Feeding Fermented *Jatropha curcas L.* Meal Supplemented with Cellulase and Phytase to Kampong Chicken

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ABSTRACT

Fermented *Jatropha curcas* meal using *Rhizopus oryzae* could decrease the fat content in the meal (5.8% Vs 0.39) and eliminated trypsin inhibitor up to 67.95 %. The decreasing of fat content indicated the elimination of the main toxic substance contained in the meal, i.e. *phorbolesters.* Most of the *phorbolesters* could be extracted with the oil fraction of the *Jatropha curcas* meal. Hopefully, this treatment could destroy the toxic jatropha curcas meal to a high quality meal as poultry feed. However, the fiber and phytate content in the meal were still high. This experiment was conducted to study the effects of using fermented *Jatropha curcas* meal treated with cellulase and phytase in the kampong chicken diet as to increase the growth and decrease the mortality rate. Two hundred kampong chickens were used in this experiment and reared from day old up to 10 weeks of age. The data analyzed with a Completely Randomized Design with 5 treatment diets and 4 replications, with 10 birds in each replicate. The experimental diets were: T0 (the control diet, without *Jatropha curcas* meal), T1 (the diet contained 5% untreated *Jatropha curcas* meal), T2 (the diet contained 5% fermented *Jatropha curcas* meal + cellulase 200 ml/ton of feed), T3 (the diet contained 5% fermented *Jatropha curcas* meal + 1000 FTU phytase), and T4 (the diet contained 5% fermented *Jatropha curcas* meal + cellulase 200 ml/ton + 1000 FTU phytase). The parameters observed were feed consumption, body weight gain, final body weight, feed conversion ratio, and mortality rate. The results showed that there were no significant differences on the parameters observed due to the treatments. However, feeding untreated *Jatropha curcas* meal in the diets (T1) decreased the body weight gain approximately 10.52% and the final body weight approximately 10.13% as compared to that of the control (T0). Feeding fermented Jatropha curcas meal supplemented with cellulase + phytase(T4) yielded the final body weight and feed conversion ratio similar to those the control (T0) diet. The final body weight of the chickens fed T0, T1, T2, T3 and T4 were 955.08 g/bird, 858.33 g/bird, 872 g/bird, 935 g/bird, and 951.25 g/bird, respectively. The feed conversion ratio of the chickens fed T0, T1, T2, T3 and T4 were 2.93, 3.51, 3.49, 3.20, and 2.89, respectively. The the feed consumption per bird during 10 weeks period of experiment 2567.53 g, 2663.76 g, 2752.32 g, 2685.05g, and 2520.5 g, for chickens fed T0, T1, T2, T3 and T4 respectively. There was no mortality observed in all treatments.

Key words: fermented Jatropha curcas meal, growth, mortality, kampong chicken

INTRODUCTION

Jatropha curcas (physic nut or purging nut) is a drought-resistant shrub or tree belonging to the Family *Euphorbiaceae*, which is cultivated in Central and South America, South-East Asia, India and Africa (Schmook and Seralta-Peraza, 1997). The seeds of physic nut are a good source of oil, which can be used as a diesel substitute (Becker and Makkar, 1998). 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 curcas* also provides a meal which may serve as a highly nutritious protein suplement in animal feed if the toxins and antinutrients present in the meal are removed. The meal has high trypsin inhibitor and lectin activities, which could be inactivated by heat treatment. In addition, high concentration of antimetabolic, metal-chelating and heat-stable factor, phytic acid, has been reported in *Jatropha curcas* meal (Makkar *et al*., 1998). Apart from these, phorbolesters that are present at high levels in the kernels have been identified as the main toxic agent responsible for toxicity (Makkar *et al*., 1997). Untreated *Jatropha curcas* meal was toxic to rats, mice and ruminants (Becker and Makkar, 1998) as well as to poultry (Sumiati *et al*., 2007). 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)

Martinez-Herrera *et al*. (2006) used different treatments to decrease or neutralize the antinutrients present in the meal. Trypsin inhibitors were easily inactivated with moist heating at 121^{o}C for 25 min. Extraction with ethanol, followed by treatment with 0.07%$NaHCO_3$ considerably decreased lectin activity. The same treatment also decreased the phorbolester content by 97.9% in seeds. Sumiati *et al*. (2007) conducted various treatments (physical, combination of chemical + physical, and biological) to detoxify Indonesian *Jatropha curcas* meal as poultry feed. The treatments used in this experiment were: (1) heat treatment using autoclave at 121^{o}C during 30 min.; (2) adding NaOH 4%, followed by autoclaving at 121^{o}C during 30 min.; (3) fermentation using *Rhizopus oligosporus*. The results of this experiment showed that all treatments decreased the curcin or lectin activities, Increased protein utilization efficiency, retention of calcium and phosphorus, and increased metabolizable energy values of meal. Fermentation using *Rhizopus oligosporus* was the best method to detoxify the toxins and thus increasing the nutrititive value of the *Jatropha curcas* meal for poultry.

