

Original Research Paper

Nutritional Evaluation and Effectiveness of Detoxified *Jatropha curcas* Kernel Meal in Finishing Pig Diets on Biogas and Methane Production from Slurry

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Abstract: The purpose of this work was to evaluate Phorbol Ester's (PE), Total Fenols (TF), Total Tannins (TT), Condensed Tannins (CT), Saponins Content (SPN), amino acids profiles and apparent digestibility of detoxicated *Jatropha curcas* meal in finishing pigs diets, as well as biogas and methane production by the manure from cross-bred pigs fed this meal. Apparent digestibility coefficients of Dry Matter (DM), Crude Energy (CE), Crude Protein (CP), Crude Fiber (CF) and Ether Extract (EE) were evaluated. Twenty crossbred pigs with 70±1.95 kg initial weight were allotted in individual metabolic cages, according to body weight in a Complete Randomized Block Design with ten replications per treatment. On the last day of the adaptation phase of the digestibility experiment, the manure were collected and put it in bottles with hydraulic retention time of seven days for further evaluation of biogas and methane produced in liters and liters % of total solids. The content of PE, TF, TT, CT, SPN, were, respectively, 0.06 mg/g, 26.08 and 10.43 equivalent gram of tannic acid, 0.05 equivalent gram of leucocyanidin and 0.005%. The results of apparent digestibility coefficients were 83.80% (DM), 3.500 kcal/kg (CE), 13.45% (CP), 1.5% (CF) and 2.3% (EE). Regarding the generation of biogas and methane of manure from pigs fed detoxified *Jatropha curcas* meal, biogas production (1201.4, 1089.3 mL/g Total Solids) and methane (246.7 and 218.2 mL/g Total Solids) from manure weren't significantly different ($p>0.05$) between the treatments. So, concludes that detoxicated *Jatropha curcas* meal is a promising by-product due its nutritional composition and metabolic response which may be used in finishing pig diets without affecting the generation of renewable energy.

Keywords: Biodiesel, Biogas, By-Product, Finishing Pigs, Nutritional Composition

Introduction

The feed spend is about 70% of average costs of swine production for slaughter in Brazil (Giroto and Santos Filho, 2000). In this context, the financial success of any production is directly related to the cost of the feed ingredients. Considering that the diets are constituted basically of corn and soybean meal, which

present constant price instability, it's clear the need to search for new alternatives that can replace economically these ingredients. Thus, the research involving the use of alternative feeds, especially the by-products or waste resulting from the industrial processing of agricultural products and of modern practices of agricultural mechanization, are gaining attention (Gomes, 2006).

The growing global concern about the environment and the search for energy renewable sources put the biodiesel on the spotlight of interests. Several countries, including Brazil, are seeking the path of technological domain of biofuel, both in agronomic and industry levels, which should cause a strong impact on the Brazilian economy and on the social inclusion policies of this country (Abdalla *et al.*, 2008).

Brazil has nearly two hundred species of oil plants and palmaceae for oil production and biodiesel (Beltrão, 2006). *Jatropha curcas* is considered promising for used in animal feed due its high oil content (25-40%), higher than the other oilseeds used in the biofuels market (Arruda *et al.*, 2004).

The production of biodiesel generates some by-products, which can be the focus of more detailed analysis. The use of *Jatropha curcas* meal in animal feed arouses the interest of many researchers and it could be used in animal feed, provided that appropriate treatments should be performed for the reduction or elimination of bioactive molecules, such as phorbol esters (Neiva Junior *et al.*, 2007).

The chemical analysis and feeding tests are the first items to determine the potential and nutritional value of a food to be used on animal nutrition. Different animal species metabolize food differently; being this variation quantified upon the determination their digestibility coefficients. The digestibility of a feed is defined as the skill with which the animal digests and absorbs the nutrients and energy it contains. The digestibility determination of an ingredient is the first concern when it comes to evaluating their potential for inclusion in rations for animal (Pezzato *et al.*, 2004). Thus, the purpose of this study was to determine the chemical composition, phorbol esters, total phenols, total tannins, condensed tannins, saponins content, amino acid profile, the apparent digestibility of detoxified *Jatropha curcas* meal (*Jatropha curcas* L.) for finishing pigs and to evaluate the biogas and methane generation from the manure obtained from pigs fed this product.

