Detoxification of *Jatropha Curcas* Meal as Poultry Feed

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Abstract

The increasing of Jatropha curcas cultivation as raw material of biodiesel in Indonesia leads to increase Jatropha curcas meal as byproduct. Besides being a source of oil, Jatropha also provides a meal which may serve as a highly nutritious protein suplement in animal feed if the toxins are removed. Curcin and phorbol ester are the main toxic components contained in the Jatropha curcas meal. This experiment was conducted to study the effects of various treatments (physical, chemical, biological) of Jatropha curcas meal on curcin, protein utilization efficiency, metabolizable energy values, retention of calcium and phosphorus. The treatments used in this experiment were: (1) heat treatment using autoclave at 121°C during 30 minutes; (2) adding NaOH 4%, followed by autoclaving at 121°C during 30 minutes; (3) fermentation using Rhizopus oligosporus. To determine the protein utilization efficiency, retention of calcium and phosphorus as well as metabolizable energy values, 23 broilers of 35 days of age were used in this experiment. The chickens were placed in individual cages and the excreta were collected during four days. The experimental diets used in this experiment were: BD (100% basal diet), T0 (80%BD + 20% untreated Jatropha curcas), Tphys (80%BD + 20% heat treated Jatropha curcas), Tchem (80%BD + 20% chemical treated Jatropha curcas), Tbiol (80%BD + 20% biological treated Jatropha curcas). The data of curcin was analysed descriptively and the others data were analysed using analyses of variance. The results showed that physical, chemical as well as biological treatments decreased the curcin of Jatropha curcas meal up to 66.7%, 77.8% and 22.2%, respevtively, compared to the untreated one. All of the treatments increased the protein utilization efficiency with the values of 12.1% (T0), 36.6% (Tphys), 44.5% (Tchem) and 48.2% (Tbiol). The retention of calcium were 22.2% (T0), 28.8 % (Tphys), 31.2% (Tchem) and 38.4% (Tbiol). The retention of phosporus were 26.0% (T0), 51.5% (Tphys), 43.2% (Tchem) and 50.5% (Tbiol). The metabolizable energy (MEn) values were 2505.2 kcal/kg (T0), 3055.9 kcal/kg (Tphys), 3017.5 kcal/kg (Tchem) and 3152.5 kcal/kg (Tbiol). The conclusion of this experiment is that biological treatment (fermentation using Rhizopus oligosporus) is the best method to detoxify the toxins and thus increasing the nutrititive value of the Jatropha curcas meal for poultry.

Keywords: *Jatropha curcas* meal, detoxification, nutritive value, broilers

Introduction

The increasing of *Jatropha curcas* cultivation as raw material of biodiesel in Indonesia leads to increase *Jatropha curcas* meal as byproduct. So far, cultivation area of *Jatropha curcas* in Indonesia is about 35,000 hectares, and the target of Indonesia is to plant the *Jatropha curcas* on about 1 million hectares of land. Besides being a source of oil, *Jatropha* also provides a meal which may serve as a highly nutritious protein suplement in animal feed. *Jatropha curcas* meal (fully defatted) has a crude protein content of between 53-63% and about 90% of this is present as true protein (Aderibigbe *et al.*, 1997). Makkar *et al.* (1998) reported that pepsin digestibility nitrogen in *Jatropha curcas* meal was very high (93-95%), suggesting high availability of protein to animals. However, both seed and oil have been found to be toxic to animals. El-Badawi *et al.* (1995) reported high mortality and severe pathological changes in Brown Hisex chicks fed diet containing 0.5% *Jatropha curcas* seed. Feeding *Jatropha curcas* meal at the level of 5% in the diet to the broilers reduced feed consumption, caused 100% mortality at the age of 22 days and it damaged the liver as well as kidney (Sumiati *et al.*, 2007). Ahmed and Adam (1979) studied the sequential development of the clinical signs an lesions in the organs of desert sheep and Anglo-Nubian goats dosed with *Jatropha curcas* seeds at 0.005, 0.5 and 1 g/kg/day. Diarrhoea, reduce water consumption, dehydration, sunken eyes, inappetence and ioss in condition were the important signs of poisoning in the sheep and goats.

