Evaluation of Bio-detoxification of *Jatropha curcas* Seed Cake and Cottonseed Cake by Basidiomycetes: Nutritional and Antioxidant Effects

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Abstract

Poultry and swine are the major proportion of the livestock industry in terms of output value. To meet the growing need for protein sources in these sectors, the use of biomasses coming from agro-industrial residues can be an interesting option in the future years. This study aimed to evaluate the capacity of seven basidiomycetes to grow, detoxicate, increase protein content, and its antioxidant activity when grew in pure *Jatropha* seed cake (JSC) and cottonseed cake (CSC) biomasses and mixtures containing 50% of lignocellulosic biomasses from coconut husks and *Acrocomia aculeata* (macauba cake). Results showed that five basidiomycetes were able to grow in these substrates. *F. hepatica*, *P. lecomtei*, and *P. pulmonarius* presented the highest bio-detoxification capacity. All treatments showed a reduction in total phenolic compounds and antioxidant activity, but treatments with coconut husks showed lower reductions. Results also indicated that there were molecules produced by basidiomycetes responsible for antioxidant activity other than phenolic compounds. These results indicated that basidiomycetes could detoxify JCS and CSC biomasses, suggesting their possible use in animal feed and that the addition of coconut husks in JSC and macauba cake in cottonseed cake could promote greater colonization by fungi.
Keywords  Macauba cake · Coconut husks · Bio-detoxification · Antioxidant activity · Basidiomycetes

Statement of Novelty

Globally, there is an increasing demand for sustainable protein sources for animal feed. The use of agro-industrial wastes, such as biomasses from *Jatropha curcas* seed cake (JSC), cottonseed cake (CSC), appears as a viable, economic and ecological alternative. However, JSC and CSC contain toxic compounds that can impair animals’ digestibility. Bio-detoxification with macro-fungi is a cheap and efficient process to reduce the concentration of these substances and to improve protein content, besides generating edible mushrooms and lignocellulosic enzymes. Our work evaluated the colonization of seven different basidiomycetes in pure and mixed biomasses with lignocellulosic agro-industrial wastes aiming to select a suitable one for animal feed, focusing on reducing producer’s cost and avoiding environmental disposal.

Introduction

As the human population grows, the demand for food increases proportionately. To help address the global food challenge, researchers have developed alternatives sources of animal feed for poultry and swine industries, the most consumed meats in the world. Among these new options, oil press cakes have been widely used as sources of proteins and carbohydrates.

Amongst the biomasses with potential for use in animal nutrition, *Jatropha curcas* seed cake and cottonseed cake, residues of oil extraction, both oilseeds suitable for biodiesel production, are good candidates especially due to their high protein content. However, toxic compounds such as phorbol esters and free gossypol, present respectively in Jatropha cake and cottonseed cake, make these materials unsafe for animal feed. Phorbol esters can act either acutely inducing an intense inflammatory response, or chronically, inducing tumor development [1]. Free gossypol (FG) can cause infertility and toxicity in young ruminants and monogastric animals [2]. As an alternative to enable the use of these biomasses, colonization with macrofungi (basidiomycetes) offers the advantages of being a low-cost process to biodeconticate these substrates as well as the production of...
value-added products, such as edible mushrooms and lignocellulosic enzymes [3]. Besides, the cultivation of mushrooms in agro-industrial wastes can improve their protein content, produce bioactive molecules such as phenolic compounds, polysaccharides, sterols, and reduce lignin and cellulose content, improving their digestibility for animal feed [4]. A good balance of carbon–nitrogen is important to promote the better growth of fungi [5]. So, the combination of Jatropha or cottonseed cakes as protein sources with coconut husks or Acrocomia aculeata (Jacq.) Lodde ex Mart. (palm) cake as a carbohydrates source is interesting for basidiomycetes cultivation since the last ones make the carbon–nitrogen balance increases. Finally, the carotenoids of Acrocomia aculeata and the high phenolic content of coconut husks might help the antioxidant activity of final biomasses products [6].

Basidiomycetes are a class of fungi with species that produce visible fruiting bodies commonly known as mushrooms [7]. They have high commercial value due to their nutritional composition and for being an alternative source of quality proteins and essential amino acids [8, 9] as well as because some species present medicinal properties. Among basidiomycetes used for bio-detoxification purposes, some species stand out. Agaricus subrufescens (syn. A. blazei, A. brasiliensis), also known in Brazil as “Sun mushroom” is widely consumed for its medicinal activities [10] being known for preventing a range of diseases such as cancer, diabetes, arteriosclerosis, and others [11]. Lentinula edodes is popularly known as Shiitake and it is the second most-consumed mushroom in the world. Shiitake has high nutritional value and medicinal properties, being pointed as a good choice to reduce cholesterol, modulation of the immune system, and by its anti-inflammatory properties [12]. Schizophyllum commune is one of the most common mushrooms in nature. Although usually classified as a non-edible species due to its fibrous texture, it is traditionally consumed in Mexico [13].

S. commune gained pharmaceutical visibility after the discovery of its derived polysaccharide, Schizophyllan, which has anticancer activity [14].

Another species of basidiomycetes applied in bio-detoxification is Pleurotus spp., popularly known as oyster mushroom. P. ostreatus is widely consumed, while P. eryngii and P. pulmonarius are less consumed. These two species present, however, anticancer, anti-inflammatory, and antimicrobial properties already recognized, mainly for their polysaccharides, peptides, proteins, triterpenes, and nucleotides [15]. Fistulina hepatica is another basidiomycete known for its medicinal properties. Its antimicrobial and antioxidant activities have already been studied and polysaccharides and phenolic compounds have been identified as bioactive molecules [16, 17]. Panus lecomtei is a mushroom traditionally consumed in Brazilian indigenous communities and Japan. Little is known about this species, however, some authors [18, 19] have already reported that P. lecomtei has anticancer and antimicrobial activities. Finally, Ganoderma lucidum also called Reishi mushroom, the “immortality mushroom”, has traditionally been used in Chinese medicine to prevent hepatitis, nephritis, hypertension, and asthma, and also to stimulate the immune system [20]. The bioactive compounds identified in G. lucidum include triterpenoids, polysaccharides, nucleotides, sterols, and proteins; being these molecules responsible for anticancer, antimicrobial, anti-inflammatory, antioxidant, antifungal, and anti-viral properties of the species [21]. Nowadays, global concern about antibiotic resistance and residues in animal products has resulted in the search for alternatives to growth promoters and veterinary drugs (such as prebiotics, probiotics, and other feed additives), focus on reducing the use of antimicrobials in animal production [22].

Therefore, this work aimed to evaluate growing, detoxifying efficiency, protein gain, and improvement of antioxidant activity of four basidiomycetes with medicinal properties (Agaricus subrufescens, Schizophyllum commune, Fistulina hepatica, and Ganoderma lucidum), and three edible mushrooms (Lentinula edodes, Pleurotus spp, and Panus lecomtei) cultivated in pure biomasses of Jatropha curcas seed cake and cotton seed cake and combined with coconut husks and macauba cake biomasses. Results obtained here can be used in poultry and swine industries as a guide to select biomasses suitable for animal feed enriched with bioactive compounds, reducing meat-producing costs, and avoiding the disposal of these agro-residues in the environment.