Materials and Methods

Chemical Analyses

This work was development in the premises of the Animal Nutrition Laboratory of the Nuclear Energy in Agriculture Center (CENA) of University of São Paulo, São Paulo, Brazil. The Detoxified *Jatropha curcas* Meal (DJCM) used was collected in a biodiesel industry producer, being later industrially detoxified.

The chemical composition analysis were performed according to AOAC (2005) and the amino acid profile obtained by HPLC process. The concentrations of phorbol esters were analyzed following the method by Makkar *et al.* (1997), the contents of total phenols and

total tannins analyzed by Folin-Ciocalteu, condensed tannins (proanthocyanidins) by the butanol-HCl method (Makkar, 2000) and the concentration of saponins analyzed according to Makkar *et al.* (2007).

Apparent Digestibility and Experimental Set Up

Ten crossbred pigs, barrows, with average weight of 70±1,95 kg were used, distributed in a randomized block design in function of body weight in two treatments: The Reference Diet (RD) and Test Diet (TD), with five replicates (blocks), with an animal for metabolic cage (experimental unit).

Before the experimental period, feed intake tests to determine the maximum level of inclusion of DJCM in reference diet were performed, so that feed intake was not prejudice in the excreta collection period. Thus, the TD was composed of 92.3% of the RD and 7.7% of DJCM. The reference diet was formulated in accordance with the requirements proposed by Rostagno *et al.* (2005) as shown in Table 1.

The partial excreta collection method was used and chromic oxide (Cr₂O₃) employed as a marker added to the experimental diets at a concentration of 0.05%. The animals were weighed before and after the trial, which lasted thirteen days with seven days of adaptation and measurement of voluntary feed intake, three days for adaptation and maintenance of flow of marker and three days of excreta collection. The quantities of diets supplied were calculated according to the metabolic weight (PV^{0.75}) and a proportion of 1:1 water and diet was supplied to the animals twice a day, at 08:00 and 16:00.

In adaptation period, the animals were encouraged to consume the maximum of feed aiming at avoiding leftovers and in the collection period the quantities of diets in each treatment were based on lower consumption among the animals of the block, obtained in the adaptation period.

From the average feed intake in the adaptation period, was established the Amount Restricted Feed (ARF) provided during the days of excreta collection, according to the following calculations:

$$ARF = I * Metabolic Weight$$

Where:

$$\begin{aligned} ARF &= \text{Amount restricted feed (kg)} \\ I &= \text{Index} = \text{average feed intake of} \\ &\quad \text{the days (kg)/Metabolic weight} \\ \text{Metabolic Weight (kg)} &= (\text{live weight})^{0.75} \end{aligned}$$

The index (I) was calculated for all animals, however, was adopted the lowest value of I, to calculate the ARF. Although the I value standardization, ARF were evaluated individually, also calculated being in function on the weight of each animal. Water was provided ad libitum throughout the experimental period.

Excreta samples from the rectum were collected twice daily (08:00 and 16:00) being these immediately placed in plastic bags, identified and frozen. After, these samples were thawed and homogenized in a single composite sample. These were dried in trays at appropriate ovens of forced air circulation at 65°C for 72 h, milled in mill through sieve riddled of 1 mm. The excreta and feed samples were analyzed for dry matter, crude energy, crude protein, crude fiber, ether extract and chromium oxide content.

Chromium oxide percentage in the samples was done in Instrumentation Nuclear Laboratory of Nuclear Energy in the Agriculture Center (CENA) of University of São Paulo by technique of X-ray fluorescence for dispersive energy (Zucchi and Nascimento Filho, 1995; Nascimento Filho *et al.*, 1997). The apparent digestibility coefficients of RD and TD were calculated according to Serrano (1989) and from this, determined the apparent digestibility coefficients of dry matter, crude energy, crude protein, crude fiber and ether extract of DJCM using the equation proposed by Matterson *et al.* (1965).

Biogas and Methane Production

On the last day of adaptation phase to the cage in digestibility experiment, the pigs manure were collected and added in proportion of 250 mL of excreta's, 250 mL of urine and 615 mL of water in PET bottles previously identified with total volume of 3,125 mL with rubber stoppers adapted to screw caps, sealed with silicone glue to prevent the gas produced was lost, placed in oven of forced ventilation at 40°C without agitation and with

Retention Hydraulic Time (RHT) of seven days. These conditions were observed by Souza *et al.* (2005) as being ideal for biogas production by pigs manure, simulating the conditions of a batch bio digester, without continuous flow of effluents, for biogas and methane (CH₄) quantification. Two treatments were used, RD and TD (7.7% inclusion of DJCM), through ten bottles (replicates) for each treatment.

