂ production during the feed fermentation. The results for the jatropha cake may be due to the high quantity of fat that coats the jatropha fibers. This high quantity of fat consequently causes difficulties for the rumen microbial attack on the feed. Subsequently, this difficulty caused antinutritional effects for the microorganisms, thus causing inefficiency and reducing the availability of cations by combining with the fatty acids (Becker et al., 2014). Overall, this reduces the degradability of the cakes and can reduce the production of gases.

The main pathway for methanogenesis requires CO₂ and H₂, and methanogenesis occurs at greater production rate of H₂. During this fermentation, the production of acetic and butyric acids increases (Williams, Fisher, Berrisford, Moate, & Reynard, 2014). The CH₄ production presented by the cottonseed cake may have occurred because of the poor release of H₂ in the *in vitro* environment. The production of CO₂ from the cottonseed cake was low; therefore, the methane production was lower. Moringa cake was hemagglutinating and bactericidal to the rumen.

Ruminants use VFAs as a source of energy (Martin, Morgavi, & Doreau, 2009); the production of VFA is directly influenced by the amount of structural and non-structural carbohydrates. As ethyls, increase for fat, propionate increasingly contributes to the production of butyrate. Butyrate is used as the main supplier of energy for the animal, thus resulting in the tables of the chemical composition and the production of acetate, propionate and butyrate. Sunflower and castor bean cakes showed increasing levels of acetate. These greater levels of acetate can be explained by the amount of NDF and ADF, which contributes to the formation of acetate and butyrate production. Increases in NDF and ADF increase the VFAs, H₂ and CO₂ produced. These greater concentrations increase methane production.

The cottonseed cake had a greater acetate production. Horner, Coppock, Schelling, Labore, and Nave (1998) showed that diets with cottonseed increases in the concentration of acetate 15-30%. This increase is a result of the fermentation of the cottonseed fiber, which is highly digestible and produces high amounts of acetate. However, the moringa cake produced less acetate and butyrate for all addition levels. This lower production occurred because moringa cake have bactericidal lectins that bind to carbohydrates, lipopolysaccharide and teichoic acid. Therefore, the presence of these lectins cause decreased ruminal flora, reduced fermentation and decreased production of acetate and butyrate (Martínez-Fernández et al., 2014). This oilseed press cake also decreases the production of propionate. This decrease may have occurred because the moringa cake display cationic properties. According to Olivares-Palma et al. (2013), moringa will kidnap H₂ thus decreasing the production of propionate (glucose + 2 H₂) and further reduce the production of methane.

The sunflower and jatropha cake showed a decrease in acetate production because pH increased lipids in the diet. Lipids cover fibrous feed, reducing the degradability, production of acetate and production of propionate. The ether extract assists in the removal of the H₂ + free rumen by biohydrogenation. Removing the H₂ process will decrease the production of propionate.

The low pH values can be justified by the presence of soluble carbohydrates contained mainly in the sugarcane, which have been slightly degraded in the rumen. According to Owens and Goetsch (1993), the low amount of acetate and high concentration of propionate directly influences the pH. The acetate:propionate ratio increased with increased levels of these cakes. Therefore, the pH was initially low, and with the addition of cakes, the pH values increased. Whereas low pH values are not suitable for the maintenance of ruminal microflora, these values are not notable below 70% substitution levels of sugarcane with cakes.

Conclusion

According with *in vitro* tests the moringa (70%) and cottonseed (50%) cakes promote (70%) highest reduce greenhouse gases promoting reduction in energy loss. The highest acetate concentration was 70% cottonseed cake, propionate concentration with 30% cottonseed and butyrate concentration with 50% moringa in sugarcane replace.

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