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In vitro degradability and total gas production of biodiesel chain byproducts used as a replacement for cane sugar feed

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ABSTRACT. This study aimed to determine the in vitro degradability of dry matter and the total gas production of oil seed press cake from biodiesel production (*Gossypium hirsutum* L., *Helianthus annuus* L., *Ricinus communis*, *Moringa oleífera* L. and *Pinhão manso curcas* L.) at four different levels of replacement (0, 30, 50, and 70%) for cane sugar (*Saccharum officinarum* RB.) in ruminant feed. Inocula were prepared using the ruminal fluid of three Holstein cows, and data were collected after 48 hours of incubation. The byproducts of *Moringa* had the highest degradability, and castor presented the lowest values at all evaluated levels of replacement. Castor bean byproduct showed the highest total gas production, cotton showed the lowest production, and the byproduct of *Moringa* at the 70% level showed the best ruminal fermentation results. These results demonstrate that the use of oil seed press cake from biodiesel production (*Helianthus annuus* L. and *Ricinus communis*) can replace cane sugar in ruminant feed.

Keywords: fermentation, ruminant, alternative foods, methane.

Degradabilidade *in vitro* e produção de gás total de coprodutos da cadeia do biodiesel em substituição a cana-de-açúcar

RESUMO. Objetivou-se determinar a degradabilidade *in vitro* da matéria seca e produção de gás total dos coprodutos da produção de biodiesel (*Gossypium hirsutum* L., *Helianthus annuus* L., *Ricinus communis*, *Moringa oleífera* L. e *Jatropha curcas* L.) sobre a produção de gases em quatro diferentes níveis (0, 30, 50 e 70%) de substituição à cana-de-açúcar (*Saccharum officinarum* RB.) na alimentação de ruminantes. Os inóculos foram produzidos com o liquido ruminal de três vacas holandesas, as amostras foram incubadas e as coletas dos dados foram feitas após 48 horas de incubação. Dentre os coprodutos testados o que apresentou maior degradabilidade foi o da moringa e o que apresentou a menor foi o da mamona em todos os níveis avaliados. Para a produção total de gás o coproduto que apresentou a maior produção de gás foi o da mamona e o que obteve a menor produção foi o do algodão, sendo que o coproduto da moringa no nível de 70% foi o que apresentou os melhores resultados de fermentação ruminal seguido dos coprodutos do girassol e da mamona, podendo substituir a cana-de-açúcar na alimentação dos ruminantes.

Palavras-chave: fermentação, ruminantes, alimentos alternativos, metano.

Introduction

The growth of farm cattle for meat and milk production has led to increased research on animal nutrition using alternative foods because the forages that comprise the main ingredients in ruminant feed may not meet the growing demand. Thus, the study of byproducts from biodiesel production as an alternative for animal feed allows for an increased nutritional value of the diet at a low cost and increases the competitiveness of the supply chain by reducing prices. In Brazil, a large amount of agricultural byproducts are produced, and agribusiness products with potential use in animal feeding, especially those from the biodiesel chain (pies and sharps); can be used as sources of nutrients for animals for immediately degradation in the rumen.

However, when using agricultural industry byproducts as alternative food, it is necessary to evaluate these materials with respect to the performance and health of the animals (AZEVEDO et al., 2013). The proper use of the byproducts further enables the production chain of biodiesel, although it is necessary to obtain knowledge of certain factors such as the storage and the need for treatments to improve their nutritional value, thereby reducing the deleterious effects towards ruminal microorganisms as well as the consequent losses that may occur in dairy farming or cutting (MIZUBUTI et al., 2011).

Specific tests of the feed supplied to ruminants have been developed to determine the *in vitro* gas production. The in vitro gas production technique of (BUENO et al., 2008) is based on ruminal fermentation simulation in flasks inoculated with rumen micro-organisms and has been used to study the effects of foods containing bioactive factors with respect to the ruminal degradability of the organic matter.

The aim of this study was to determine the *in vitro* degradability of dry matter and the total gas production of byproducts of cotton (*Gossypium hirsutum* L.), sunflower (*Helianthus annuus* L.), castor bean (*Ricinus communis*), moringa (*Moringa oleifera* L.) and pinhão manso (*Pinhão manso curcas* L.) resulting from biodiesel production to replace cane sugar (*Saccharum Officinarum* RB.).