The toxic or antinutrient compound contained in the *Jatropha curcas* include curcin-a lectin, phenol, tannin, phytate, trypsin inhibitors, saponin and phorbol esters (Francis *et al.*, 2006). Curcin and phorbol ester are the main toxic components contained in the *Jatropha curcas* meal. The meal of

Jatropha curcas has a high activity of trypsin inhibitor and lectin but these can be reduced by heat treatment (Aderibigbe *et al.*, 1997). Phorbol esters is not possible to be destroyed by heat treatment because they are heat stable and can withstand roasting temperature as high as 160°C for 30 min, however, it is possible to reduce its concentration in the meal by chemical treatments (Makkar and Becker, 1997).

This research therefore was conducted to investigate the effects of various treatments (physical, chemical, biological) of *Jatropha curcas* meal on curcin concentration, protein utilization efficiency, metabolizable energy values, retention of calcium and phosphorus in the broilers.

Material And Methods

Jatropha curcas Meal Sample

Jatropha curcas meal sample was obtained from a biodiesel industry at Kudus, Central of Java. The meal was made from the seed (with shell) of the *Jatropha* and pressed using expeller machine. Chemical composition of the sample was analysed at Faculty of Animal Science, Bogor Agricultural University (Table 1).

Table 1. Chemical composition of *Jatropha curcas* used in this experiment*

Component	%
Dry matter	88.82
Crude protein	18,40
Crude fiber	32.81
Ether extract	20.62
NFE	4.36
Ash	12.63
Ca	0.56
P	0.67

* Analysed at laboratorium of Feed Science and Technology, Faculty of Animal Science, Bogor Agricultural University (2006)

Detoxification Procedures

Physical (heat) Treatment

This treatment was conducted according to the modified method of Aregheore *et al.* (2003). Appoximately 1500 g of the *Jatropha* meal placed in an autoclave at 121°C for 30 min. The autoclaced sample was removed and washed with distilled water four times, and then the sample was dried in an oven at 60°C for 48 hours prior to milling.

Chemical Treatment

This treatment was carried out according to the modified method of Aregheore *et al.* (2003). Approximately 1500 g of the *Jatropha* meal placed in the tray and mixed with 4% sodium hydroxide (NaOH) to form a paste. This was followed by heating in an autoclave at 121°C for 30 min. The autoclave sample was removed and washed with distilled water four times, and then the sample was dried in an oven at 60°C for 48 hours prior to milling.

Biological (fermentation) Treatment

This treatment was carried out according to the modified method of Aisjah *et al.* (1998). Approximately 1500 g of the *Jatropha* meal was steamed for 30 min, and then the sample was removed and allowed to cool at room temperature. This was followed by adding 10% steril water (w/w), and 0.6% (w/w) *Rhizopus oligosporus*. The sample was placed in the plastic tray with 2-3 cm of thickness and covered with wrapping plastic, and then it was incubated for 3-4 days at room temperature. The wrapping plastic was holed at the first 24 hours of incubation. The sample was dried in an oven at 60°C for 24 hours prior to grinding.

Curcin Assay

Curcin concentration in the untreated and treated samples was analysed using HPLC. Approximately 1 g of sample was weighed, and it was followed with saphonification process for 24 hours at 21°C. After that, 10 ml of ascorbic acid was added to the sample and followed with heating at 100°C for 30 min. The sample was allowed to cool at room temperature, and then 100 ml isopropilene was

added and the sample was stirred and evaporated to form a pellet. The sample was diluted using hexan and injected to the HPLC with the condition as followed: coulom at 180°C-185°C, detector at 220°C, injector at 200°C.

In Vivo Experiment

This experiment was conducted to determine protein utilization efficiency, metabolizable energy values, retention of calcium and phosphorus of untreated as well as treated *Jatropha curcas* meal.

Treatment Diets

Untreated as well as treated meal was mixed with basal diet (Table 2). The treatment diets were: BD (100% basal diet), T0 (80%BD + 20% untreated *Jatropha curcas)*, Tphys (80%BD + 20% heat treated *Jatropha curcas*), Tchem (80%BD + 20% chemical treated *Jatropha curcas*), Tbiol (80%BD + 20% biological treated *Jatropha curcas*).

