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Bio-Detoxification of Jatropha Seed Cake and Its Use in Animal Feed

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1. Introduction

Biodiesel production using the seed oil of *Jatropha curcas* L. (physic nut), as a raw material, results in large amounts of solid residue, called Jatropha seed cake. This seed cake contains lignocellulosic compounds, water, minerals and proteins [1-3]. However, it also contains toxic compounds and anti-nutritional factors [1-3]. The detoxification and reuse of this seed cake is very important for adding economic value, and also reduces potential environmental damage caused by improper disposal of this by-product.

The toxicity of Jatropha seed is mainly attributed to a group of diterpene esters called phorbol esters. These esters are present in high concentrations in toxic seed varieties but in lower concentrations in a non-toxic seed variety from Mexico [4]. Phorbol esters activate protein kinase C, a key signal transduction enzyme released in response to various hormones and developmental processes in most cells and tissues [5,6].

In addition to the phorbol esters, there is also a toxic protein called curcin in Jatropha seed cake. This protein has two polypeptide chains and is able to inhibit protein synthesis [7]. Curcin is a ribosome-inactivating protein and promotes mucosal irritation and gastrointestinal hemagglutinating action [8].

Phytic acid (myo-inositol hexaphosphoric acid) and tannins are considered anti-nutritional factors because they inhibit the absorption of proteins and minerals [9-11]. Phytic acid is a

compound formed during seed maturation [12]. The seed of *J. curcas* has a high concentration of phytic acid, up to 10% of its dry matter [2]. Tannins are polyphenols water-soluble and polar solvents [13]. The tannin content in the seeds of *J. curcas* is low, representing only 3% of its dry weight [13].

Detoxification of the *Jatropha* seed cake could allow its use as a protein-rich dietary supplement in the animal feed [1,14,15].

The use of residue or by-products in animal nutrition can minimize expenditures on the development of food sources, such as soybean, cotton and wheat meals, without causing undesirable effects on the overall production system. However, it is first necessary to know the nutritional value and effects of the by-product's inclusion in animal diets.

Some studies have used physical and chemical treatments to detoxify *Jatropha* seed [2,16,17]. These methods have been effective but require the use of chemicals that may result in other the presence of other residues. Conversely, bio-detoxification does not require the application of any chemical compounds. It may also reduce the concentrations of phorbol esters and anti-nutritional factors to non-toxic levels [18].

2. Methodology

2.1. Microorganism, fungal growth conditions and inoculum production (spawn)

The isolate Plo 6 of *P. ostreatus* used in this study belongs to a culture collection from the Department of Microbiology at the Federal University of Viçosa, MG, Brazil. *P. ostreatus* was grown in a Petri dish containing potato dextrose agar culture medium at pH 5.8 and incubated at 25 °C. After seven days, the mycelium was used for inoculum production (spawn) in a substrate made of rice grains [19]. The rice was cooked for 30 min in water with a ratio of 1:3 rice: water (w/w). After cooking, the rice was drained and supplemented with 0.35% CaCO₃ and 0.01% CaSO₄. Seventy grams of rice was packed into small glass jars and sterilized in an autoclave at 121 °C for 1 h. After cooling, each jar was inoculated with 4 agar discs (each 5 mm in diameter) containing the mycelium. The jars were then incubated in the dark at room temperature for 15 d.

2.2. Substrate and inoculation

The *J. curcas* seed cake was obtained from an industry of biodiesel (Fuserman Biocombustíveis, Barbacena, Minas Gerais State, Brazil).

To select the most suitable substrates for lignocellulolytic enzyme production, we conducted preliminary experiments with *Jatropha* seed cake and various lignocellulosic residues. We tested *P. ostreatus* growing on *Jatropha* seed cake with different percentages of eucalyptus sawdust, eucalyptus bark, corncobs, and coffee husks [20]. The addition of these agroindustrial residues was necessary to balance the carbon and nitrogen ratio, which might benefit mycelial growth [21-23].

The compositions selected for biological detoxification were based on the results of the above preliminary experiments (Table 1). The substrates were humidified with water to 75% of their retention capacity. Then, 1.5 kg of each substrate was placed in polypropylene bags and autoclaved at 121 °C for 2 h. After cooling, the substrates were inoculated with 75 g of spawn and incubated at 25 °C. Samples from non-inoculated autoclaved bags were kept as controls.

Substrates	Mass substrates (kg)	
	Jc	Agroindustrial residue
Jatropha seed cake (Jc)	20	0
Jc + 10% eucalypt bark (JcEb10)	18	2

Table 1. Substrate compositions used for *Pleurotus ostreatus* growth

2.3. Chemical composition of the substrates and enzymatic assays

The phorbol ester contents were analyzed by high performance liquid chromatography (HPLC), as previously described [2]. A standard curve was made using solutions of phorbol-12-myristate 13-acetate (Sigma Chemical, St. Louis, USA) at concentrations from 0.005 to 0.5 mg mL⁻¹.

To determinate the dry mass, 1.5 kg of the substrate was dried at 105 °C until a constant weight was obtained.

The levels of tannins and phytic acid were quantified by a colorimetric method [24,25].

The laccase and manganese peroxidase activities were measured using 2,2'-azino-bis-3-ethylbenzotiazol-6-sulfonic acid [26] and phenol red solution [27] as substrates, respectively. Xylanase and cellulase activity was calculated by measuring the levels of reducing sugars produced by the enzymatic reactions [28,29]. Phytase activity (myo-inositol hexakisphosphate phosphohydrolase) was determined using the Taussky-Schoor reagent [30].

The level of reducing sugars was determined by the dinitrosalicylic acid (DNS) method (99.5% dinitrosalicylic acid, 0.4% phenol and 0.14% sodium metabisulfite). A standard curve was made with D-glucose, with concentrations from 0.5 to 1.5 g L⁻¹ [31].