**Materials and Methods**

**Plant Material**

Four different biomasses for basidiomycetes growth were used in this experiment: pure Jatropha seed cake (JSC) and cottonseed cake (CSC), and in mixtures containing 50% of lignocellulosic biomasses from coconut husks (CH) and Acrocomia aculeata (macauba cake-MC). Seeds from Jatropha curcas were provided by Embrapa Cerrados (Planaltina – DF, Brazil). After mechanical extraction of oil, the cake was dried for 30 min, at 100 °C, in a rotatory dryer. Cottonseed cake was provided by Farmotec Industry (Bauru – MG, Brazil).

Coconuts husks were obtained from local sellers (Brazil – DF, Brazil), crushed, and subsequently oven-dried for 1 week, at 60 °C. Macauba (Acrocomia aculeata) fruits were provided by Agrotech/Soleá Company (João Pinheiro – MG, Brazil). Macauba mesocarp had its oil extracted mechanically, and residual solid biomass was dried for 30 min, at 100 °C.
Media Preparation and Basidiomycetes Cultivation

Fungi tested were Agaricus subrufescens (strain CC414), Lentinula edodes, Schizophyllum commune (strain FPB117), Pleurotus pulmonarius (strain EF88), Fistulina hepatica (strain CC102), Panus lecomtei (strain CC40), and Ganoderma lucidum (strain CC351). Samples were obtained from the Microorganisms and Microalgae Collection (Coleção de Microorganismos e Microalgas Aplicados Bioferreiraria – CMMABio) from Embrapa Agroenergy (Brasília – DF, Brazil). Strains were preserved in potato dextrose agar plates (PDA) at 4 ºC. The inoculum was prepared by transferring disks of 5 mm of diameter from the preserved cultures to another PDA plate, and incubating it in a BOD incubator, for 7 days, at 28 ºC.

Growth medium assayed were pure Jatropha seed cake (JSC), pure cottonseed cake (CSC), Jatropha seed cake with coconut husks (JSC + CH), cottonseed cake with coconut husks (CSC + CH), Jatropha seed cake with macauba cake (JSC + MC), and cottonseed cake with macauba cake (CSC + MC). In each 10 cm jar (225 ml of volume), 15 g of each biomass was weighted, totaling 30 g for mixtures. For JSC and CSC puree, 30 g were added to each jar. Biomasses humidity was adjusted to approximately 65% by adding water. After that, biomasses were sterilized in an autoclave for 45 min, at 121 ºC. After cooling, 4 disks (5 mm of diameter) of each basidiomycete’s inoculum were added to each jar. Jars without inoculum were incubated at the same conditions to be used as uncolonized control samples. Cultures were prepared in triplicate. Jars were incubated for 15 days at 28 ºC, in a BOD incubator. After incubation time, cultures were dried with air circulation for 48 h, at 40 ºC, milled, and stored at − 20 ºC until further analysis.

Growth Rate

During the 15 days of incubation time, every 03 days, mycelium growth measurements were assayed. The growth rate in cm/day for each fungus, at each different biomass, was determined at the end of the cultivation period. Basidiomycetes that were not able to grow in the biomasses were removed for further analysis.

Phorbol Ester and Free Gossypol Quantification

Phorbol ester quantification in JSC was performed in triplicate. An adaptation from the original protocol described in the literature [23] was applied. Thus, methanol was added (15 mL) to falcon tubes containing 3 g of cultures grown with jatropha seed cake and then crushed with Teflon sticks for 1 min. After that, solution was ultrasonicated (USC480A, Unique, SP, Brazil) at an ice bath for 3 min. A centrifugation procedure at 9.000 rpm for 8 min was done. The supernatant was transferred to a round bottom flask and was evaporated in a rotary evaporator under vacuum (Rotavapor R210/215, Büchi Labortechnik, Flawil, Swiss), at 40 ºC. Phorbol esters were resuspended with 5 mL of methanol and centrifuged at 14.000 rpm for 8 min at 8 ºC. Chromatographic analysis was performed at a UPLC (Ultra-Performance Liquid Chromatography) System (Acquity UPLC H-Class System, Waters, Massachusetts, USA) with a PDA detector, using a reverse-phase column (Waters, Acquity UPLC HSS-T3), pre-column (VanGuard HSS-T3) at 45 ºC. An elution gradient of 0.05% trifluoroacetic acid (30%), acetonitrile (49%), and methanol (21%) was used. The initial flow was 0.4 mL/min and, the final flow was 0.6 mL/min. The detector was adjusted at 280 nm. A calibration curve was performed using a standard solution of phorbol myristate acetate (PMA) (Sigma-Aldrich). The results were plotted on this curve to obtain phorbol esters content.

Free gossypol (FG) quantification was performed according to a method previously described in the literature [24]. In a falcon tube, 1 g of milled and dried samples was added. The extraction was performed with two sequential steps in a cold ultrasonic bath: the first one with 3 mL of ultrapure water and the second one with 7 mL of acetone. The tubes were centrifuged at 9.000 rpm, for 5 min, at 8 ºC and finally, 1 mL of supernatant was transferred to 2 mL sample vials. A Water Acquity UPLC H-Class system (Waters, Milford, USA), with a PDA detector using a reverse-phase column C18 (100 × 2.1 mm, 2.6 μm, KINETEX®), was used to conduct the chromatography analysis. Using a standard solution of gossypol (≥ 95% purity HPLC, Sigma-Aldrich) a calibration curve was performed. The results were plotted on this curve to obtain free gossypol content.

The degradation rate of both phorbol ester and free gossypol was assayed considering 100% as the amount of these toxic compounds in the uncolonized biomasses (control).

Ergosterol Content

Ergosterol, a primary sterol present in cell membranes of filamentous fungi is widely used to estimate fungal biomass [25]. Ergosterol quantification was performed in triplicate, applying a method adapted from a published protocol [26]. Briefly, methanol (10 mL) and ethanol (5ML) were added to the dried milled culture (400 mg) with 2 g of KOH in the assay tubes. Saponification was carried out in a water bath at 70 ºC for 30 min. After cooling of samples, water (5 mL) and hexane (10 mL) were added. Tubes were ultrasonicated (USC480A, Unique, SP, Brazil) for 1 min and, supernatants were collected. Extraction was performed twice using 5 mL of hexane each time. Extracts were pooled and rota-evaporated (Multivapor Büchi, Flawil, Swiss) in a bath temperature of 40 ºC. Extracts were resuspended in methanol and centrifuged at 13.500 rpm for 5 min. Quantification was
performed by injecting extracts in a UPLC system (Acquity UPLC H-Class System, Waters, Massachusetts, USA) with a PDA detector and a 2.6 µm C18 column (Kinetex, Torrance, CA, USA). A calibration curve was made with an ergosterol standard (Sigma-Aldrich), and the results of the samples were plotted on this curve to obtain ergosterol content.

**Crude Protein Content**

In a Perkin Elmer AD-6 Auto Balance (Massachusetts, EUA), 2 mg of milled and dried colonized biomasses were weighted. The nitrogen content was quantified with a CHN (Carbon, Hydrogen and, Nitrogen) analyzer (2400 CHNS/O Series II, PerkinElmer, Massachusetts, EUA). The carrier gas used was helium. Acetanilide (C = 71.09%, N = 10.36%, H = 6.71%) was used to calibrate the instrument. For crude protein content, nitrogen content was multiplied by the standard default conversion factor of 6.25. This analysis was performed in triplicate [27].