Material and methods

An experiment was conducted at the Experimental Field José Henrique Bruschi, Coronel Pacheco, which is owned by Embrapa Dairy Cattle and located in the Zona da Mata of Minas Gerais – Minas Gerais State. The coordinates are 21° 33' 22 south latitude and 43º 06' 15 west longitude, and the altitude is 414 m. The climate is Cwa (mesothermal), according to the Köppen classification, with an annual average rainfall of 1.600 mm. The average annual temperature is 22.5ºC with an average relative humidity approximately 77%.

The sugar cane used as a control for the in vitro incubations was cut at 365 days of age. The biodiesel byproducts of cotton (*Gossypium hirsutum* L.), sunflower (Family Asteraceae; *Helianthus annuus*), castor (Family Euphorbiaceae; *Ricinus communis*), moringa (Family Moringaceae; *Moringa oleifera* Lam.) and pinhão manso (*Pinhão manso curcas*) were derived from the processing of vegetable oil extracts.

The substrates consisting of the material and the byproducts were pre-dried in an oven with forced air at 55°C for 48 hours. To determine the dry matter (DM), the materials were processing in a Wiley mill equipped with a screen with 1.0 mm perforations, and ash (ASH) was obtained at 105°C and crude protein (CP) was determined by the kjeldahl method according to the procedures described by (SILVA; QUEIROZ, 2002)., the neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were determined according to the (VAN SOEST, 1994) method, and lignin (LIG) and ether extract (EE) were determined using an ANKOM®XT10 system. The total carbohydrates (TC) were calculated suing the equation 100 - (CP + EE + ASH) as described by (SNIFFEN et al., 1992).

In vitro incubation diets were formulated by replacing cane sugar with byproducts in the following proportions: 100/0, 70/30, 50/50, and 30/70 (cane sugar / byproduct). Subsequently, 0.5 g dry matter (DM) of the diet was weighed using an ANKOM® (F57) bag, placed into 60 mL glass bottles and sealed; six replicates per treatment were prepared.

The inoculum for incubation was obtained from three Holstein cows having an average weight of 600 kg. The cows were cannulated in the rumen, and the inoculum was transferred to preheated thermos flasks at 39°C. Samples were taken immediately to the laboratory, where they were homogenised, filtered through two layers of gauze and kept in a water bath at 39°C under CO_2 saturation until the other solutions (buffer, macro- and micro-nutrients, resazurin solution and half B) were added to the culture medium. The ruminal fluid and buffer solution were mixed at a proportion of 5:1.

The inoculum (30 mL) was then transferred to incubation vials, which were sealed and then shaken in an orbital shaker at 120 oscillations per minute in an incubator at 39°C. Profiles of cumulative gas production in vitro in each vial were measured and after 48 hours incubation using a graduated displacement apparatus ml water. From the percentage of gas production, we calculated the volume corresponding to the cumulative gas production within 48 hours after the fermentation process, the values were subsequently corrected for $g{\star}DM^{-1}$.After 48 hours of incubation in ANKOM® bags, wastes were removed, placed on ice to stop fermentation and then washed with abundant water and dried in an oven at 55°C for 48 hours. The in vitro degradability of dry matter (DMD) was obtained by the difference in weight between the dry matter of the sample before and after incubation.

The experimental design used a randomised 5 x 4 factorial arrangement (byproduct levels x replacement). When the variable was independent of the main factors of the byproducts, we applied the mean test, and replacement levels determined the most representative regression model. Those variables where the effect of the main factors was dependent, the regression model that best represented the data was applied to the byproducts according to the level of substitution of sugar cane. Additionally, the effect of the byproducts at each level of substitution was subjected to the mean test. When choosing the regression models that best represented the behaviour of the data, we considered the level of significance using the (SAS, 2003).

Results and discussion

The results of the chemical composition analyses are shown in Table 1.

Table 1. Chemical composition (g kg^{-1}) of sugar cane of byproducts from the production of biodiesel.

Byproducts	DM	CP	NDF	ADF	ADL	EE	ASH	IVDMD	TC	NFC
Sugar cane	270.4	22.5	518.2	362.2	40.4	11.9	48.5	554.6	917.1	128.5
Cotton	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	27.6
Castor	912.6	420.2	423.3	383.4	154.4	43.8	42.3	497.1	493.6	70.3
Moringa	901.2	577.6	202.7	80.5	10.3	84.8	49.8	791.3	287.8	85.1
Pinhão Manso	920.7	356.9	391.4	334.5	43.4	110.6	79.5	571.3	453.0	61.6

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

The cane sugar contained 22.5 g kg^{-1} of CP and 11.9 g kg^{-1} of EE, corroborating the results reported by Magalhães et al. (2006) and Abdalla et al. (2008). The byproducts from moringa and cotton presented the highest CP and in vitro digestibility of dry matter. The highest levels of neutral detergent fibre, acid detergent fibre and ash byproduct were observed in the byproduct of castor oil followed by that of sunflower. Additionally, the byproduct of pinhão manso contained greater amounts of lignin and lipids. The degradability of dry matter (DMD) was found to be dependent on the effect of the byproducts used to replace cane sugar (Table 2).