Sumiati *et al*. (2008) fermented Indonesian *Jatropha curcas* meal using *Rhizopus oryzae* and it could decrease the fat content in the meal (5.8% Vs 0.39) and eliminated trypsin inhibitors up to 67.95 %. The decreasing of fat content indicated the eliminating of the main toxic substance content in the meal, i.e. *phorbolesters.* Most of the *phorbolesters* could be extracted with the oil fraction of the *Jatropha curcas* meal. Hopefully, this treatment could destroy the toxic jatropha curcas meal to a high quality meal as poultry feed. However, the fiber and phytic acid content in the meal were still high. Poultry can not digest fiber, especially cellulose, even the fiber could interfere other nutrients contained in the feed. Sing (2008) reported that phytic acid is an anti-nutritional constituen of plant derived feeds. As a reactive anion, it forms a wide variety of insoluble salts with mineral including phosphorus, calcium, zinc, magnesium and copper. Pyitic acid is also known to form complexes with protein and proteolytic enzymes(pepsin and trypsin). Because of the lack of endogenous phytase enzymes that hydrolyze phytic acid: phosphorus, calcium, protein and other phytic acid bound nutrients are less available to poultry. This experiment was conducted to study the effects of using fermented *Jatropha curcas* meal using *Rhizopus oryzae* supplemen-ted with cellulase and phytase in the kampong chicken diets on the growth and mortality rate.

MATERIALS AND METHODS

Jatropha curcas Meal Sample

Jatropha curcas meal sample was obtained from Surfactant and Bioenergy Research Center, Bogor Agricultural University. Chemical composition of the sample was analyzed at the Faculty of Animal Science, Bogor Agricultural University (Table 1).

Table 1. Chemical composition of untreated and fermented *Jatropha curcas* meal*

Component	Untreated *J. curcas*	Fermented *J. curcas*
Dry matter, %	84.99	94,01
Ash, %	5.63	5,95
CP, %	24.71	22,39
EE, %	5.8	0,39
CF, %	32.58	44,22
NFE, %	16.27	21,06
Ca, %	1.00	0,68
P, %	0.99	0,35
GE, kcal/kg	3893	3984
Pytic acid, %**	10.18	7,45

* The nutrients were analyzed at the Laboratory of Feed Science and Technology, Faculty of Animal Science, Bogor Agricultural University;

** Phytic acid was analyzed at the Animal Research Institute, Bogor, Indonesia.

Fermentation Procedures

In this experiment, the culture that usually used to ferment soybean in Indonesia to make a food called tempe, was used as source of *Rhizopus oryzae*. This culture was used to ferment *Jatropha curcas* meal. The procedure of *Jatropha curcas* meal fermentation can be seen on Figure 1.

Feeding Trial Using Kampong Chickens

Two hundred kampong chickens were used in this experiment and reared from day old up to 10 weeks of age. A completely Randomized design with 5 treatment diets and 4 replications, with 10 birds in each replicate was used in this experiment.