2.4. Digestibility of Jatropha seed cake and ammonium production in rumen liquid measured *in vitro*

To analyze the suitability of the chosen substrates (Table 1) in animal feed, we determined their levels of dry matter (DM), organic matter (OM), crude protein (CP), mineral matter (MM), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), non-fiber carbohydrates (NFC), hemicellulose (HEM), cellulose (CEL) and lignin according to previously described methodology [32,33].

The *in vitro* dry matter digestibility (IVDMD) was determined according to a previous method [34], with some modifications. One liter of rumen fluid was collected from fistulated cattle kept at the Department of Animal Science, Federal University of Viçosa, about two hours after feeding. The animals' diet consisted primarily of grass and corn silage. The rumen digesta was filtered through 4 layers of gauze and the liquid fraction was stored in capped plastic flasks and refrigerated. Ruminal fluid was incubated at 39 °C for 30 min to suspend feed particles and precipitate protozoa, which allowed ruminal bacteria to be collected anaerobically from the middle of the flasks. The IVDMD assay was performed in two steps. In the first step, 350 mg of each substrate sample, harvested before and after colonization by *P. ostreatus*, was incubated with a mixture of 4 mL of ruminal fluid and 32 mL of McDougall buffer. This procedure was performed in anaerobic bottles with a continuous flow of carbon dioxide (CO₂). Bottles were then sealed with rubber stoppers and aluminum closures and incubated for 48 h at 39 °C at 120 rpm. In the second step, after incubation, the filtered material was placed in pre-dried and weighed porcelain filters and washed with hot water four times or until complete removal of all McDougall solution. Next, we added 70 mL of a detergent solution, and the samples were autoclaved for 15 minutes at 121 °C. After heat treatment, the filters were washed again with hot water until complete removal of the detergent solution, and then washed using 10 mL of pure acetone. The filters were heated to 105 °C for 16 h or overnight. After that, the filters were placed in a desiccator, and the dried mass was measured on an analytical balance.

To analyze the production of ammonia, the samples were incubated under the same conditions as described for the IVDMD process. These samples were placed into two flasks containing buffer, rumen fluid and the substrates samples (Table 1) harvested before and after fungal colonization. Ammonia quantification was determined using ammonium chloride as an indicator and absorbance was measured in a spectrophotometer (Spectronic 20D) at 630 nm [35].

2.5. Animal assay

The experiment was conducted in the Goat Experimental Section from the Department of Animal Science at the Federal University of Viçosa - MG, BRAZIL. Twenty-four healthy female Alpine goats weighing 20 ± 1.5 kg, with a mean age of five months, were used. This experiment was performed after the *Jatropha* seed cake had been bio-detoxified by *P.ostreatus*Plo6 [20].

2.5.1. Experimental design

The experimental trial lasted 72 days. During the first 12 days, animals were allowed to adapt to the experimental diet. The data were collected during the following 60 days.

The animals were kept in individual confinement stables (1.5x2.0m) equipped with food and water systems. The stables had fully slatted floors adapted for the total collection of feces and urine. Water was provided *ad libitum*. The daily food consumption was quantified by subtracting the total offered feed.

The diets were formulated to meet the nutritional requirements of goats with a starting body weight of 20 kg and a daily weight gain of 100g [37]. The feed contained an average of 12% crude protein.

The treatments consisted of the detoxified substrates at four levels: 0, 7, 14 and 20% (based on total dry matter) in forage hay Tifton-85 (*Cynodon* spp). The ratio of forage: concentrate was 30:70 (Table 2).

Ingredient	Bio-detoxified jatropha seed cake (% dry mass)			
	0	7	14	20
Forage hay Tifton-85	33.18	31.26	31.26	31.25
Jatropha seed cake bio-detoxified	0.00	6.97	13.93	19.90
Maize flour	57.20	55.98	51.84	47.11
Soybean meal	8.37	4.57	1.76	0.53
Sodium chloride	0.20	0.20	0.20	0.20
Calcareous	0.95	0.92	0.90	0.90
ADE vitamins	0.08	0.08	0.08	0.08
Micromineral mixture*	0.03	0.03	0.03	0.03
Sodium bicarbonate	0.40	0.40	0.40	0.40
Chemical composition (%)				
Dry mass (DM)	84.65	84.75	84.80	84.81
Crude protein (CP)	12.84	12.05	11.73	11.89
Ether extract (EE)	3.38	3.25	3.06	2.87
Neutral detergent fiber (NDF)	41.00	42.46	44.42	45.94
Lignin	2.55	4.04	5.67	7.06
Calcium	0.19	0.22	0.26	0.30
Phosphorus	0.27	0.28	0.30	0.32
Net energy (NE, Mcal/kg)	1.87	1.72	1.63	1.54

Table 2. The chemical composition and ingredient proportions of the diet

The feed was supplied twice a day as a complete mixture to allow intake of approximately 10% of the offered amount. The amount was based on the intake of the previous day.

To determine *in vivo* digestibility and nitrogen balance, we collected total feces and urine for five days. Ten percent of the total excretion was sampled. Urine was stored in plastic bags containing 20 mL of sulfuric acid (40% v:v).

The fecal metabolic nitrogen level (Nmet.fecal) was calculated according to previously established methods [37]. The amount of undigested nitrogen (Nund) was calculated as the difference between fecal nitrogen (Nfecal) and Nmet.fecal. To determine the fraction of urinary nitrogen of endogenous origin (Nuend), a previously published equation was used [38]. From the difference between the urinary nitrogen and endogenous urinary nitrogen (Nuend) levels, we calculated the exogenous urinary nitrogen (Nuexo). Nitrogen balance (NB) was estimated with the following equation: $NB = N \text{ ingested} - [Nund + Nuexo]$. The biological value of protein was calculated according to previous methods [39].