Each bottle was dimensioned for storing two liters of biogas and permit the biogas produced quantitation, through the use of a pressure gauge (transducer-model PDL800-LANA-CENA/USP) adapted of Theodorou *et al.* (1994) and graduated syringes, where two first days was held instrument calibration and preparation of the response curve. During the seven days, the pressure exerted by gases was measured for subsequent determination of the biogas volume of each bio digester (bottle). After each time of reading of gases production, a gas aliquot was stored in test tubes previously identified and subjected to vacuum where the determination of the methane levels (CH₄) were done using a gas chromatograph model CG-2014 GAS CHROMATOGRAPHIA (Shimadzu). The equipment calibration was performed with standard gas supplied by White Martins. In addition, total solids levels (AOAC, 2005) of the waste incubated in each bio digester were evaluated. Considering the effective bio digester volume of 2,000 mL, the biogas and methane volumetric total production were determined, as well as daily thereof production in mL/g of total solids. The design was randomized blocks, with two treatments and ten replicates per treatment.

Table 1. Nutritional composition of the reference diet

Ingredients	Reference ration
Corn	81.35
Soybean meal (45%)	16.07
Di calcium phosphate	0.82
Limestone calcitic	0.65
Vitamin C supplement ¹	0.40
Mineral supplement ²	0.10
Salt	0.40
L-Lysine (78%)(HCl)	0.15
Copper sulphate (25%)	0.06
Nutritional calculated values	
Metabolizable energy, kcal/kg	3230.00
Crude protein, %	14.19
Digestible methionine, %	0.22
Digestible methionine + cystine, %	0.45
Total lysine, %	0.76
Digestible lysine, %	0.68
Digestible threonine, %	0.45
Calcium, %	0.53
Total phosphorus, %	0.44
Available phosphorus, %	0.25

¹Amountsper kg diet: Vit. A-2520 UI; vit. D3-540 IU; vit. E -9.9 IU; vit. K3-0.72 mg; thiamine-404 mcg; riboflavin-1.98 mg; pyridoxine-404 mcg; vit. B12-8.1 mcg; folicacid-225.2 mcg; Pantothenic Acid-6.3 mg; Niacin-12.6 mg; growth promoter-10 mg; Se-0.24 mg; ²Amountsper kg diet: Cu -9 mg; Fe -81 mg; I -0.9 mg; Mn-54 mg; Zn-135 mg

Statistical Data Analysis

The statistical analysis of the data was performed using SAS ® v.9.2 (Statistical Analysis). SAS LAB procedure was used to verify data adequacy to the linear model and then the variables obtained were submitted to analysis of variance using the PROC GLM.

Results and Discussion

In relation to bioactive molecules analyzed in DJCM (Table 2), phorbol esters content (0.06 mg/g) is lower than that found for Makkar *et al.* (1997), who evaluated the content of these in nontoxic to the animals varieties (0.11 mg/g), by Rakshit *et al.* (2008) evaluating DJCM in diet for rats (0.2 mg/g).

Chivandi *et al.* (2006), evaluating the effect of different methods of detoxification in the *Jatropha curcas* meal with 0.8 mg/g of phorbol esters, observed significant losses in pigs blood parameters. The phorbol esters mimic the Diacylglycerol (DAG) action, inactivating the protein kinase C, which regulates different pathways signal of translation and other metabolic activities by acting as co-carcinogen and causing cellular and biochemical effects in many different animal species (Goel *et al.*, 2007) and studied extensively in different animal models like goats, sheep, mice, rats and fish when fed with phorbol ester-containing feeds (Adam and Magzoub, 1975; Makkar *et al.*, 1998). The phorbol esters content depends on the oil residue present on pie or meal after the processing (Rakshit *et al.*, 2008), which justifies the values obtained in this study, since this is a specific research and the oil was almost completely extracted to obtain the meal and this passed over by the detoxification process.

The total phenols and total tannins values are similar to those reported by Makkar *et al.* (1998), but the content of condensed tannins is different from those observed by the same authors, that evaluating toxic and nontoxic varieties of *Jatropha curcas* not found condensed tannins in both varieties. It is important noting that there are factors that affect the tannin concentration in plants, among which stand out climate, plant mineral nutrition and growth stage (Waterman and Mole, 1994).