In an initial assessment of the levels of substitution of forage using byproducts, it was observed that the level of DMD in 30% moringa byproduct did not differ significantly from the byproducts of cotton and sunflower, and they did not differ from the byproducts of castor and pinhão manso. At the 50 and 70% levels, the DMD of the byproducts of cotton, castor and pinhão manso did not differ, and the byproduct Moringa had higher results at these two levels followed the byproduct of sunflower.

An analysis of the levels of each byproduct showed that the DMD decreased with increasing byproduct replacement by cotton, castor and pinhão manso ($p < 0.05$). However, replacement using moringa byproduct showed the best DMD result at all levels of replacement, followed by sunflower byproduct, for which a DMD increase of 21% was observed at the 50% level.

When observing the equations for the four levels of substitution for each byproduct, it is apparent that with the exception of the sunflower byproduct ($p > 0.05$), all replacements showed a linear behaviour. Only the byproduct of moringa showed an increasing linear equation.

Considering the total gas production (Table 3), the factors were dependent. The moringa byproduct at the 30% replacement level presented a significantly higher total gas production than the byproducts of cotton and castor beans, and these results did not differ from those of the sunflower and pinhão manso byproducts. At the 50% replacement level, the gas production rates from byproducts of castor oil and moringa were very similar and differed significantly from those of the other byproducts.

The cotton byproduct showed a lower gas production. At the 70% level of replacement, the castor bean byproduct showed the highest gas production and differed significantly from all byproducts except for the results of the sunflower byproduct, which also did not differ from that of cotton, moringa and pinhão manso.

With an increasing level of substitution of each byproduct, the gas production values for cotton and sunflower byproducts show decreased levels at 30 and 50% replacement and an increased production level at 70% replacement.

Table 2. Average contents (%) of dry matter degradability (DMD) and the regression equations of the byproducts of biodiesel production at different levels of replacement for cane sugar.

Byproducts	Level of substitution				Equation	R^2	P-value
	0%	30%	50%	70%			
Cotton	47.76	43.67^{ab}	39.58^{c}	36.25^{c}	$\hat{y} = 48.065 - 0.166x$	0.8009	< 0.0001
Sunflower	47.76	44.76^{ab}	46.11^{b}	42.80^{b}	$\hat{y} = 45.3617$	0.1659	> 0.05
Castor	47.76	39.59^{b}	35.22^{c}	33.96^{c}	$\hat{y} = 46.764 - 0{,}203x$	0.8139	< 0.0001
Moringa	47.76	47.27^{a}	57.97^{a}	55.16^{a}	$\hat{y} = 46.827 + 0{,}138x$	0.4062	0.0008
Pinhão Manso	47.76	41.18^{b}	35.33^{c}	33.84^{c}	$\hat{y} = 47.361 - 0{,}208x$	0.8517	< 0.0001

*Means followed by different letters in the same column differ significantly at 5% based on the Tukey test.

Table 3. Mean values of the total gas production (mL g DM^{-1}) and regression equations of the byproducts of biodiesel production at different levels of replacement for sugar cane after 48 hours of in vitro incubation in culture medium.

Byproducts	Level of substitution				Equation	R^2	P-value
	0	30%	50%	70%			
Cotton	87.81	69.79^{b}	49.70^{c}	52.92^{b}	$\hat{y} = 85.743 - 0.551x$	0.6634	< 0.0001
Sunflower	87.81	72.48^{ab}	61.22^{b}	68.71^{ab}	$\hat{y} = 88.535 - 0.892x$	0.4579	0.0003
Castor	87.81	69.07^{b}	80.56^{a}	79.47^{a}	$\hat{y} = 79.234$	0.0294	> 0.05
Moringa	87.81	84.03^{a}	80.85^{a}	60.25^{b}	$\hat{y} = 91.622 - 0.356x$	0.6981	< 0.0001
Pinhão Manso	87.81	72.46^{ab}	66.87^{b}	63.69^{b}	$\hat{y} = 85.759 - 0.347x$	0.8464	< 0.0001

*Means followed by different letters in the same column differ significantly at 5% based on the Tukey test.

