

Degradation of Phorbol Esters on the *Jatropha curcas* Linn. Seed by Biological Detoxification

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Abstract. The application of fermentation is one of methods to increase food quality biologically. Availability of *Jatropha curcas* residual from oil factory be focused as a soybean meal or fish meal replacement. On the other hand, *J. curcas* residuals possess a toxic compound as well. This study aimed to examine the effect of *Aspergillus niger* on the nutrition and harmful content of *J. curcas* as a potential ingredient of feed. In brief, *J. curcas* residual was fermented with a detoxification method at 3 d, 5 d, and 7 d. Crude protein, fat, and crude fiber content were assessed to discover the biological responses of *J. curcas* post-fermentation while phorbol ester was evaluated to toxic content post-detoxification. The results showed that crude protein and fat content were highest on 7 d post-fermentation but it was no significant difference ($p > 0.05$). While crude fiber content showed significant difference which the 3 d fermentation had the highest content of fiber. For phorbol ester content, 3 d fermentation showed a better result than the control group ($p < 0.05$). The present findings suggest that *A. niger* is recommendable as starter to reduce fiber and toxic content of *J. curcas* residual at 3 d fermentation.

Keywords: Fermentation, nutrient, protein substitution, residual, waste to feed

1 Introduction

The feed is one of the factors that influence the growth of farmed fish. Intensive cultivation relying on commercial feed. Sources of protein in the diet comes from animal protein and vegetable protein [1, 2]. The primary source of vegetable protein feed is generally taken from soy flour, which has about 34.39 % protein content [3]. According to [4], the use of

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soy flour in commercial diets ranged between 10 % to 25 %. However, soybean prices are high now and still be imported [5, 6]. According to [7], that the cost of imported soybean in Indonesia about IDR 11 342 kg⁻¹. The increase in soybean prices has resulted in an increased cost of commercial feed [8]. It is not followed by the value of fish, affecting the sustainability of fish farming in Indonesia. One alternative to reduce the use of soy flour that is to take advantage of other feed ingredients such as *Jatropha curcas* seed.

The availability of *J. curcas* seed meals in many countries, especially Indonesia, is very abundant due to the increased production of JCO – *J. curcas* oil as an energy source instead of petroleum [9–13]. Amount of 1 t of dry beans will produce 200 L to 300 L of oil, the residue in the form of a *J. curcas* seed meal at 700 kg to 800 kg [14]. It can be utilized as feed ingredients because of the availability in abundant amounts, relatively cheap, easily digested by fish, have good nutritional content (protein), and do not compete with humans needs [15, 16].

According to [17], the crude protein content within skinned *J. curcas* seed meal in Indonesia is 19 % to 21 %, while the crude protein in the *Jatropha* seed meal without the husk is around 45 % to 50 %. Additionally, composition and percentage of amino acids, and minerals almost the same as other grains. The nutrient content makes *J. curcas* seed meals is considerable potential as a partial replacement of local feed ingredients soy flour. However, *J. curcas* seed meal is not commonly used because it has a toxic substance that is phorbol ester [18–20]. It causes lysis of fish erythrocytes that interfere with the formation of red blood cells when it is consumed by fish [21, 22]. However, the toxic substances can be minimized by detoxification. Several methods of detoxification has done, both physically (heating using an autoclave for 30 min, 121 °C), chemically (extraction using methanol), and biologically (fermentation using various molds) [22–25].

Further research is needed to biologically detoxification by fermentation using *A. niger* because it is grow fast saprophytic mold and harmless due to not produce mycotoxins. The results of the study on *J. curcas* detoxification is expected to be applied to an alternative feed ingredient safety in fish farming.

The purpose of this study is as follows: i) To determine the effect of the biological detoxification by fermentation using *A. niger* on the content of toxic substances and nutrients in the seed meal of *J. curcas* L. ii) To get the best fermentation time on seed meal of *J. curcas* L. which gives the best results in the biological detoxification using *A. niger*.

2 Material and Methods

2.1 Experimental design

The present study used an experimental approach with a Completely Randomized Designed (CRD). This research applied three treatments and three replications.

T1: 3 d fermentation of *J. curcas* seed meals by *A. niger*

T2: 5 d fermentation of *J. curcas* seed meals by *A. niger*

T3: 7 d fermentation of *J. curcas* seed meals by *A. niger*

2.2 *A. niger* preparation

The preparation of *A. niger* followed [26] with some modification. The fungus

A. niger was inoculated to Erlenmeyer contained 100 mL media of sterile bean sprouts. Inoculum of *A. niger* was incubated in the shaker incubation for 72 h at room temperature.