The chemical compositions of feeds, orts and feces as a percentage of DM, MM, OM, CP, EE, NDF, ADL and NFC were determined according to previous methodology [32, 33, 40]. The total protein level in the bio-detoxified Jatropha seed cake was calculated from the total nitrogen content by applying the correction factor 4.38. The net energy (NE) was obtained by a previously reported equation [41].

The blood samples were collected in the morning, before supplying the feed, by jugular puncture and vacuum tubes. Blood was stored with and without the anticoagulant EDTA. After this procedure, the tubes were refrigerated and sent to a laboratory for blood biochemical analysis to determine the hemogram compounds. This analysis included the numbers of erythrocytes, hemoglobin, hematocrit and leukocytes. In the Blood serum was analyzed creatinine, alkaline phosphatase, urea and total protein.

2.6. Statistical analyses

The experiments on phorbol ester degradation, anti-nutritional factors, *in vitro* digestibility of Jatropha seed cake, and production of liquid ammonia in the rumen were of a randomized design with 5 replicates each. The resulting data were subjected to analysis of variance (ANOVA), and the mean values were compared by Tukey's test ($p < 0.05$) using Saeg software (version 9.1, Federal University of Viçosa).

The experiments on animals were distributed in a completely randomized design with six replicates per diet condition. The resulting data were subjected to analysis of variance (ANOVA) and regression analysis ($p < 0.05$). Regression models (linear, quadratic or cubic) were fitted to the observed significance (5% level of probability) using the REG procedure (SAS 9.0).

3. Results

After 15 days of inoculation, *P. ostreatus* Plo 6 completely colonized the substrates (Figure 1). This illustrates the ability of this fungus to grow in the presence of both phorbol esters and anti-nutritional factors.

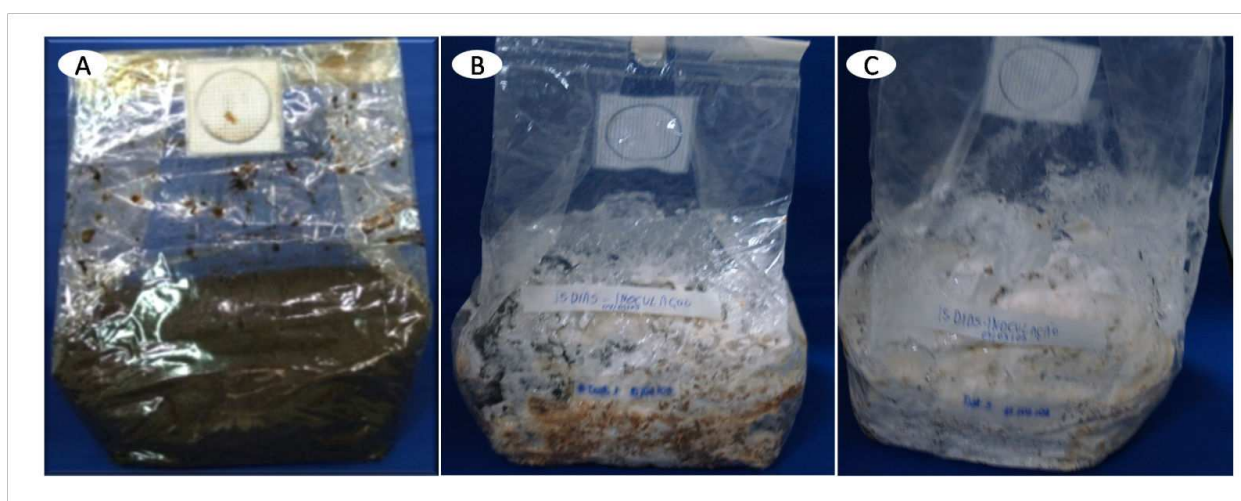


Figure 1. Mycelial growth of *P. ostreatus* Plo 6 in substrate containing varying percentages of Jatropha seed cake. Before the inoculation(A) and 15 days after inoculation: (B) Jatropha seed cake (Jc) and (C)Jc + 10% eucalyptus bark (JcEb10).

3.1. Phorbol ester degradation

Autoclaving the substrates (at 121 °C) reduced the phorbol ester content by an average of 20% (Figure 2). However, these compounds were not degraded at 160 °C for 30 min [17]. Moreover, the addition of sodium hydroxide and sodium hypochlorite combined with heat treatment was able to reduce only 25% of the phorbol concentration [42].

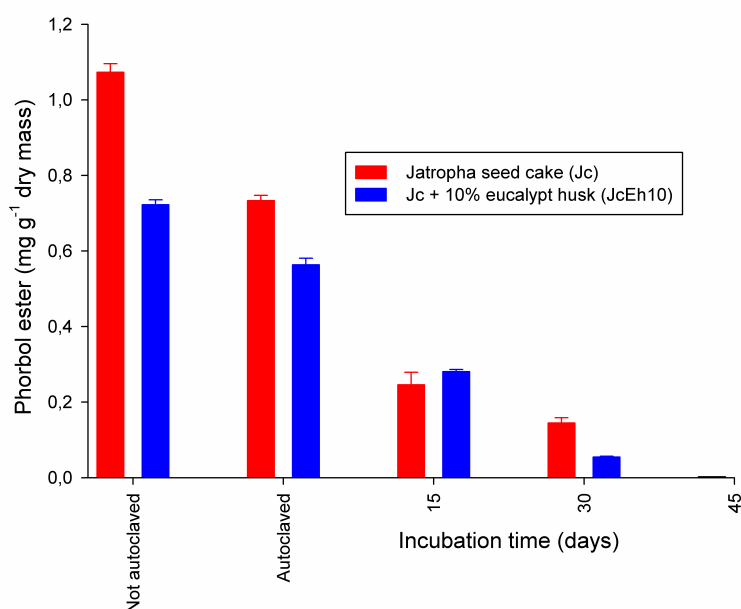


Figure 2. Phorbol ester degradation by *Pleurotus ostreatus* Plo 6 in substrates with varying proportions of Jatropha seed cake (Table 1).