Tannins can affect negatively the feed intake, digestibility and the performance of animals. These effects depend greatly on the amount, the tannin type ingested and the animal tolerance, being more prominent in non-ruminants. Levels above 1% of condensed tannins in the diet, well above the values obtained in this study, affect mainly the intake, digestibility of protein and of the essential amino acids (McDonald *et al.*, 1995).

The saponins content observed (0.005%) is similar to those reported by Makkar *et al.* (1998) assessed the

nutritional content of seeds and meal of detoxified *Jatropha curcas* from non-toxic varieties in Mexico and even to those observed by Belewu and Sam (2010) who evaluated the content of different bioactive molecules of the DJCM by different microorganism's strains. The saponins are glycosides present in most part of the diets ingredients for animals. Is characterized by a bitter taste, capacity to form foam in aqueous solution, cause hemolysis and, also, of complexing in steroid. Moreover, their anti-nutritional effects also are related to changes in the permeability of the intestinal mucosa, inhibiting the transport of some nutrients, facilitating the absorption of other compounds (Francis *et al.*, 2001).

In relation to amino acids analyzed of the DJCM (Table 3), the contents were slightly below those found by Makkar *et al.* (1998), that reported values of arginine (12.9%), cystine (1.58%), phenylalanine (4.89%), histidine (3.08%), Isoleucine (4.85%), leucine (7.5%), lysine (3.4%), methionine (1.76%), tyrosine (3.78%), threonine (3.59%), tryptophan (1.31%), valine (5.3%). According to these authors, the chemical composition of *Jatropha curcas* varies depending on the source, being similar to soybean meal, presenting only minor amount of lysine and higher value of sulfur amino acids.

The CE of DJCM determined in this study was 5,900 kcal/kg and the apparent digestibility coefficient of energy was 59.6%, from the value of digestible apparent energy of 3,500 kcal/kg (Table 4). Thus, DJCM can be considered an excellent source of energy, since the digestible energy value (3,500 kcal/kg) is similar to corn (Rostagno *et al.*, 2005) and other cereals considered energy sources, such as the low tannin sorghum, triticale and the wheat meal, which have average digestible energy values of 3,794 (Ferreira *et al.*, 1997), 3,236 (Furlan *et al.*, 1999; Radecki and Miller, 1990) and 3,300 kcal/kg (Lima and Viola, 2001), respectively.

Regarding the CP content and their digestibility, value of 51.1% were observed, result considered excellent for protein sources. However, the apparent digestibility coefficient was only 26.3% with digestible protein value of 13.5%. These values are inferior when compared with digestible protein values of other oilseed meals considered as protein sources and oil source for biodiesel production.

In Turkey, Boguhn *et al.* (2010) evaluated the digestibility of DJCM amino acids and concluded that for young turkeys is higher compared with other ingredients to animals of the same species, therefore, can use it as an ingredient in diet.

In addition to soybean meal, worldwide used as ingredient protein in the animal diets, some foods considered by-products, such as cotton meal, peanut meal and canola meal, have respectively 25, 44 and 26% of digestible protein for pigs (Rostagno *et al.*, 2005).

Table 2. Dry matter content and bioactive molecules found in detoxified *Jatropha curcas* meal (DJCM)

Components	DJCM
Dry Matter (100°C), %	96.500
Phorbol esters, mg/g	0.060
Phenols total ² ,	26.080
Total tannins ² ,	10.430
Condensed tannins ³ ,	0.050
Saponins, %	0.005

¹Analyses carried at the Animal Nutrition Laboratory of Nuclear Energy in Agriculture Center of University of São Paulo, São Paulo, Brazil (LANA/CENA/USP) and in the Animal Production Center in the Tropics and Subtropics, University of Hohenheim, Stuttgart, Germany.

²Results in equivalent gram of tannic acid.

³Results in equivalent gram of leucocyanidin.

Table 3. Amino acidic profile in detoxified *Jatropha curcas* meal (DJCM)¹

Amino acid ¹ , % in DM	DJCM
Alanine	2.19
Arginine	5.94
Aspartic acid	4.16
Glycine	1.98
Isoleucine	1.93
Leucine	2.93
Glutamic acid	7.44
Lysine	1.24
Cystine	0.71
Methionine	0.76
Phenylalanine	1.81
Tyrosine	1.28
Threonine	1.53
Tryptophan	0.50
Proline	1.83
Valine	2.30
Histidine	1.09
Serine	2.14

¹Values obtained by means of HPLC in the animal nutrition laboratory of the CBO Analysis, Campinas/SP, Brazil.