Table 2. Composition and nutrients content of basal diet

Ingredients	Composition (%)
Yellow corn	66,0
Soybean meal	28,5
$CaCO_3$	2,5
Palm oil	0,7
DCP	1,2
Premix	1,0
DL- Methionine	0,1
Total	100
Calculated nutrients content *	
MEn (kcal/kg)	2907
Crude Protein (%)	18,15
Crude Fiber (%)	3,45
Ca (%)	1,05
P- avail. (%)	0,35

* Based on nutrients content of NRC (1994)

Table 3. Nutrients content of treatment diets

Ransum Perlakuan	BD[1)]	T0	Tphys	Tchem	Tbio
Dry matter (%)	88,11	88,25	89,52	88,80	89,54
Ash (%)	5,9	7,25	5,27	5,61	5,74
Crude Protein (%)	17,97	18,06	16,15	16,77	18,33
Crude fiber (%)	3,49	9,35	12,74	14,76	9,15
Ether extract (%)	2,51	6,13	4,77	3,29	5,40
NFE (%)	58,24	47,46	50,59	48,38	50,91
Ca (%)	1,95	1,69	1,65	1,64	1,66
P total (%)	0,87	0,85	0,75	0,67	0,85
Gross Energy (kcal/kg)	4204,0	4337,8	4452,8	4294,2	4343,8

BD (100% basal diet), T0 (80%BD + 20% untreated *Jatropha curcas)*, Tphys (80%BD + 20% heat treated *Jatropha curcas*), Tchem (80%BD + 20% chemical treated *Jatropha curcas*), Tbiol (80%BD + 20% biological treated *Jatropha curcas*)v

Animals, Experimental Design and Housing

This study was carried out according to modified method of Sibbald (1980). Twenty three (23) broilers (5 weeks of age) were used in this study. Each of broiler was placed in metabolic cage. All of the broilers were fasted for 24 hours, and then 20 broilers were fed the treatment diets during 4 days. Another 3 broilers were further fasted for 24 hours to obtain endogenous exreted nutrient. During fasting period, water was offered *ad libitum*. Excrement (faeces + urine) of broilers fed test diets were collected during 4 days and the excrement (faeces+urine) of broilers of endogenous one were collected during 24 hours. The excrement was weighed, and then it was put in the refrigerator for 24 hours. The excrement was removed and melted at room temperature, followed with drying in an oven at 60°C for 24 hours. The excrement was ground prior to analyse. Dry matter, crude protein, gross energy, Ca and P of the excrement were analysed.

Completely randomized design was used in this experiment (5 treatments, 4 replications). Data obtained were analysed using analyses of variance (ANOVA) and if there were any significant difference, the data were further tested using least significant different test (Steel and Torrie, 1995).

Results And Discussion

Chemical Composition of *Jatropha curcas* meal

The chemical composition of *Jatropha curcas* meal used in this study is presented in Table 1. Crude protein contained in the meal was 18.40%, it was lower than that of the meal of the *Jatropha* from Cape Verde (56.4% crude protein) or Nicaragua (61.2% crude protein) that reported in Makkar *et al.* (1998). This was happened due to difference in oil processing, *Jatropha curcas* meal from Indonesia was made from the seed with shell, while the meal reported in Makkar *et al.* (1998) was made from dehulled seed. Crude fiber contained in the meal used in this experiment was very high (32.81%), and it is not so good as poultry feed. Crude fat of the meal was still high.

Effect of Treatments on Curein Concentration of the *Jatropha curcas* Meal

Table 4. presents the concentration of curcin obtained from various treatments:dry heat, adding sodium hydroxide, and fermentation treatments. The curcin concentration of the untreated meal was 0.09%. All of the treatments decreased curcin concentration with the values of 0.03% (dry heat treatment), 0.02% (sodium hydroxide treatment), and 0.07% (fermentation treatment). This results indicated that curcin is heat labil and destroyed by the chemical treatment. The same result was reported by Aregheore *et al.* (2003) that concentration of lectin(curcin) in heat-treated meal was significantly lower ($P<0.05$) than in the untreated meal. Chemical treatment consequently, removed all traces of lectin in the various meal (Aregheore *et al.*, 2003).

Table 4. Curcin concentration of untreated and treated *Jatropha curcas* meal*

Treatments	Curcin (%)
Untreated	0.09
Physical(dry heat at 121oC for 30 min)	0.03
Chemical (adding 4% NaOH. Followed by heating at 121oC for 30 min)	0.02
Biological (fermentation using *Rhizopus oligosporus*)	0.07

* Analysed at Laboratorium of Post Harvest, Institute of Agricultural Research and Development, Bogor (2006)

The decreasing curcin of the biological (fermentation) treated meal using *Rhizopus oligosporus* was not as high as the other treatments, because the steam temperature used in this experiment was lower than that in autoclave. This result showed that curcin was destroyed by heat (high temperature).