In this study, *P. ostreatus* degraded 99% of the phorbol ester after a 45-day incubation (Figure 2). This rate of degradation was higher than rates observed when chemical deodorization, de-acidification, or bleaching agents were applied to *J.curcas* oil and seed cake [43]. With the exception of bleaching, none of the above chemical processes were effective in reducing the amount of phorbol esters in *J. curcas* seed [44].

The ability of *P. ostreatus* to depolymerize lignin (Figure 3) explains the observed phorbol ester degradation (Figure 2). The degradation of other organic compounds such as chlorophenols and aromatic hydrocarbons also occurs due to depolymerization by laccase and MnP activity [45,46]. The activities of these enzymes of *Phanerochaete* sp [47] and *P. ostreatus* [48] have also been reported to cause dye discoloration in the textile industry and the elimination of pollutants. However, other enzymes may have also influenced degradation of the toxic compounds. Higher cellulase and xylanase activities (Figure 3) were observed between the 15th and 30th incubation days, as indicated by a 58% and 85% degradation of phorbol ester, respectively. However, on the 15th day of incubation we observed lower phorbol ester degradation and lower ligninase activity in the substrate containing eucalyptus bark (Figures 3). This result supports the hypothesis that phorbol ester degradation occurs because of co-metabolism by the enzymes responsible for lignin depolymerization.

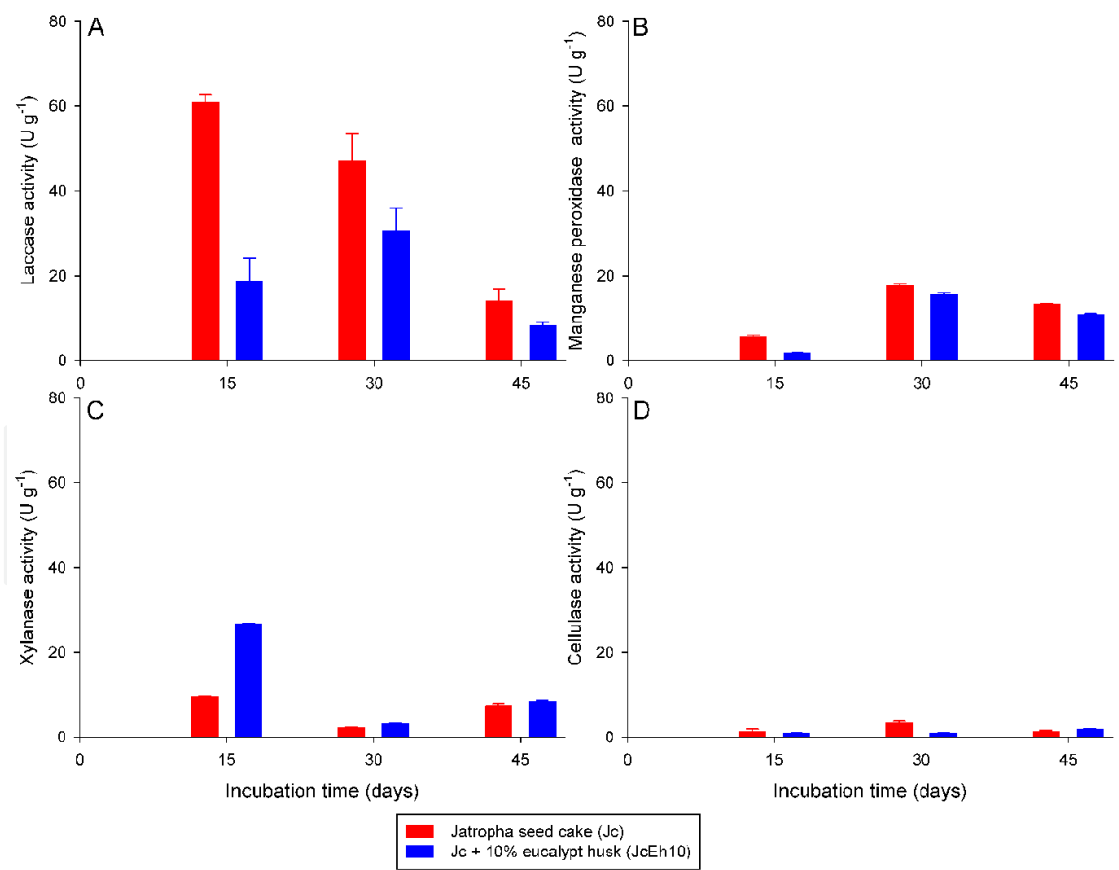


Figure 3. Lignocellulolytic enzymes activity of *Pleurotus ostreatus* Plo 6 in substrates with varying proportions of Jatropa seed cake (Table 1).

After 45 d of substrate incubation with *P. ostreatus*, the residual average phorbol ester concentration was $1.8 \times 10^{-3} \text{ mg g}^{-1}$ dry mass (Figure 2). This concentration is much lower than the 0.09 mg g^{-1} of phorbol esters found in the non-toxic variety of *J. curcas* [17].

3.2. Degradation of anti-nutritional factors

Tannin concentrations observed in the seed cake (Figure 4) are similar to those previously reported in the fruit peel of *J. curcas* [4]. The greatest concentration of this compound was observed in the eucalyptus bark substrate (Figure 4). This may have been due to the prior presence of tannins in the eucalyptus bark [49].