**Preparation of Extracts**

Samples extracts were produced to perform antioxidant analysis. Extracts of milled and dried colonized biomasses were obtained in triplicate, as reported below to each sample, 80% ethanol was added (1:20 w/v), and the solutions were placed in an ultrasonic bath (room temperature) (USC480A, Unique, SP, Brazil) for 20 min. After that, samples were shaken at 180 rpm for 30 min, and finally centrifuged at 4,000 rpm for 10 min. Then, the supernatant was collected, filtered with Whatman no. 41 filter paper, and evaporated (Rotavapor R210/215, Büchi Labortechnik, Flawil, Swiss) with bath temperature of 40 °C. Residual extracts were freeze-dried (K120, Liotop, São Carlos, SP, Brazil) and stored at −20 °C in the dark until analyses.

**Antioxidant Activity**

Antioxidant activity was evaluated in extracts resuspended with methanol (5 mg/mL) by two different methods of free radical scavenging activity, one with radical DPPH- (2,2-diphenyl-1-picrilhidrazyl) and the other with ABTS +• (2,2’-azino-bis 3-ethylbenzotiazolin-6-sulfonate) radical.

DPPH radical antioxidant activity was performed as described by previous authors [28], with modifications. The reaction was performed in a microplate, by adding 22 µL of extract to 200 µL of DPPH solution (150 µM). Samples absorbance was read at 520 nm (Molecular Devices, Spectramax 190, California, USA). Results of the samples were plotted on a calibration curve performed with Trolox (6-hydroxy-2,5,7,8-tetrametilchroman-2-carboxylic acid) at 0.5 mg/mL.

A method described by previous authors [29] and adapted by others [30] was applied to analyze ABTS +• antioxidant activity. ABTS +• solution was prepared by adding 88 µL of potassium persulfate solution (140 mM) to 5 mL of ABTS solution (7 mM), kept overnight in the dark. This solution was diluted in methanol (approximately 1:40 v/v) till the absorbance at 734 nm reached 0.70. In a microplate, the extract was added (10µL) to 290 µL of ABTS +• solution and incubated at 30 °C, for 6 min, in the dark. The absorbance of the samples was read at 734 nm (Molecular Devices, Spectramax 190, California, USA). Results of the samples were plotted on a calibration curve, performed with Trolox (6-hydroxy-2,5,7,8-tetrametilchroman-2-carboxylic acid) at 0.5 mg/mL, to obtain antioxidant activity.

**Total Phenolic Compounds (TPC)**

Phenolic compounds quantification was carried out according to a previous method with adaptations [31]. Extracts were resuspended with ethanol 80% (3 mg/mL). Thus, 500 µL of Folin–Ciocalteau (FC) reagent were added to 100 µL of extract diluted in 7 mL of distilled water. Samples were vortexed and incubated for 8 min at room temperature. Then, 1.5 mL of Na₂CO₃ 20% (w/v), 900 µL of water were added to the tubes and incubated for 2 h, at room temperature in the dark. The sample’s absorbance was read at 765 nm in 1 mm cuvettes (Agilent, Cary 60, California, USA). TPC content was obtained by plotting the results of the samples on a calibration curve, performed with Gallic Acid (0.5 mg/mL).

**Statistics**

All essays were performed in triplicate. The data were submitted to analysis of variance (ANOVA). In the case of p < 0.05, the data were submitted to the Tukey test. Statistics analyses were performed using RStudio software [32] and graphs were made using GraphPrism 8 (GraphPad Software, La Jolla, California, EUA). Pearson’s correlation test was performed grouping in one block, all experiments with JCS, and another with all experiments with CSC (p < 0.01). An analytic Hierarchy Process (AHP) was used to rank the most promising treatments.

**Results**

**Growth Rate in Jatropha Seed Cake (JSC), and Cottonseed Cake (CSC)**

The results of the growth rate (GR) showed that A. subrufe-scens and, L. edodes were not able to grow in the jars with biomasses containing Jatropha seed cake (JSC), Jatropha seed cake with coconut husks (JSC + CH), and Jatropha
seed cake with macauba cake (JSC + MC) (Fig. 1). For substrates containing cottonseed cake (CSC), cottonseed cake with coconut husks (CSC + CH), and cottonseed cake with macauba cake (CSC + MC), *A. subrufescens* was also unable to grow, while *L. edodes* showed a lower growth rate (Fig. 2). Considering the inability of both species (*A. subrufescens* and *L. edodes*) to grow in the tested substrates, they were excluded from further analysis.

All the other basidiomycetes species, *S. commune*, *P. pulmonarius*, *F. hepatica*, *P. lecomtei*, and *G. lucidum*, were capable to grow in jars for 15 days. *P. lecomtei* and *S. commune* presented the highest growth rate, respectively, in Jatropha seed cake (JSC) (Fig. 1a) and cottonseed cake (CSC) (Fig. 2a), showing a GR of 0.66 and 0.69 cm/day. These two species also showed higher GR in JSC + MC (Fig. 1c) and CSC + MC (Fig. 2c), differing statically from the other 05 basidiomycetes species assayed.

As for substrates containing coconut husks (CH), in JSC + CH, *S. commune*, *F. hepatica*, and *P. lecomtei* presented the highest growth rate (0.56, 0.51, and 0.46 cm/day, respectively), statically differing from the other tested species (Fig. 1b). In CSC + MC, diverse growth rates were obtained (Fig. 2b) with *P. lecomtei* and *S. commune* presenting higher GR (0.59 and 0.58 cm/day, respectively), followed by *F. hepatica* (0.34 cm/day).

**Phorbol Ester, Gossypol, Ergosterol, and Crude Protein Contents**

**Jatropha Seed Cake (JSC)**

Results of phorbol ester content in JSC indicated that the control sample (uncolonized) contained 1.16 mg/g of phorbol ester, while mixtures of JSC + CH and JSC + MC presented 0.42 mg/g and 0.47 mg/g, respectively. Colonized samples presented phorbol ester contents in JSC varying from the lowest value of 0.02 mg/g to the highest amount of 0.19 mg/g, identified respectively, in *P. lecomtei* and *S. commune* colonization. In JSC + CH colonized samples, phorbol ester values varied from 0.02 mg/g for *P. lecomtei* and *P. pulmonarius* to 0.24 mg/g for *G. lucidum* colonization. In JSC + MC, values varied from 0.01 mg/g for *P. lecomtei* and *P. pulmonarius*, and 0.06 mg/g for *S. commune* and *F. hepatica* colonization (Table 1). Finally, in colonized JSC substrates degradation percentage ranged from 53.8%
for *G. lucidum* (JSC + CH) to 98.2% for *P. lecomtei* (JSC) (Table 1).

Ergosterol content analysis in uncolonized biomasses of JSC, JSC + CH, and JSC + MC showed contents of 52.5, 40.9, and 35.5 ppm, respectively. Meanwhile, in colonized materials, ergosterol was quantified in contents up to 1268.7 ppm (JSC + MC) and 1261.4 ppm (JSC + CH) for *S. commune*, followed by colonization of *P. pulmonarius* in JSC + CH (763.1 ppm) and JSC + MC (694.1 ppm) biomasses. Overall, for all other species, JSC biomass containing CH presented higher ergosterol content when compared to JSC and JSC + MC (Table 1).