Effect of Treatments on Protein Utilization Efficiency

The data of intake, exretion and protein utilization efficiency of diets consisted various treated meal were presented in Table 5. The protein intake of diet contained untreated meal was the lowest. All of treatments increased protein intake compared to the untreated meal. The chemical and fermentation treatments significantly ($P<0.01$) increased protein intake compared to the untreated meal and the protein intake of fermentation treated meal was the highest and statistically the same as basal diet. The protein utilization efficiency was improved by the treatments and the fermentation treatment gave the highest value. This results indicated that curcin is not the main toxin to interfere the protein utilization, but maybe the other toxin, phorbol esters, and fermentation treatment could destroy this toxin.

Table 5. Intake, Excretion and Protein utilization efficiency of basal diet and diets contained *Jatropha curcas* meal

Parameters	Diets				
	BD	T0	Tphys	Tchem	Tbiol
Protein Intake (g/bird)	37.59^{A*}	12.06^{C}	17.19^{BC}	29.78^{B}	43.19^{A}
ProteinExcretion (g/bird)	$9.89^{AB}1$	8.74^{B}	10.50^{AB}	17.41^{AB}	22.56^{A}
Protein utilization efficiency (%)	73.76	12.05	36.58	44.53	48.16

* Values in the same row with differents superscript differ significantly ($P<0,01$)

Effect of Treatments on Retention of Calcium and Phosphorus

Table 6 presents the intake, exretion and retention of Calcium and Phosphorus of diets contained various treated meal. The treatments increased intake of Ca and P compared to the untreated meal. The fermentation with *Rhizopus oligosporus* had the highest intake of Ca and P. The retention of Ca and P increased due to the treatments, and the fermentation treatment gave the highest values. These results indicated that phytate contained in the *Jatropha meal* was broken down by the phytase enzyme yielded by *Rhizopus oligosporus*. *Jatropha curcas* meal contained high phytate (7-10%) (Makkar *et al.*, 1997). In mature seed, phosphorus is present primarily in the form of phytic acid as a complex salt of calcium, magnesium and potassium, and/or with protein. Under normal dietary conditions, phytate phosphorus is either available to, or poorly utilized by, poultry because they lack the phytase enzyme to hydrolyze the phytate and release the phosphorus (Ravindran, 1999). Piliang (2002) reported that *Rhizopus oligosporus* could synthesize phytase enzyme.

Table 6. Retention of Ca and P of basal diet and diets contained *Jatropha curcas* meal

Parameters	Diets				
	BD	T0	Tphys	Tchem	Tbiol
Calcium:					
Intake (g/bird)	3,83 ^{C*}	0,97 ^{A}	1,66 ^{AB}	2,90 ^{BC}	3,94 ^{D}
Excretion g/bird)	2,42 ^{C}	0,06 ^{A}	1,28 ^{AB}	1,95 ^{BC}	2,56 ^{D}
Retention (g/bird)	1,49	0,36	0,46	1,04	1,46
Phosphorus:					
Intake (g/bird)	1,71 ^{CD}	0,48 ^{A}	0,76 ^{AB}	1,36 ^{BC}	2,01 ^{D}
Excretion g/bird)	0,77 ^{AB}	0,47 ^{A}	0,60 ^{A}	0,92 ^{AB}	1,17 ^{B}
Retention (g/bird)	1,03	0,22	0,37	0,64	1,06

* Values in the same row with differents superscript differ significantly (P<0,01)

Effect of Treatments on Metabolizable Energy Value

Table 7 shows metabolizable energy values of diets contained various treated meal. The treatments increased nitrogen-corrected metabolizable energy (MEn) of diets contained *Jatropha meal*. Compared to the untreated meal, the treatments increased MEn with the increasing values of 22% (dry heat treatment), 20.4% (NaOH treatment), and 25.8% (fermentation treatment). The fermentation treated meal had the highest value of MEn. It could be due to the activity of protease, phytase, and lypase enzymes synthesized by the *Rhizopus oligosporus*. It was reported that *Rhizopus oligosporus* synthesize proteases enzymes (Ansori, 1989), phytase enzyme (Piliang, 2002), and lypase enzyme (Stenkraus, 1989).

Table 7. Metabolizable energy of basal diet and diets contained *Jatropha curcas* meal

Diets	MEn (kcal/kg)
BD	2891,77
T0	2505,20
Tphys	3055,92
Tchem	3017,45
Tbiol	3152,45

Conclusion

Biological (fermentation) treatment using *Rhizopus oligosporus* of the *Jatropha curcas* meal resulted in the highest values of protein utilization efficiency, retention of Ca and P, and metabolizable energy in the broilers. The curcin was not the main toxic to interfere nutrients availability of *Jatropha curcas* meal.

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