The thermal treatment of the substrates decreased the tannin concentration by 46% (Figure 4). This result was similar to that observed in vegetables after cooking or autoclaving at 121°C and 128°C for different periods of time [10].

Regardless of the substrate, tannin degradation by *P. ostreatus* Plo 6 increased as a function of the incubation time. The highest observed rate was between 15 and 30 d in the substrate with eucalyptus bark (Figure 4). A high tannin degradation rate was also observed in *Pleurotus* sp. cultivated in coffee husk for 60 d [50]. The degradation of tannin is related to tannase activity (tannin acyl hydrolase). This enzyme's activity in polyphenol degradation has been reported in *Aspergillus* and *Penicillium* [51]. Thus, *P. ostreatus* can degrade the tannins in Jatropha seed cake.

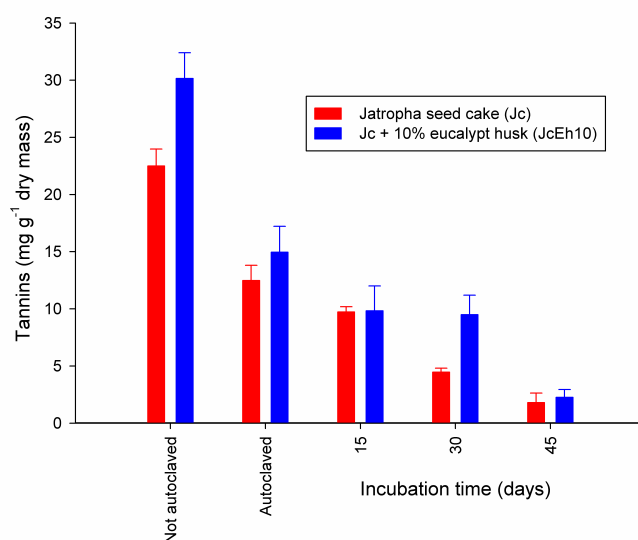


Figure 4. Tannin degradation by *Pleurotus ostreatus* Plo 6 in substrates with different proportions of Jatropha seed cake.

Although phytic acid is considered to be heat-stable [52], the amount of phytic acid decreased by 20% after sterilization of the substrates, (Figure 5). A degradation of 50% of this anti-nutritional factor was also been observed in legumes subjected to autoclaving at 121°C for 90 min [10].

Phytase activity by *P. ostreatus* caused a 95% decrease of phytic acid in the substrates (Figure 5). A high degradation rate of this anti-nutritional factor by microbial phytase has previously observed in culture medium containing rapeseed meal that has phytic acid content between 2% to 4 % of the dry mass [53]. The presence of this enzyme has also been observed in *Aspergillus* sp [9], *Agaricus* sp, *Lentinula* sp and *Pleurotus* sp [54]. Thus, *P. ostreatus* degrades the phytic acid that is present in Jatropha seed cake and thereby increases its potential for use in animal feed.

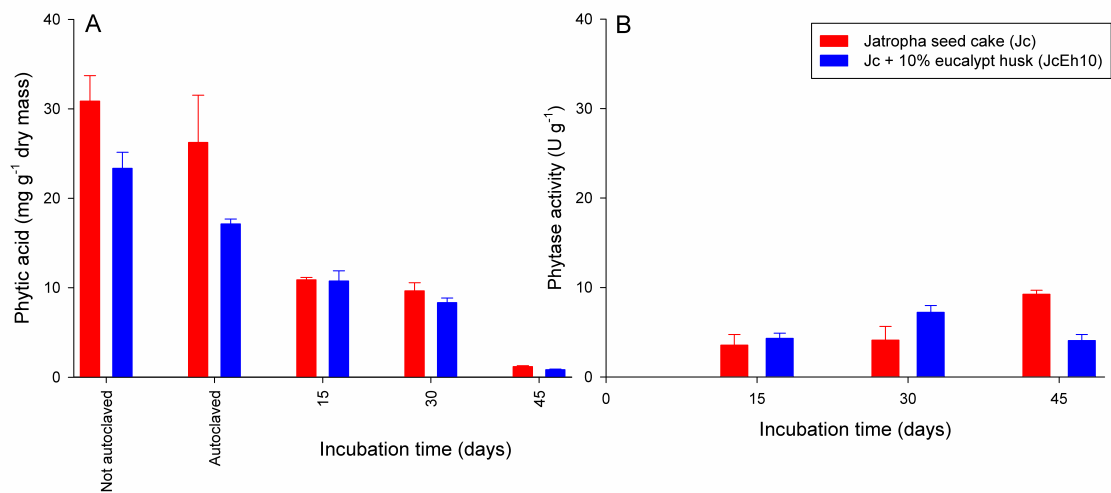


Figure 5. Phytic acid degradation (A) and phytase activity (B) by *Pleurotus ostreatus* Plo 6 in substrates with varying proportions of Jatropha seed cake.

3.3. Digestibility of Jatropha seed cake and ammonium production in rumen liquid *in vitro*

Many agro-industrial residues contain a higher content of fibers, of low digestibility, than proteins, vitamins and minerals. The colonization or fermentation of these by-products by microorganisms, especially lignocellulosic fungi, can efficiently and affordably increase their digestibility and nutritional value [55]. This procedure has been used successfully in cotton waste [56] by colonization with *Brachiaria* sp [57].

Before fungal colonization, we observed higher levels of CP, lignin, ADF and EE in the Jatropha seed cake (Table 3). These data show the importance of adding eucalyptus bark to balance carbon and nitrogen and decrease the fat content, thus resulting in improved fungal growth. Furthermore, these data confirm the potential of using the bio-detoxified seed cake as a source of protein and lipids in ruminant diets [58]. The use of foods rich in these nutrients in animal diets is important because (a) the proteins are the main source of nitrogen and amino acids, and (b) lipids can reduce the production of methane by the rumen [59]. For every 1% increase in the amount of fat added to the diet, there is a 6% reduction in methane

emissions by ruminant animals. This reduction in methane production may be due to a negative effect on the lipid protozoa and methanogenic archaea [60].