Since CH and MC biomasses have low protein content, higher crude protein content was identified in uncolonized pure JSC biomass rather than in the mixtures. Uncolonized biomasses mixtures presented different protein contents: 27.9 g/100 g in JSC, 11.4 g/100 g in JSC + CH, and 16.9 g/100 g in JSC + MC. Usually, the protein content of the cultivation medium increases because of the syntheses of fungal protein and also due to the consumption of lignocellulosic material during growth, leading to an effect of protein concentration due to depletion of other components. The crude protein (CP) content of colonized JSC biomass ranged from 20.8 g/100 g for *P. pulmonarius* to 30.1 g/100 g for *G. lucidum*. For JSC mixed coconut husks (CH), crude protein content varied from 17.3 g/100 g for *S. commune* to 19.7 g/100 g for *F. hepatica*. For JSC + MC, CP data ranged from 18.7 g/100 g for *P. pulmonarius* to 22.5 g/100 g for *F. hepatica* (Table 1).

Although presenting average ergosterol content, which is an indicator of fungi growth, *P. pulmonarius* and *S. commune* species were incapable to concentrate protein by bioconversion in pure JSC, showing decreases of −16.9 and −25.7%, respectively, in the protein content of colonized biomass (Table 1). The coefficient r of Pearson between initial protein content and % of the increased protein was inversely proportional (r = −0.88; P < 0.01). For all other species, lower conversions were identified in JSC, being 1.2, 7.6 and, 7.8%, respectively, for *P. lecomtei*, *F. hepatica*, and *G. lucidum*. Higher protein bioconversion was identified in JSC + CH biomass. Although there was no statistical difference between fungi treatments, an increase of 72.0% was identified for *F. hepatica* colonization, followed by an increase of 66.5% for *G. lucidum, 56.6% for P. pulmonarius*, 51.5% for *S. commune*, and 47.5% for *P. lecomtei* (Table 1).

In JSC + MC biomass, the most notable increase was 33.7% in *F. hepatica* colonized material, while *P. pulmonarius* showed the smallest increase (10.8%).

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![Figure 2](image-url)

**Fig. 2** Growth rate (cm/day) of seven basidiomycetes colonized in cottonseed cake, cottonseed cake with coconut husks, and cottonseed cake with macauba cake during 15 days in 10 cm jars. CSC: Cottonseed cake; CH: Coconut husks; MC: Macauba cake. Means followed by the same letter are not different by Tukey test (p < 0.05).
The results of FG content in uncolonized biomasses varied between 2170.8 ppm (in CSC) and 607.2 ppm (in CSC + MC). In colonized biomasses, FG content varied from 59.4 ppm for S. commune to 423.0 ppm for P. pulmonarius. In the CSC + CH biomasses mixture, FG content ranged from 59.4 ppm for S. commune to 423.0 ppm for P. pulmonarius. For CSC + MC fungi treatments, FG content ranged from 676.7 to 1291.0 ppm, for F. hepatica and P. lecomtei, respectively. For CSC + MC fungi treatments, FG content ranged from 769.6 to 2321.0 ppm, respectively, identified for P. lecomtei and S. commune (Table 2).

Overall, higher protein contents were identified in pure CSC, followed by CSC + MC and CSC + CH biomasses. Protein contents for uncolonized treatment were 27.6 g/100 g, 12.3 g/100 g and, 15.1 g/100 g respectively for CSC, CSC + CH, and CSC + MC biomasses mixtures. The protein content of fungi treated CSC varied from 621.7 for F. hepatica to 1665.6 ppm for S. commune. For CSC + CH fungitreatments, protein content varied from 676.7 to 1291.0 ppm, for F. hepatica and P. lecomtei, respectively. For CSC + MC fungi treatments, protein content ranged from 769.6 to 2321.0 ppm, respectively, identified for P. lecomtei and S. commune (Table 2).

The highest protein bioconversions were identified for F. hepatica and P. lecomtei at CSC + MC, 44.7 and 41.6%, respectively; followed by S. commune and P. pulmonarius at CSC + CH, 36.5 and 36%, respectively. Lower protein contents for uncolonized treatment were 27.6 g/100 g, 12.3 g/100 g and, 15.1 g/100 g respectively for CSC, CSC + CH, and CSC + MC biomasses mixtures. The protein content of fungi treated CSC varied from 621.7 for F. hepatica to 1665.6 ppm for S. commune. For CSC + CH fungitreatments, protein content varied from 676.7 to 1291.0 ppm, for F. hepatica and P. lecomtei, respectively. For CSC + MC fungi treatments, protein content ranged from 769.6 to 2321.0 ppm, respectively, identified for P. lecomtei and S. commune (Table 2).

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Cottonseed Cake (CSC)

The results of FG content in uncolonized biomasses varied between 2170.8 ppm (in CSC) and 607.2 ppm (in CSC + MC). In colonized biomasses, FG content varied from 59.4 ppm for P. pulmonarius to 423.0 ppm for S. commune colonization in CSC. In CSC + CH, FG content ranged from 90.56 ppm with F. hepatica colonization to 658.4 ppm with S. commune colonization. In CSC + MC samples, FG content varied from 3.5 ppm for P. lecomtei colonization, to 454.6 ppm with S. commune (Table 2).

Higher percentages of FG degradation were identified in CSC + MC biomass for P. lecomtei, F. hepatica, and G. lucidum colonization with percentages of 99.4, 98.9, and 93.9%, respectively. Meanwhile, F. hepatica and P. lecomtei showed higher degradation in CSC + CH, 88.5 and 87.1%, respectively. The lowest percentages of FG degradation were identified for S. commune growing in CSC + CH (16.5%) and in CSC + MC (25.5%) (Table 2).

Ergosterol content in uncolonized biomasses were 47.3, 37.7, and 51.7 ppm, respectively, at CSC, CSC + CH, and CSC + MC. In colonized material is expected that the ergosterol content increases since it is a primary sterol present in the cell membranes of filamentous fungi used as a biomarker of fungi growth. The highest ergosterol content was 2.321 ppm, identified in S. commune at CSC + MC, in agreement with the visual growth rate observed in this specific experiment. In CSC biomass treated with fungi, ergosterol content varied from 621.7 for F. hepatica to 1665.6 ppm for S. commune. For CSC + CH fungitreatments, ergosterol content varied from 676.7 to 1291.0 ppm, for F. hepatica and P. lecomtei, respectively. For CSC + MC fungi treatments, ergosterol content ranged from 769.6 to 2321.0 ppm, respectively, identified for P. lecomtei and S. commune (Table 2).

Overall, higher protein contents were identified in pure CSC, followed by CSC + MC and CSC + CH biomasses. Protein contents for uncolonized treatment were 27.6 g/100 g, 12.3 g/100 g and, 15.1 g/100 g respectively for CSC, CSC + CH, and CSC + MC biomasses mixtures. The protein content of fungi treated CSC varied from 30.4 g/100 g for P. pulmonarius to 32.4 g/100 g for F. hepatica. In the CSC + CH biomass mixture, protein content ranged from 14.0 g/100 to 16.8 g/100 g, respectively for G. lucidum and S. commune. Finally, in CSC + MC biomass, protein content varied from 18.8 g/100 g to 21.9 g/100 g for P. pulmonarius and F. hepatica, respectively (Table 2).

The highest protein bioconversions were identified for F. hepatica and P. lecomtei at CSC + MC, 44.7 and 41.6%, respectively; followed by S. commune and P. pulmonarius at CSC + CH, 36.5 and 36%, respectively. Lower protein
conversions were identified in CSC + MC biomass for *G. lucidum* and *P. pulmonarius*, 7.7 and 10.8%, respectively (Table 2).