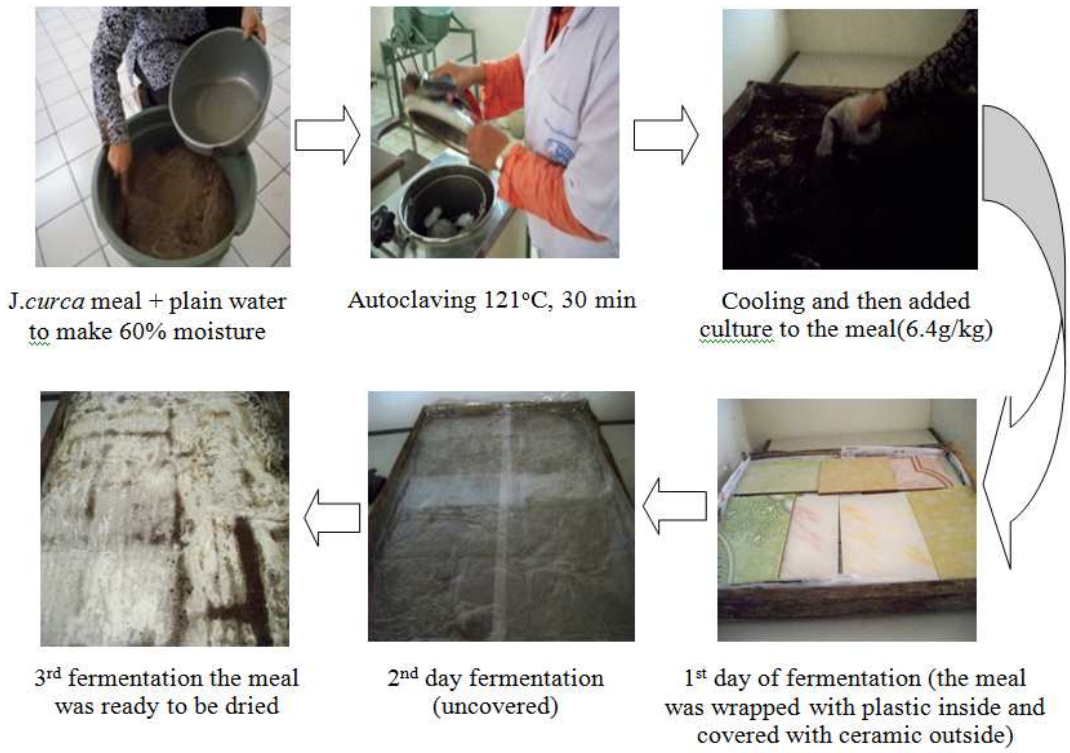

Figure 1. The Procedure of *Jatropha curcas* Meal Fermentation

Table 2. The composition of the experimental diets

Ingredient	Treatment				
	T0	T1	T2	T3	T4
			…..%.....		
Yellow corn	51.23	53.21	53.21	53.21	53.21
Rice bran	20.50	15.00	14.50	14.50	14.50
Soybean meal	17.00	16.50	16.50	16.50	16.50
Untreated *J.curcas* meal	0	5.00	0	0	0
Fermented *J.curcas* meal	0	0	5.00	5.00	5.00
MBM	7.50	7.00	7.00	7.00	7.00
Palm oil	3.00	2.50	3.00	3.00	3.00
Salt	0.10	0.10	0.10	0.10	0.10
Vit-min mix	0.50	0.50	0.50	0.50	0.50
Dl-methionine	0.173	0.187	0.187	0.187	0.187
Cellulase, ml/ton			200	0	200
Phytase, FTU/kg[1)]			0	100	100
Calculated composition[2)]					
ME, kcal/kg	2855.64	2862.71	2865.11	2865.11	2865.11
CP, %	18.23	18.39	18.26	18.26	18,20
nPP, %	0.61	0.56	0.56	0.56	0.56
Na, %	0.14	0.13	0.13	0.13	0.13
Lysine, %	0.83	0.83	0.82	0.82	0.82
Methionine, %	0.36	0.37	0.37	0.37	0.37
Meth + cystine,%	0.62	0.62	0.62	0.62	0.62

[1)] DSM Nutrition Product;
[2)] Nutrient compositions based on Leeson and Summers calculation (2005).

Table 3. The average feed consumption of kampong chicken during 10 weeks of experiment (0-10 weeks of age) (g/bird)

Replication	Treatment				
	T0	T1	T2	T3	T4
1	2760.31	2430.12	2769.88	2396.72	2522.78
2	2693.89	2791.88	2769.88	2801.55	2513.04
3	2411.07	2.695.62	2.743.38	2792.74	2264.2
4	2404.83	2737.42	2726.13	2749.19	2781.99
Average	2567.53	2663.76	2752.32	2685.05	2520.50
SD	186.26	160.67	21.47	193.58	211.45

Table 4. The average body weight gain of kampong chicken during 10 weeks of experiment (0 - 10 weeks of age) (g/bird)

Replication	Treatment				
	T0	T1	T2	T3	T4
1	877.80	920.33	802.97	651.93	942.47
2	984.10	613.47	812.77	1001.87	918.53
3	973.43	855.20	944.50	1016.10	930.60
4	842.10	906.53	791.70	934.83	875.40
Average	919.36	823.88	837.99	901.18	916.75
SD	70.26	143.05	71.53	169.90	29,25

The experimental diets were: T0 (the control diet, without *Jatropha curcas* meal), T1 (the diet contained 5% untreated *Jatropha curcas* meal), T2 (the diet contained 5% fermented *Jatropha curcas* meal + cellulase 200 ml/ton of feed), T3 (the diet contained 5% fermented *Jatropha curcas* meal + 1000 FTU phytase), and T4 (the diet contained 5% fermented *Jatropha curcas* meal + cellulase 200 ml/ton + 1000 FTU phytase). The composition of experimental diets is presented on Table 2. The experimental diets were fed to 2 weeks old up to 10 weeks old in order to minimize the mortality. During the two weeks of the experiment (0- 2 weeks of age), the chicks were fed commercial diets.