In the ruminant diet, proteins and amino acids supply nitrogen for microbial protein production. Proteins synthesized by microorganisms of the rumen have a higher nutritional value than dietary protein. According to Alemawor et al. [61], the low level of protein in the skin of cocoa limits its use as animal feed. In this context, increasing the CP in Jatropha seed cake by colonization with *P. ostreatus* (Table 3) increases its potential for use in animal feed.

Components (g 100g ⁻¹)	Jatropha seed cake (Jc)		Jc + 10% eucalypt bark (JcEb10)	
	0 (control)	45 days	0 (control)	45 days
Dry mass (DM)	95.027 ^{ab}	96.243 ^A	95.028 ^{ab}	96.076 ^A
Organic matter (OM)	93.304 ^{aA}	91.136 ^B	92.738 ^{ba}	91.972 ^B
Crude protein (CP)	11.438 ^{ab}	13.158 ^A	11.075 ^{ba}	11.264 ^A
Ether extract (EE)	17.929 ^{aA}	7.563 ^B	16.214 ^{ba}	7.097 ^B
Non-fiber carbohydrates (NFC)	63.937 ^{bb}	70.915 ^A	65.259 ^{ab}	73.800 ^A
Neutral detergent fibre (NDF)	49.217 ^{ab}	53.920 ^A	49.445 ^{ab}	54.129 ^A
Acid detergent fibre (ADF)	37.549 ^{aA}	35.243 ^B	34.442 ^{bb}	37.363 ^A
Lignin	20.890 ^{aA}	16.558 ^B	16.902 ^{ba}	12.246 ^B
Hemicellulose	21.669 ^{ba}	14.279 ^B	25.331 ^{aA}	17.022 ^B
Cellulose	25.661 ^{ba}	23.837 ^B	32.058 ^{aA}	22.030 ^B
Ash	6.696 ^{bb}	8.864 ^B	7.262 ^{ab}	8.028 ^A
<i>In vitro</i> digestibility	54.902 ^{bb}	77.918 ^A	60.306 ^{ab}	83.899 ^A

Table 3. The chemical composition of different proportions of Jatropha seed cake and agro-industrial residues colonized for 45 days by *P. ostreatus*

Ether extract content in substrates also decreased after incubation with *P. ostreatus* (Table 3). The reduction was independent of substrate and averaged 57%, suggesting that the fungus may have used lipids as a nutrient source. This reduction also contributes to the use of Jatropha residue in ruminant diets because it is typically recommended that EE represent less than 10% of a diet's dry matter.

After inoculation with *P. ostreatus* Plo 6, the substrates showed an increase of DM and CP and a reduction of organic material (Table 3). This is similar to observations made in cocoa husks fermented with *P. ostreatus* [61] and in *Jatropha curcas* kernel cake fermented with *Aspergillus niger* and *Tricholoma longibrachiatum* [62]. These data suggest two biological processes: (a) the uptake or absorption of organic matter by the fungus, resulting in the production of proteins and mycelial growth (increased dry weight); and (b) the mineralization or degradation of organic matter resulting in an increase in mineral content (ash) and NFC (Table 3). Assimilation of organic material resulting in an increase in crude protein was also found in

P. sajor-caju grown in cotton waste [56]. Degradation or mineralization of organic matter was also observed in a culture of *P. ostreatus* on eucalyptus bark [63].

We observed an increase in carbohydrates after inoculation with *P. ostreatus* (Table 3). This increase confirms the degradation of more complex compounds such as lignin, cellulose and hemicellulose by *P. ostreatus* (Table 3). Degradation of these compounds in the *Jatropha* seed cake substrate contributed to an increase in dry matter digestibility (Table 3). An increase in digestibility after lignin degradation has also been shown after cultivation of *Phanerochaete chrysosporium* on cotton stalks [64], *P. sajor-caju* in agroindustrial residue [57] and *P. ostreatus* on eucalyptus bark [63].

Therefore, colonization of *Jatropha* seed cake by *P. ostreatus*, with or without addition of eucalyptus bark, was shown to increase the nutritional value and *in vitro* digestibility of this by-product from the biodiesel production chain.

3.3.1. Ammonia production by microorganisms in the ruminal liquid

The ruminant's microorganisms are large and genetically diverse, consisting of bacteria, fungi, protozoa and viruses [65]. These microorganisms contribute to the fermentation of substrates that have low solubility (e.g., plant material rich in fiber) in organic acids, methane, ammonia, acetate, lactate, formate, ethanol, propionate, CO₂ and H₂ [66].

The ammonia production observed in this study can be considered low (Figure 6). The production of this compound by rumen ammonia-producing bacteria may vary from 33 to 159% of the dry mass depending on the bacterial species and protein content of the diet [67]. Rumen microorganisms are capable of incorporating a large portion of the produced ammonia by deamination of amino acids and hydrolysis of nitrogen compounds. However, when the rate of deamination exceeds the rate of assimilation, protein catabolism results in the undesirable and inefficient process of high ammonia production and low retention of nitrogen [68]. This undesirable process can be characterized by the loss of protein through the excretion of nitrogen as urea in the animal's urine [69]. According to previous studies, the rate of dietary protein degradation is directly proportional to the ammonia production and protein nitrogen loss. Therefore, low ammonia production by microorganisms in the rumen fluid demonstrates that the substrates colonized with *P. ostreatus* exhibit good digestibility and that the rate of ammonia uptake is not exceeded (Figure 6).