The highest protein increments were identified in JSC + CH treated with *F. hepatica* (72%) followed by JSC + CH with *G. lucidum* (66.6%) and JSC + CH, treated with *P. pulmonarius* (56.6%). For CSC, the highest protein increments, after fungi colonization, were at 34 and 36% in JSC + CH, treated with *P. pulmonarius* and *F. hepatica*, respectively, as well as in CSC + MC treated with *Panus lecomtei* and *F. hepatica*, with increments of 41.6 and 44.7%, respectively.

### Antioxidant Activity and Phenolic Compounds

#### Jatropha Seed Cake

Results showed that antioxidant activity decreased in all treatments compared to uncolonized (control) biomasses. It could be related to oxidative enzymes produced during fungi growth. Therefore, the best treatments would be those presenting lower losses in the ability to react with DPPH and ABTS free radicals. In DPPH assay, TEAC from JCS colonized varied between 2.4 (which was also the lowest data among all biomasses and basidiomycetes tested) and 31.0 μg/mg of extract for *P. lecomtei*, and *S. commune*, respectively. In JCS + CH biomass, TEAC results ranged from 12.8 to 56.0 μg/mg (which was also the highest data among all biomasses and basidiomycetes tested) of extract for *P. lecomtei*, and *P. pulmonarius*, respectively. In JSC + MC treatment, TEAC ranged from 15.9 μg/mg of extract identified for *P. pulmonarius* to 27.8 μg/mg of extract for *F. hepatica* (Table 3).

In ABTS +• assay, TEAC varied between 18.4 and 62.4 μg/mg of extract, for *P. pulmonarius* and *G. lucidum*, respectively in JCS treatments. In JCS + CH treatment, TEAC ranged from 23.2 to 97.2 μg/mg of extract for *P. lecomtei* and *P. pulmonarius*, respectively. *P. lecomtei* and *G. lucidum* presented respectively, 6.4 and 55.1 μg/mg of extract in JSC + MC, with remaining sample results falling in between these values. From all colonized biomasses, in Jatropha seed cake, antioxidant activity from *P. pulmonarius*, in JCS + CH treatment, presented the highest values between all other colonized biomasses, although not differing statistically to other treatments (Table 3).

Total phenolic compounds in uncolonized biomasses were higher in pure JCS when compared to JCS with mixtures (JSC + CH, and JSC + MC) (Table 3). TPC presented a higher reduction for colonized treatments. No TPC data was detected for *P. pulmonarius* and *P. lecomtei* at this treatment. The only treatment that presented TPC similar to untreated materials was JCS + CH biomass with *G. lucidum*, respectively.

### Table 2

| Treatment   | Biomass          | Gossypol (ppm) | Gossypol degradation (%) | Ergosterol (ppm) | CP (g/100g d.w.) | CP difference (%) |
|-------------|------------------|----------------|--------------------------|------------------|------------------|-------------------|
| Uncolonized | CSC              | 2170.8 ± 7.6Aa | –                        | 47.3 ± 2.3Da     | 27.6 ± 2.4Bb     | –                 |
|             | CSC + CH         | 788.6 ± 10.0Ab | –                        | 37.7 ± 0.7Db     | 12.3 ± 1.1Cb     | –                 |
|             | CSC + MC         | 607.2 ± 10.5Ac | –                        | 51.7 ± 10.7Da    | 15.1 ± 2.5Bb     | –                 |
| *S. commune* | CSC              | 423.0 ± 94.6Ab | 80.5 ± 3.4Ba             | 1593.3 ± 259.7Ab | 30.9 ± 1.4Ab     | 11.7 ± 5.1Bb     |
|             | CSC + CH         | 658.4 ± 20.6Ac | 16.5 ± 2.6Cb             | 1132.0 ± 103.5Ac | 16.8 ± 1.5Ac     | 36.5 ± 12.2Aa    |
|             | CSC + MC         | 454.6 ± 28.0Ab | 25.5 ± 6.6Cb             | 2321.0 ± 99.0Ab  | 20.3 ± 0.8Ab     | 18.5 ± 16.7Ab    |
| *P. pulmonarius* | CSC            | 59.4 ± 12.2CDB | 97.3 ± 0.1Aa             | 808.2 ± 23.7Rc   | 30.4 ± 2.6Ab     | 34.3 ± 5.5Ab     |
|             | CSC + CH         | 344.8 ± 37.0CDA | 56.3 ± 4.7Rc             | 1111.9 ± 203.5Ab | 16.7 ± 1.9Ab     | 36.0 ± 15.7Aa    |
|             | CSC + MC         | 70.5 ± 0.9Ab   | 88.4 ± 0.2Ba             | 1267.5 ± 96.0Ab  | 18.8 ± 2.8Ab     | 19.8 ± 9.3Ab     |
| *F. hepatica* | CSC              | 57.76 ± 3.4Da  | 97.3 ± 0.2Rs             | 621.7 ± 25.8Ca   | 32.4 ± 2.3Aa     | 17.0 ± 8.4Ab     |
|             | CSC + CH         | 90.56 ± 42.7Da | 88.5 ± 5.4Ab             | 676.7 ± 33.7Ca   | 16.5 ± 2.1Ac     | 34.0 ± 16.7Aa    |
|             | CSC + MC         | 6.5 ± 11.3C   | 98.9 ± 1.9Aa             | 844.7 ± 200.6Ca  | 21.9 ± 1.6Ab     | 44.7 ± 10.7Aa    |
| *P. lecomtei* | CSC              | 65.7 ± 17.0CDA | 95.5 ± 0.8Aab            | 819.7 ± 234.8Ca  | 31.7 ± 1.8Ab     | 44.8 ± 6.5Ab     |
|             | CSC + CH         | 102.1 ± 45.6Daa| 87.1 ± 5.8Ab             | 1291.0 ± 120.1Ab | 15.3 ± 1.2Rb     | 24.0 ± 9.8AbAb   |
|             | CSC + MC         | 3.5 ± 7.4Cfa  | 99.4 ± 1.0Aa             | 899.8 ± 208.9Ca  | 21.4 ± 3.5Ab     | 41.6 ± 23.1Aa    |
| *G. lucidum* | CSC              | 206.8 ± 66.1C  | 90.5 ± 1.7Aa             | 1090.6 ± 278.9Ba | 30.9 ± 4.1Aa     | 11.7 ± 14.7Ab    |
|             | CSC + CH         | 339.4 ± 37.0C  | 57.0 ± 4.7Bb             | 871.9 ± 25.5Rc   | 14.0 ± 1.0Bc     | 13.5 ± 8.0Ab     |
|             | CSC + MC         | 37.2 ± 7.3Bc  | 93.9 ± 3.6Aba            | 869.4 ± 20.5Ca   | 20.8 ± 2.3Ab     | 7.7 ± 3.0Aa     |

d.w.: dried weight; CP: crude protein; CSC: cottonseed cake; CH: coconut husks; MC: macauba cake. Means followed by the same letter are not different by Tukey test (p < 0.05). Capital letters represent differences between treatments in the same biomass and lowercase letters represent differences between biomasses in the same treatment.
with 31.8 μg of GAE/mg of extract. Phenolic compounds are usually related to antioxidant activity in plant materials, but in fungi-treated biomasses, this was not observed. The Pearson correlation analysis revealed no correlation between TPC and DPPH• assay or with the ABTS +• assay.