Table 4. Chemical composition, crude energy, apparent digestibility coefficients (CDap) and apparent digestible nutrient (NDap) of Detoxified *Jatropha Curcas* Meal (DJCM) for finishing pigs

Centesimal fractions	Chemical composition	CDap, %	NDap
Dry Matter, %	96.5	86.8	83.80
Crude energy, kcal/kg	5,900.0	59.6	3,500.00
Crude Protein, %	51.1	26.3	13.50
Crude fiber, %	4.3	34.5	1.50
Ether Extract, %	21.9	10.2	2.24

¹Analyses carried at the Animal Nutrition Laboratory of Nuclear Energy in Agriculture Center of University of São Paulo, São Paulo, Brazil (LANA/CENA/USP).

Table 5. Biogas and methane production in mL/g of total solids of pigs manure fed with Reference Diet (RD) and reference diet + 8% DJCM (RD8%)

	RD	RD8%	P	CV (%)
Biogas (mL/g of TS)	1,201.37	1,089.27	Ns	25.2
Methane (mL/g of TS)	246.71	218.16	Ns	29.8

P-coefficient of probability. CV-coefficient of variation

Thus, it's evident that DJCM, while possessing high level of crude protein in its composition, this nutrient is hardly been tapped by the pigs. Probably, the presence of

bioactive molecules, such as protease inhibitors, saponins, tannins and phytates impaired the nutrient digestibility, which shows that the meal used in this study wasn't fully

detoxified. Another possible explanation for the low crude protein digestibility coefficient observed in this study, may be due to low inclusion of the DJCM in the diet due low acceptance by the animals.

Regarding the crude fiber content, the value of 4.3% was observed with apparent digestibility coefficient of 34.5% with the digestible fiber value of 1.1%. It's verified that the DJCM has lower content of crude fiber and digestibility higher when compared to the values obtained in cotton meal (25%) (Rostagno *et al.*, 2005). In relation to the ether extract content, the value of 21.9% were observed with the digestibility apparent coefficient of 10.2%, providing the digestible ether extract value of 2.2%. The nutritional value of some foods depends mainly on the content, nutrients biological availability and further the presence and levels of toxic substances or bioactive molecules, which can alter this composition or become unavailable some of these nutrients (Pezzato, 1995).

The mean values of volumetric cumulative production per gram of biogas and methane in total solids are shown in Table 5. The production curves of biogas and methane (mL/g of total solids) by the pigs manure, well as the accumulated production of biogas and methane (mL) are shown in the graphs 1 and 2, respectively.

In studies of Miranda (2005) average potentials of biogas production of 0.489; 0.566 and 0.768 m³/kg of Total Solids (TS) were verified in biodigesters supplied with excretas of pigs, subjected to temperature 35°C and Retention Hydraulic Time (RHT) of 30 days, these results are lower than those found in this study.

The biogas (1201.4; 1089.3 mL/g of total solids) and methane (246.7 and 218.2 mL/g of total solids) production from the finishing pigs manure fed with reference diet and diet containing DJCM are compatible with those observed by Souza *et al.* (2005), evaluated different retention hydraulic times and incubation temperatures of pigs manure. The methane proportion in relative to the biogas generated was of 20.5 and 20.35% for the treatments RD and RD8%, values well below of 60 to 70%, theorized by Miranda (2009) as normal. This happened, probably, due to the low retention hydraulic time (7 days) exercised in this study, corroborating Lucas Junior (1994) that assessing the effects of retention hydraulic times of 50, 30, 20 and 15 days, in the case of bio digestion anaerobic of pigs manure, found that the highest efficiency is obtained at RHT of 30 days, with worst use of the manure in the RHT of 15 days.

Conclusion

Detoxified *Jatropha curcas* Meal (DJCM) can be used in finishing pig diets without affecting the generation of renewable energy by its manure. Although the DCJM has shown low apparent digestibility of the nutrients for pigs, the biogas and methane generation wasn't compromised.

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Author Contributions

All the authors equally contributed in this work and the article.

Conflict of Interest

There is no conflict of interest.

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