The highest ammonia production was observed in substrates colonized by *P. ostreatus* (Figure 6). The increased production of ammonia may be due to the higher amount of crude protein in these substrates (Table 3). This shows that the colonized substrates have a greater capacity to be degraded by rumen microorganisms than those that were not colonized. This higher capacity can be a result of the following: (a) the presence of toxic compounds which inhibit microbial growth in substrates without fungal colonization or (b) a reduction in lignocellulosic compounds by enzymatic action resulting in compounds (e.g., NFC, CP and NDF) contributing to the growth and metabolism of microorganisms (Table 3). This degradation/mineralization of lignocellulosic compounds is also reflected in the increase in dry matter and ash content (Table 3).

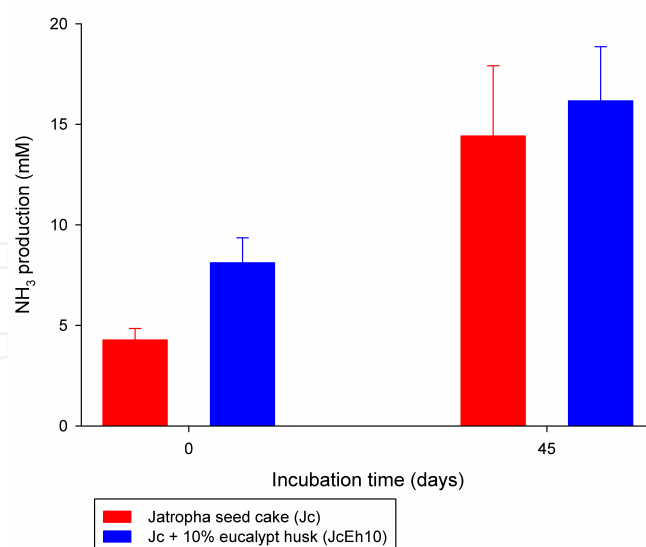


Figure 6. Ammonia concentration in batch cultures containing ruminal liquid and the substrates evaluated (Table 1). Substrates were harvested before and after 45 days of colonization by *Pleurotus ostreatus* Plo 6.

Finally, it is important to note that *P. ostreatus* Plo 6's reduction in levels of phorbol esters (99%) and ammonia (Figure 6) did not inhibit the development of rumen bacteria. This result confirms the detoxification of Jatropha seed cake by *P. ostreatus* and again highlights the importance of fungal colonization in the preparation of the cake for use in animal feed.

3.4. Animal assay

3.4.1. Food intake, digestibility and nitrogen balance

The intake of dry matter and nutrients was influenced by the different amounts of detoxified Jatropha seed cake in the diet (Table 4). The DM intake (% BW) and NDF showed a quadratic response ($P < 0.05$), and there was a positive linear effect ($P < 0.05$) on DM intake (g/kg BW^{0.75}). The DM, OM and CP increased linearly ($P < 0.05$) and no changes were observed in either the EE and NFC consumption by animals (Table 4).

The increase in DM intake may be attributed to a reduction of the energy values in the experimental diets (Table 2). In this sense, the animals ate more DM to reach their energy requirements. Thus, we can infer that the consumption and palatability of the diets was not restricted by the inclusion of detoxified Jatropha seed cake, although it increased DM intake by the animals. In prior experimental animals, the replacement of soybean meal by Jatropha seed cake resulted in a decrease in DM ingestion, which was attributed to the presence of anti-nutritional factors [70]. In this study, the maximum intake of DM and NDF was 3.68 and 1.67% of BW, respectively. This was not enough to promote the rumen fill effect. In diets with a low energy level, animals tend to exceed the consumption limit of 1.2% of BW, offsetting any food energy deficiency [66].

The increase in the DM intake resulted in increases in intakes of OM and CP. However, this increase had no effect on the overall consumption of EE and NFC. These results support the theory of compensation in DM intake by animals on diets with a low concentration of energy.

Intake (g d ⁻¹)	Jatropha seed cake (% dry mass)				Regression	R ²	CV (%)
	0	7	14	20			
Dry mass (DM)	701.34	719.90	826.27	891.35	Y = 681.609+10.058x	0.94	13.67
DM (%BW)	2.91	2.84	3.19	3.68	Y = 2.901-0.030x+0.003x ²	0.99	10.33
DM (g/kgBW ^{0.75})	64.41	63.61	71.89	81.65	Y = 61.327+0.883x	0.83	10.42
Neutral detergent fiber(%BW)	1.17	1.19	1.38	1.67	Y = 1.170-0.009x+0.001x ²	0.99	9.83
Organic matter	673.35	696.55	795.80	854.01	Y = 657.092+9.545x	0.95	13.62
Crude protein	91.40	87.55	97.29	106.09	Y = 87.469+0.791x	0.72	14.10
Ether extract	23.54	24.10	26.10	26.06	ns	--	14.07
Non-fiber carbohydrates	272.74	274.36	304.92	307.44	ns	--	16.10
Digestibility (%)							
Dry mass	74.16	68.10	65.40	62.46	Y = 73.328-0.565x	0.97	7.24
Organic matter	74.88	69.02	65.90	63.06	Y = 74.136-0.577x	0.97	6.83
Crude protein	67.92	54.10	52.14	46.70	Y = 65.305-0.984x	0.89	10.53
Ether extract	80.40	77.62	78.61	76.92	ns	--	5.18
Neutral detergent fiber	69.32	64.19	60.55	58.05	Y = 68.774-0.560x	0.98	7.44
Non-fiber carbohydrates	82.46	78.50	75.75	73.75	Y = 82.050-0.432x	0.98	7.99

Table 4. Consumption and apparent total tract digestibility of dry matter and nutrients in goats fed with bio-detoxified Jatropha seed cake

The inclusion of increasing levels of detoxified Jatropha seed cake promoted a linear reduction ($P < 0.05$) in the digestibility of DM, OM, CP, NDF and NFC diets tested (Table 4). The exception was EE digestibility, which did not show significant variation and had average values of 78.39%. This reduction in dry matter digestibility can be attributed to an increase in passage rate as a function of consumption, resulting in the shorter digestion time of nutrients in the gastrointestinal tract [66]. This effect is associated with the highest possible lignin concentration of the experimental diets.