### Tables

#### Table 3

| Treatment          | Biomass | DPPH• (μg Trolox/ mg extract) | ABTS +• (μg Trolox/ mg extract) | TPC (μg GA/mg extract) |
|--------------------|---------|-------------------------------|---------------------------------|------------------------|
| Uncolonized        | JSC     | 109.4 ± 1.0^Aa                | 147.9 ± 9.3^Ac                  | 49.3 ± 4.5^Aa          |
|                    | JSC + CH| 77.3 ± 3.1^Ab                 | 202.9 ± 0.8^Aa                  | 36.7 ± 1.7^Ab          |
|                    | JSC + MC| 80.8 ± 2.5^Ab                 | 176.4 ± 5.5^Ab                  | 30.8 ± 3.2^Ab          |
| S. commune         | JSC     | 31.0 ± 13.7^Ba                | 40.4 ± 12.5^Ba                  | 20.5 ± 0.3^Ba          |
|                    | JSC + CH| 15.0 ± 4.7^Ca                 | 64.5 ± 7.0^BCa                  | 6.8 ± 1.2^Bb           |
|                    | JSC + MC| 17.8 ± 1.6^BCa                | 19.5 ± 9.5^Ca                   | 7.7 ± 1.5^BCh          |
| P. pulmonarius     | JSC     | 17.9 ± 4.1^BCa                | 18.5 ± 8.8^Bb                   | 10.5 ± 1.7^Ca          |
|                    | JSC + CH| 56.0 ± 5.5^Ba                 | 97.2 ± 23.6^Bb                  | 8.7 ± 2.5^Bb           |
|                    | JSC + MC| 15.9 ± 3.6^Ca                 | 27.0 ± 13.6^Cb                  | 0.0 ± 0.0^Bb           |
| F. hepatica        | JSC     | 8.0 ± 3.9^BCa                 | 29.3 ± 8.3^Bb                   | 4.0 ± 2.3^Ch           |
|                    | JSC + CH| 14.5 ± 2.6^Ca                 | 43.7 ± 8.1^BCab                 | 10.2 ± 1.7^Ba          |
|                    | JSC + MC| 27.8 ± 5.2^Ba                 | 55.1 ± 6.3^Ca                   | 9.2 ± 2.7^Bab          |
| P. lecomtei        | JSC     | 2.4 ± 0.2^Cb                  | 42.1 ± 3.7^Ba                   | 0.0 ± 0.0^Cb           |
|                    | JSC + CH| 12.3 ± 4.4^Cab                | 23.2 ± 3.2^Ca                   | 14.0 ± 0.7^Ba          |
|                    | JSC + MC| 23.6 ± 1.1^BCa                | 6.4 ± 0.9^Ca                    | 0.0 ± 0.0^Bb           |
| G. lucidum         | JSC     | 19.1 ± 6.2^BCa                | 62.4 ± 13.6^Ba                  | 17.3 ± 3.0^Bab         |
|                    | JSC + CH| 54.3 ± 1.7^Ba                 | 48.7 ± 8.7^BCa                  | 31.8 ± 6.8^Aa          |
|                    | JSC + MC| 26.5 ± 5.2^BCa                | 35.3 ± 9.5^Ca                   | 4.0 ± 0.3^CDb          |

TPC: Total phenolic content; GA: Gallic acid; JSC: Jatropha seed cake; CH: coconut husks; MC: macauba cake. Means followed by the same letter are not different by Tukey test (p < 0.05). Capital letters represent differences between treatments in the same biomass and lowercase letters represent differences between biomasses in the same treatment.

### Discussion

In the current population growth scenario, which demands an increase in food, agro-industrial residues management appears as an important concern for researchers. Focused on that, they have developed strategies to combine the use of these residues to improve animal feed, reduce meat production costs, and prevent the disposal of this waste in the environment. Among potential residues to be applied for these purposes, Jatropha curcas cake (JSC) and cotton seedcake (CSC), both byproducts from the oil extraction industry, show interesting options, considering their high protein content and the potential for improving animal feed. The results obtained with CSC + CH biomass colonized with G. lucidum, S. commune, and F. hepatica, 56.6, 56.2, and 52.6 μg of GAE/mg of extract, respectively (Table 4).

Uncolonized biomasses, CSC and CSC + CH presented higher GAE values, 21.3 and 24.3 μg of GAE/mg of extract, respectively, when compared to CSC + MC treatment (16.0 μg of GAE/mg of extract). Among colonized treatments, total phenolic compounds (TPC) were higher in CSC treated with G. lucidum (14.0 μg of GAE/mg of extract), in CSC + CH treated with F. hepatica (14.5 μg of GAE/mg of extract), and in CSC + MC treated with F. hepatica (17.0 μg of GAE/mg of extract). For S. commune, P. pulmonarius, P. lecomtei, and G. lucidum, TPC was not detected (Table 4).
content. However, these biomasses present some toxic compounds that can impair animal’s digestion. The application of biodegrading microorganisms capable of degrading these toxic metabolites represents a promising strategy for the elimination of these substances [1] enabling the use of these biomasses as nutritious animal feed, in addition to the potential for exploitation of edible mushrooms production. Here, we evaluated the capacity of 7 basidiomycetes species, also known as white-rot fungi, to detoxicate different protein-rich biomasses and those biomasses combined with coconut husks (CH) and *Acronomia aculeata* (macauba cake—MC). The data obtained here showed that *A. subrufescens* and *L. edodes* were able to grow in lignocellulosic wastes, as described by some authors [34, 35] who reported growing in composts such as sawdust and wheat straw. These two species, however, were not able to grow in pure JCS and CSC biomasses or combined with CH and MC. The cultivation of *A. subrufescens* has being described as complex, demanding N supplementation during all growth besides requiring strict environmental controls [36] which, may explain why no growth was achieved in the biomasses tested here. For *L. edodes*, as identified here, some authors have previously shown a remarkable growth reduction when cultivated in oak-wood sawdust, a lignocellulose-rich biomass, suggesting that nitrogen content could be a growth-limiting factor for shiitake [37, 38]. All other 05 fungi species grew in the assayed biomasses. Some authors have also reported the growth of white-rot fungi species, in pure biomasses of JSC and CSC and combined with other cellulosic sources for *Pleurotus* species (Oyster mushrooms), including *Pleurotus pulmonarius* [24, 39, 40], *Schizophyllum* [41], *Fistulina hepatica* [42], *Panus lecomtei* [3], and *Ganoderma lucidum* [3, 43].