The co-products of castor oil and moringa showed a decreased gas production at the 30% level, and the maximum production was obtained at the 50% level; however, the output decreased at the 70% level. Except for the castor byproduct, which did not affect the gas production ($p > 0.05$), the other co-products decreased the gas production with an increasing level of replacement, corresponding to decreasing linear equations.

The lipid and protein compositions of cane sugar were low when compared to the other co-products. Protein deficiency decreases the extent of degradation by rumen microorganisms, thereby decreasing the digestibility of foods and the utilisation of nutrients by the animal. Moreover, a decrease in animal production can cause damage to various systems of ruminants (e.g., digestive, reproductive and immune systems). The ether extract of cane sugar was excessively low. The amount of ruminant lipids should not be excessively high because it causes changes in ruminal fermentation.

The degradability in the rumen is directly related to the quality of animal feed and is influenced by associative effects of the consumption level and passage rate. Certain anti-nutritional factors can directly affect the weight gain of the animal, such as the presence of of saponins and ricin in the byproduct of castor beans. These factors also influence the degradation of by products (ABDALLA et al., 2008; FARIAS et al., 2012). In addition, the high amounts of NDF in the castor and cotton byproducts possibly interfered with the degradation of these materials. The observed values for the byproduct of moringa can be explained by the greater amount of CP and NFC and smaller amount of NDF, which favours degradability.

Having low values of in vitro dry matter degradability (DMD), the sunflower by product may have been influenced by the low amount of CNF contained in this byproduct. These values were consistent with those reported by Tonissi et al. (2010). The composition of pinhão manso includes saponins, which are anti-nutritional factors that can alter the rumen microorganisms and hinder the digestion of fibre. Moreover, the sunflower and pinhão manso byproduct have high levels of ether extract covering the fibre that may hinder the attack of food by the ruminal microorganisms; an anti-nutritional effect can also cause a deficiency of certain microorganisms and reduce the availability of cations by combining with fatty acids (OLIVEIRA et al., 2013).

The total gas is the sum of all the gases produced in the rumen (carbon dioxide, methane, acetate, propionate and butyrate). Thus, the total gas production for the cotton byproduct may have shown the observed behaviour due to both a low degradability, its high content of CP and the high amount of protein, thereby resulting in the formation of ammonium bicarbonate, which together with the CO_2 will reduce the total gas production (OLIVARES-PALMA et al., 2013). Additionally, a higher gas production was observed at the 30% replacement, although this can be justified by the presence of lower levels of coproducts. For the sunflower and castor bean byproducts, the total gas production could have been influenced by the high amount of NDF, thereby increasing the production of gas. The data for the sunflower byproduct are consistent with those presented by Mizubuti et al. (2011), who analysed the byproducts of the biodiesel production chain and reported a gas production of approximately 72 mL g DM^{-1}. In this study, a higher total gas was presented by the sunflower byproduct within 48 hours of production. Additionally, the byproduct of castor oil, similar to the byproduct of pinhão manso, contained ricin, which is an anti-nutritional factor that may influence the production of gas.

The byproduct of moringa, showing the greatest degradability, should therefore have produced the largest gas production; however, this plant is considered by some researchers to have antimicrobial properties, which might have caused a reduction in gas production. According to Coelho et al. (2009), in addition to being antimicrobial, moringa can also contain larvicidal lectin, which is responsible for these characteristics.

The byproduct of pinhão manso showed a decrease in the total gas production. Two possible explanation for this observation include the presence of anti-nutritional components (saponins) that damage the ruminal microorganisms and the high concentration of lipids, which can lead to an inhibition of the formation of gas by decreasing the fermentation of structural carbohydrates (BERCHIELLI et al., 2006).

Conclusion

The byproducts of oil seed press cake from biodiesel production (*Gossypium hirsutum* L., *Helianthus annuus* L., *Ricinus communis*, *Moringa oleifera* L. and *Pinhão manso curcas* L.) can replace cane sugar without causing damage to ruminal microorganisms. The coproduct of moringa at the 70% level showed the best ruminal fermentation results followed by sunflower and castor at the 30% level. These byproducts can replace cane sugar in ruminant feed. To confirm these findings determined in vitro, it is necessary to conduct studies in vivo.

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