In relation to nitrogen metabolism, significant effects on Nuendo, nitrogen balance and the biological value of protein from the level of detoxified Jatropha seed cake added were not observed (Table 5). The intake of nitrogen, excretion of Nfecal, Nmet.fecal, Nundig, Nuexo and urinary nitrogen were influenced in a linear manner at the levels studied (Table 5). Losses of nitrogen in the urine and feces were 28.63 and 40.20% of the consumed nitrogen, respectively.

Variable (g d ⁻¹)	Jatropha seed cake (% dry mass)				Regression	R ²	CV (%)
	0	7	14	20			
Consumed nitrogen	14.62	14.01	15.57	16.98	$Y = 13.993 + 0.126x$	0.72	14.10
Nfecal	4.67	5.22	6.79	7.95	$Y = 4.416 + 0.169x$	0.96	20.44
Nmet. fecal	0.39	0.37	0.42	0.45	$Y = 0.372 + 0.003x$	0.72	14.14
Nund	4.28	4.84	6.37	7.50	$Y = 4.042 + 1.166x$	0.97	21.64
Urinary nitrogen	4.56	3.49	4.29	5.19	$Y = 4.495 - 0.194x + 0.011x^2$	0.70	25.38
NUend	1.80	1.86	1.90	1.80	Ns	--	8.07
Nuexo	2.77	1.63	2.39	3.39	$Y = 2.703 - 0.213x + 0.012x^2$	0.75	43.50
NB	7.58	7.53	6.80	6.09	ns	--	36.41
BVP (%)	72.54	77.91	72.70	63.86	ns	--	21.18

Table 5. Nitrogen consumption, excretion, balance and retention by goats fed with bio-detoxified Jatropha seed cake

Generally, urea concentration is correlated with ammonia content in ruminants because digestive microorganisms using nitrogen require energy for the synthesis of bacterial proteins. Most likely, there was excess of ruminal ammonia, which increased the excretion of nitrogen in the urine; thus, levels of 12% CP in the diet of growing goats can promote higher levels in waste nitrogen. Valadares et al. [71] also found an increase in nitrogen excretion in urine when they provided a similar amount of protein to zebu cattle.

Nitrogen balance (NB) and biological value did not differ between the evaluated diets. However, the positive observed values of NB suggest its use in the synthesis of tissue.

3.4.2. Blood parameters

The experimental diets did not significantly alter the blood parameters of the animals (Table 6). The resulting values were similar to those of normal goats [72]. The hemoglobin concentration was similar to that observed in goats fed with Jatropha seed cake [62].

From the leukocyte values observed in this study, it could be inferred that animals did not experience inflammation after ingesting bio-detoxified *Jatropha* seed cake (Table 6).

The absence of significant effects in the content of creatinine, alkaline phosphatase and total protein by the different levels of bio-detoxified *Jatropha* seed cake (Table6) shows that liver function was not altered in animals fed the experimental diets.

Variable	Jatropha seed cake (% dry mass)				Regression	CV (%)	Reference*
	0	7	14	20			
Hematological profile							
Erythrocytes (x 10 ⁶ mm ³)	4.93	5.16	4.81	5.01	ns	11.88	8 a 18
Hemoglobin (g dL ⁻¹)	11.61	11.55	11.60	11.82	ns	5.93	8 a 12
Hematocrit (%)	31.75	31.83	31.68	32.33	ns	6.18	22 a 38
Leukocytes (n µL ⁻¹)	12179	12405	10533	11583	ns	14	4000 a 13000
Biochemical profile							
Creatinine (mg dL ⁻¹)	0.76	0.82	0.76	0.75	ns	7.48	1 a 1.82
Alkaline phosphatase (U l ⁻¹)	173.95	154.90	186.62	163.84	ns	18.95	93 a 387
Urea (mg dL ⁻¹)	20.76	21.53	18.09	20.53	ns	14.47	21.4 a 42.8
Total proteins (g dL ⁻¹)	6.90	6.66	6.73	6.57	ns	4.71	6.4 a 7

Table 6. Hematological and biochemical blood profiles of goats fed with bio-detoxified *Jatropha* and of normal control goats

Urea levels in the blood can increase in response to diets with low energy [73]. However, we did not observe this effect.

Thus, inclusion of *Jatropha* seed cake bio-detoxified by *P. ostreatus* shows promise as an animal feed supplement because it did not result in changes to blood parameters or clinical symptoms of poisoning. This included goats fed with up to 20% of the treated residue. Conversely, ingestion of *Jatropha* residue treated with organic solvents (ethanol and hexane) caused diarrhea and other side effects in swine [74]. Similarly, diarrhea and death resulted in goats fed with *Jatropha* seed cake colonized by *Aspergillus*, *Penicillium* or *Trichoderma* [62].

4. Conclusions

The residue of *J. curcas* increases with increased biodiesel production, so it is necessary to find an appropriate usefor these residues. In this study we demonstrate the potential to

transform the residue of biodiesel containing lignocelluloses, toxic compounds, and anti-nutritional factors into animal feed. This process adds economic value to biodiesel production and avoids the improper disposal of its by-products in the environment.

The bio-detoxification of Jatropha seed cake promotes the reduction of phorbol ester levels and increases the nutritional value of this residue. The resulting alternative food can be included in amounts up to 20% (DM) in the diet of growing goats.

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