Besides the visual measurements in centimeters, another indicator of fungi colonization is the presence of ergosterol [26, 44]. This molecule, vitamin D₃ precursor, is sterol present in the cellular membranes of fungi and is commonly used as an indicator of fungal growth [25, 45, 46]. Some authors have also reported higher ergosterol levels in basidiomycetes species [46–48], showing a positive correlation between fungi growth and ergosterol content [47, 48], which corroborates the use of this molecule as a fungal biomass growth indicator. It is important to highlight that each fungi species has its growth and ergosterol content relation [26]. So, ergosterol is a good marker of growth to compare different biomasses or treatment approaches for the same species but showed inconsistencies when comparing different fungi species. Higher content of ergosterol was identified in colonized JSC biomass mixed with lignocellulosic material (CH and MC), mainly in *S. commune*, and *P. pulmonarius*. As for CSC mixed with lignocellulosic biomasses, all fungi showed higher ergosterol content, except for *G. lucidum*. These data suggest that biomasses with lignocellulosic material induced the growth of the selected basidiomycetes, with coconut

| Table 4 | Antioxidant activity and total phenolic of pure cottonseed cake, and cottonseed cake with lignocellulosic material (coconut husks and macauba cake) treated with five basidiomycetes |
|---------|-------------------------------------------------------------------------------------------------
| Treatment | Biomass | DPPH• (µg Trolox/mg extract) | ABTS +• (µg Trolox/mg extract) | TPC (µg GA/mg extract) |
| Uncolonized | CSC | 53.5 ± 1.9 | Aa | 122.1 ± 7.6 | Ab | 21.3 ± 1.2 | Aa |
| | CSC + CH | 34.6 ± 3.7 | Ab | 125.9 ± 3.3 | Ab | 24.3 ± 1.0 | Aa |
| | CSC + MC | 42.3 ± 3.3 | Aa | 147.9 ± 7.0 | Aa | 16.0 ± 2.2 | Aa |
| *S. commune* | CSC | 14.1 ± 9.1 | B | 37.2 ± 22.6 | B | 12.0 ± 3.7 | B |
| | CSC + CH | 19.5 ± 2.9 | B | 56.2 ± 7.3 | C | 9.3 ± 3.3 | Aa |
| | CSC + MC | 16.0 ± 1.2 | B | 48.2 ± 7.8 | B | 0.0 ± 0.0 | B |
| *P. pulmonarius* | CSC | 12.3 ± 2.9 | B | 23.1 ± 7.0 | B | 5.8 ± 0.7 | B |
| | CSC + CH | 11.0 ± 2.4 | C | 34.9 ± 6.8 | B | 9.2 ± 0.9 | B |
| | CSC + MC | 18.7 ± 10.4 | B | 15.3 ± 0.9 | B | 0.0 ± 0.0 | B |
| *F. hepatica* | CSC | 4.8 ± 4.6 | B | 23.1 ± 7.0 | B | 12.8 ± 2.0 | Ab |
| | CSC + CH | 20.2 ± 0.6 | B | 52.6 ± 6.4 | A | 14.5 ± 3.7 | A |
| | CSC + MC | 18.9 ± 0.6 | B | 30.8 ± 2.4 | B | 17.0 ± 2.3 | A |
| *P. lecomtei* | CSC | 4.2 ± 3.7 | B | 15.3 ± 6.3 | B | 1.7 ± 0.2 | C |
| | CSC + CH | 15.4 ± 5.5 | B | 24.6 ± 3.5 | D | 2.3 ± 1.8 | B |
| | CSC + MC | 21.3 ± 9.9 | B | 13.8 ± 2.6 | C | 0.0 ± 0.0 | B |
| *G. lucidum* | CSC | 13.1 ± 6.5 | B | 34.5 ± 6.8 | B | 14.0 ± 5.3 | Ab |
| | CSC + CH | 19.5 ± 3.3 | B | 56.6 ± 18.2 | C | 7.2 ± 4.5 | B |
| | CSC + MC | 19.5 ± 10.8 | B | 34.1 ± 4.7 | B | 0.0 ± 0.0 | B |

TPC: Total phenolic content; GA: Gallic acid; CSC: Cottonseed cake; CH: Coconut husks; MC: Macauba cake. Means followed by the same letter are not different by Tukey test (p < 0.05). Capital letters represent differences between treatments in the same biomass and lowercase letters represent differences between biomasses in the same treatment.
husks being a good additive for JSC biomass and, macauba cake for cottonseed cake. It provides a good balance of carbon–nitrogen for fungi growth [5]. Ergosterol is also a precursor of vitamin D₃, and its presence could bring benefits to animal diet. Some authors tested supplementations of 50 μg mushroom vitamin D₃/kg in the feed diet of pigs and found increased pork antioxidant status and improved pig growth performance, carcass weight, and color [49].

To be safely used in animal feed, phorbol ester and FG present, respectively, in JSC and CSC biomasses need to be destroyed. The development of biological approaches using basidiomycetes to decrease the level of toxic compounds meets fundamentals of sustainable processes [4], when compared to other detoxication methods such as chemical, physical, and enzymatic treatments. Usually, in Jatropha seed cake, levels of phorbol ester range from 1 to 3 mg/g, but according to some authors [1], tolerable levels of phorbol esters must be below 0.09 mg/g to safely be used in an animal diet. Here, levels above 0.09 mg/g were found in S. commune and P. pulmonarius in JSC, F. hepatica in JSC + CH, and G. lucidum in JSC, and JSC + CH, which implies that these treatments didn’t reach safe levels. Except for the treatments cited above, all treatments met levels of phorbol esters under the target threshold, reaching a high rate of detoxification, as much as 98.2% in pure JSC colonized by P. lecomtei. Many authors have also reported high degradation levels of phorbol ester in biomasses colonized by basidiomycetes. It has been shown [39] a reduction of 99% in phorbol concentration, in JSC incubated for 60 days with Pleurotus ostreatus, with the same results being reported after 45-day incubation [50]. Other authors studying ten different white-rot fungi, including three samples from Pleurotus sp. and Ganoderma lucidum, showed the degradation of PE in all tested fungi, with the extent of degradation varying with the fungal culture [43], as observed in this work. A decrease in the concentration of phorbol esters in Ganoderma sp. was also reported [4]. Authors have pointed that the efficiency in the phorbol esters degradation in JSC through white-rot fungi colonization could be attributed to enzymatic complexes produced during the growth process, which have high esterase activities. It suggests that phorbol ester bonds are susceptible to hydrolytic degradation [4].

As for FG content, CSC + CH biomass mixture showed higher content when colonized with S. commune, while for CSC + MC and CSC + CH treated with F. hepatica and P. lecomtei, presented the lowest values, representing up to 99.4% degradation. The European Union Directive 2002/32/EC limits FG content in cottonseed cake feedstuffs to 20 ppm for laying hens and piglets, 100 ppm for poultry and calves, and 500 ppm for cattle, goats, and sheep. So, these treated biomasses could feed even poultry. All fungi-treated biomasses presented high-rate degradation (except for those with S. commune) achieving levels allowed for ruminant’s feed. Significant reduction in FG content was also reported from other authors [24] working with Pleurotus sp., with degradation percentages reaching 100% [51]. In addition, some authors analyzing 06 basidiomycetes including P. lecomtei, F. hepatica, Ganoderma sp., and Pleurotus sp. showed that all fungi were able to reduce FG content when colonizing CSC, with degradation percentages ranging from 74 to 94% [52]. These data corroborate the capacity of white-rot fungi to biodegrade this toxic molecule.

As a consequence of fungi fermentation, an increment in protein content occurs [43], which places it as a good strategy for improving the nutritional quality of vegetable biomass to animal feed. Although fungi are not capable to fix nitrogen, increases in crude protein content were observed here. This increase occurs due to the consumption of other nutrients (like lipids and carbohydrates) during metabolism, reduction in the total amount of biomass, and protein concentration. Thus, part of biomass nitrogen is incorporated into fungi protein, and carbohydrates are converted into fungal biomass and CO₂ [53]. Data assayed here showed higher protein increments after fungi colonization in most treatments, comparing to the respective untreated material. But some treatments, as in pure JSC with S. commune and P. pulmonarius, presented a decrease in protein. It can be an indicative of protein usage in these species’ metabolism when they are in excess. According to some authors [33], ligninolytic enzymes are produced during the secondary metabolism under conditions of limited nitrogen. Since the protein concentration of pure JCS was the highest of all cultivation mediums, the consumption of carbon-rich material was probably impaired. The high protein content available during fermentation could induce fungi to use it for their metabolism.