2.3 Fermentation process and nutrient analysis

Fermentation of *J. curcas* seed meals by *A. niger* followed [20, 27, 28]. The fermentation used plastic bags. *J. curcas* seed meals were analyzed for phorbol ester and nutrients content before used. A 250 g of *J. curcas* seed meals were steamed for 1 h to reduce moisture content to 40 %. Inoculum *A. niger* as much as 6 g was added for 1 kg *J. curcas* seed meals and stirred as that of the substrate after that of the substrate temperature down about 40 °C. Substrate was put into plastic bags and placed on a plastic basket. On the next day, plastic bags were hollowed on both sides. Plastic bags were reversed daily, and fermented for 3 d, 5 d, and 7 d. Results of fermentation were dried in the oven with 60 °C and powdered. The sample was analyzed for *Phorbol ester* and nutrient contents.

Measurement of the phorbol ester based on [29, 30]. Briefly, 50 g of fermented *J. curcas* seed meals extracted by methanol 100 mL, used magnetic stirrer on the temperature 60 °C for 60 min. Sample was then filtered using paper filter to separate solution with drain mater. A dark solution was stored in a bottle. Sample was measured using liquid chromatography mass spectroscopy (LC-MS) to separate or mixes compounds based on its polarity.

2.4 Data analysis

Analysis of Variance (ANOVA) was applied to determine the differences among treatments. If it had a real impact then done by test advanced, election follow-on test would be used depending on the value of the coefficients diversity from the data.

3 Results and discussions

J. curcas seeds used in this study were obtained from the collection of the Laboratory of the Vegetable Oils University of Muhammadiyah Malang (UMM). *J. curcas* seeds were prepared as much as 10 kg peeled off the shell to get the kernel. Amounted 10 kg of *J. curcas* seeds produced 5 kg kernel, then pressed to produce the oil, and the residual pressed to shaped plates and then dried with aerated for 1 d. *J. curcas* seed meal used in the study analyzed at UMM Nutrition Laboratory has protein and fat of 20.89 % and 54.56 % respectively. The results of *J. curcas* seeds meal proximate analyses could be seen in

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Fermentation is one of food processing technology biologically using microorganisms that increase the quality of nutrition of feed such as carbohydrates, fat, protein, fiber and other organic matter [31, 32]. *A. niger*, one of the fermentation microorganisms, because can break up tannic acid into glucose and gallic acid, and have the ability producing a different type of acid i.e. oxalic acid, 2-hydroxypropanoic-1,2,3-tricarboxylic acid, gluconic acid and some types of enzymes [28, 33].

The study revealed that *A. niger* reduced Phorbol ester concentration using fermentation proces. Results showed that three days fermentation come out with the optimum time fermentation to reduce Phorbol ester concentration.

Table 1. Nutrient and phorbol ester content of fermented *J. curcas* seeds meal

Treatments (Fermentation)	Protein (%)	Fat (%)	Crude fiber (%)	Phorbol ester ($\mu\text{g g}^{-1}$)
Raw material	20.89	54.56	3.84	2.97
A : 3 d	20.18	55.58	3.59 ^b	0.625 ^a
B : 5 d	20.03	56.18	2.58 ^{ab}	4.382 ^c
C : 7 d	20.71	57.00	2.92 ^a	2.102 ^b

Phorbol ester (phorbol-12-myristate 13-acetate) is a significant poison in *J. curcas* [34, 35]. Phorbol ester contained in the seeds and roots of *Jatropha*. Phorbol esters heat resistant and are present in the oil remaining on the cake, as much as $\pm 11\%$ [19]. The content of phorbol ester before detoxification of 2.979 $\mu\text{g g}^{-1}$, after going through rehab biologically fermented with *A. niger* for three days could reduce the content of phorbol ester on *J. curcas* seeds meal. Similar study by [36], *A. niger* could aflatoxin B¹ by 58.2 % after fermentation.

4 Conclusion

Based on the results of the study concluded, i) Fermentation with *A. niger* significantly effect phorbol ester and crude fiber, but not to crude protein and fat in *J. curcas* seeds meal. ii) 3 d fermentation give the best result referring to the decrease of the phorbol ester and crude fiber in *J. curcas* seeds meal.

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