The variation in protein metabolism identified here among different fungal species was also reported by other authors [53]. They showed that after 07 weeks of colonization with P. eryngii and L. edodes, the total amount of CP increased by 23.3–30.9% in the fungal-treated wheat straw. Authors have also reported increases and decreases in CP in different fungi rather than white-rot species. Thus, treatment of JSC colonized with ascomycetes (Trichoderma longibrachiatum, Trichoderma harzianum, and Aspergillus niger) promoted an average decrease of 26% in crude protein content, as identified here for S. commune and P. pulmonarius. Increments, however, were also reported in treatments of JSC with Aspergillus niger, showing an increase of crude protein content of up to 11.3% [54, 55]. A previous study with JSC treated with fungi Absidia spina and Mucor rouxii presented increases up to 8% in crude protein content [56]. Some authors have also demonstrated that fungi-treated CSC biomasses with Candida capsuligena, Candida tropicalis, Saccharomyces cerevisiae, Aspergillus terricola, Aspergillus oryzae, and Aspergillus niger promoted an apparent crude
protein increase between 8.95 and 22.24% [57]. As white-rot fungi, basidiomycetes are very efficient organisms in degrading lignin present in lignocellulosic materials (for example, coconut husks and macauba cake). The addition of these biomasses in the cultivation of fungi benefits the balance between carbon and nitrogen that is ideal for fungal growth, besides having more protein diluted, which implies a preferred degradation, contributing to protein concentration in these biomasses [53, 58]. Finally, as reported in this work, some authors suggested that an increase in the protein content may be related to the amount of nitrogen fixed in fungi mycelia. It is also highly related to organic matter losses as well as with other factors such as temperature, pH, nature of fungi, aeration, type of substrate, and heat [59–62]. So, variations are expected among different fungi species. Free gossypol degradation and lignocellulosic biomasses promoted by basidiomycetes can also be related to an increase in protein content [57], as higher CP concentrations were, overall, identified in colonization and biomasses treatments showing greater phorbal and FG degradation. However, at the present work, no significant correlation was found between the increase of protein content and growth rate or degradation rate.

In the colonization process of wood, white-rot fungi secrete several free radicals, aiming to activate the peroxidases enzymes system for degradation. As a protection for cells, these fungi also secrete antioxidative enzymes in addition to low molecular phenolic derivatives and other organic acids in response to ROS (reactive oxygen species) produced and in response to environmental stressors [63]. In the present work, reductions in antioxidative activity and total phenolic content of colonized biomasses were identified when compared to uncolonized biomasses. Overall, extracts of treatments using CH as lignocellulosic biomass presented higher antioxidative activity than treatments of pure JCS, CSC, or with the addition of MC. As for the uncolonized biomasses, JCS presented higher TEAC concentrations, since Jatropha seed cake is known for its high antioxidative activity [64]. Differences in antioxidant activity results between ABTS and DPPH radical assays were observed. It occurs because some antioxidant agents react rapidly with peroxy radical in ABTS + • assay meanwhile, slowly, or even inactive to DPPH•. Furthermore, DPPH radical discoloration can happen to other reducing agents, contributing to discrepant results [65]. The same results were also observed by other researchers [66]. In soybean colonized by fungi, DPPH scavenging free radicals assay results varied from 0.6 to 4.4 mg TEAC/mg of extract, and one colonized sample presented TEAC values higher than uncolonized soybean (3.2 TEAC/mg of extract). Besides, other authors have reported antioxidative activities of other molecules, such as polysaccharides, isolated from *Ganoderma atrum*, *Agaricus bisporus*, *Lentinus edodes*, *Lentinus polychrous*, and *Phellinus linteus* [67, 68]. It may be the reason why there was no correlation observed between antioxidative activities and TPC at present work. We hypothesize that the assays applied here identified other substances with antioxidative activity besides phenolic compounds, like beta-glucans produced by fungi. Another work isolated from the culture broth of *Inonotus xeranticus*, and *Phellinus linteus* [69], polyphenols with antioxidative properties, corroborating that the fungal bioactive compounds have indeed, antioxidative properties, with potential application in animal food processing.

Frequently, the antioxidative activity is correlated with the phenolic content of biomasses, as the concentration of antioxidative compounds, as a response to oxidative stress and free radicals’ production, is expected to increase as fungi grow [70]. However, most of colonized biomasses in this work did not show this similarity between antioxidative activity and TPC content. Not all extracts with a higher amount of total phenolic compounds showed high antioxidative activity and vice versa. Therefore, the hypothesis that is suggested is that oxidative enzyme production by basidiomycetes related to lignin depolymerization could have affected the antioxidative capacity of the biomasses tested. It’s known that oxidases and peroxidases are the main enzymes produced being responsible for generating free radicals that can interfere in the measure of antioxidative activity [71]. Besides, the differences between antioxidative activity and total phenolic compounds results are justified by the presence of other compounds produced by basidiomycetes that can promote antioxidative activity, besides phenolic compounds. Basidiomycetes are known to produce compounds other than phenolic compounds, such as carotenoids, C and E vitamins, and β-glucans that also present antioxidative activities [72].

Choose the best treatment for detoxification and, enhancement of biomass is a multicriteria decision, and it needs to be addressed with appropriate tools. Analytic hierarchy process (AHP) was applied to establish the weight of each dependent variable. The Analytic Hierarchy Process (AHP) is a theory of measurement through pairwise comparisons and relies on the judgments of experts to derive priority scales. It is these scales that measure intangibles in relative terms. The comparisons are made using a scale of absolute judgments that represents, how much one element dominates another to a given attribute [73]. The criteria selected and weights found were degradation rate of toxic compounds (0.48), protein increase (0.19), final CP content (0.13), ergosterol content (0.09), ABTS radical assay (0.06), and DPPH radical assay (0.04). In conclusion, the most promising treatments for JSC were *P. pulmonarius* and *S. commune*; *P. lecomtei* in JCS + CH, and *F. hepatica* in JSC + MC. Treatments with pure JSC did not perform as well as those treatments. In CSC, *F. hepatica* and *P. lecomtei* in CSC + MC were the most promising treatments followed by *P. pulmonarius* in
pure CSC. In this context, these treatments can be further investigated, for their use in animal feed.

Conclusions

This study showed that most basidiomycetes fungi were able to degrade phorbol esters and free gossypol to acceptable levels for animal feed, in pure JSC and CSC, and mixed with lignocellulosic biomasses. Increases in protein content were also observed, mainly with the addition of other lignocellulosic material to culture media. Ergosterol, a precursor of vitamin D₃ was produced. This bioprocess was proved to be a safe and good choice for the enrichment of non-conventional biomasses for animal feed. *P. pulmonarius, F. roseus,* and *P. lecomtei* presented the best results for both CSC and JSC biomasses.

Antioxidant activity and TPC were reduced after fermentation, possibly due to the production of oxidative enzymes by the fungi. TPC results showed that other antioxidant compounds might also have been produced. Therefore, further studies to quantify these compounds, possibly carotenoids, vitamins E or C, β-glucans, should be conducted to identify the antioxidant source of basidiomycetes colonization.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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