The parameters observed were feed consumption, body weight gain, final body weight, feed conversion ratio, and mortality rate. The data were analyzed using analyses of variance according to Steel and Torrie (1995).

RESULTS AND DISCUSSION

The Effect of Treatments on Feed Consumption

The average of feed consumption of kampong chickens in this experiment is presented on Table 3. Feeding diets contained *J.curcas* (T0, T1, T2, T3, T4) did not affect the feed consumption. It showed that feeding 5% untreated as well as fermented *J.curcas* did not influence the feed consumption, and thus it indicated that the meal used in this experiment was from *J.curcas* seed contained low phorbolesters. Generally, the presence of phorbolesters in feed has significant effect on its acceptance (Aregheore *et al*., 2003). Sumiati *et al*. (2007) reported that feeding 5% untreated *J.curcas* meal highly significantly ($P<0.01$) reduced feed consumption of broilers. Makkar *et al*. (1998) reported that there were different varieties of J.*curcas*, non-toxic and toxic varieties. The toxic varieties contained phorbolesters up to 2.7 mg/g kernel and non-toxic ones just contained up to 0.11 mg/g kernel.

The Effect of Treatments on Body Weight Gain

Statistically, there were no significant differences among treatments in the body weight gain of kampong chickens. However, feeding 5% untreated J.*curcas* meal (T1) impaired the growth with 10.39% as compared to that the control diet (T0). Feeding fermented *J.curcas* meal supplemented with cellulose (T2) tended to increase the growth with 1.7% as compared to that of untreated *J.curcas* meal diet (T1). Supplementation of fermented *J.curcas* with phytase (T3) increased 9.38% the body weight gain, and the supplementation of cellulase and phytase to the fermented *J.curcas* diet elevated 11.27% of the body weight gain (T4). The body

weight gain of the chickens fed T4 was similar to that of the control diet (T0/without *J.curcas* meal in the diet).

These results indicated that the supplementation of cellulase in the diet containing *J.curcas* meal had a little effect on the growth of kampong chicken. It could be due to high fiber and lignin content in the meal, and thus the cellulase with concentration of 200 ml/ton feed was not effective to break down the fiber. Sumiati *et al*. (2008) reported that fermented *J.curcas* meal used in this experiment contained 44.22 % fiber and 25.8% lignin.

Phytase supplementation in the fermented *J.curcas* meal diet seemed to be effective in degrading the phytate contained in the meal. Phytase is an enzyme which hydrolyses phytic acid to inositol and inorganic phosphorus, leading to improve phosphorus utilization and overall performance of broilers (Singh *et al*., 2003b). Supplementation of cellulase and phytase in the fermented J.*curcas* meal diet (T4) gave more body weight gain as compared to that of a single enzyme supplementation (T2 and T3).

The Effect of Treatments on Feed Conversion Ratio

The average of feed conversion ratio of kampong chickens in this experiment is presented in Table 5. Feeding 5% untreated *J.curcas* meal (T1) reduced the feed efficiency with the value of 19.8% as compare to that of the control diet (T0/without *J.curcas* meal in the diet). Supplementation of cellulase did not seem to be effective in increasing feed efficiency. However, phytase supplementation in the diet (T3) increased feed efficiency 8.83%, while the supplementation of cellulase and phytase in the diet (T4) yielded the highest feed efficiency, i.e. 17.66%. These results showed that the supplementation of cellulose and phytase enzymes gave higher effect on feed efficiency as compared to that of a single enzyme supplementation.

The Effect of Treatments on Final Body Weight

Feeding 5% untreated J.*curcas* meal (T1) decrease the final body weight 10.14% as compared to that of the control diet (T0/without J.*curcas* in the diet). The supplementation of the enzymes to the diets contained fermented J.*curcas* meal tended to raise final body weight of kampong chicken. Supplementation cellulose and phytase in the diet (T4) yielded the final body weight similar to that of the control diet (T0). This data indicated that phytase was effective to degrade phytic acid content in the meal. There were several studies which indicated that microbial phytase supplementation increases body weight gain, feed intake and feed efficiency in broiler chikhens (Singh and Khatta, 2002; Singh *et al*., 2003a). A significant improvement in the growth performance of broiler chickens, as a result of phytase supplementation, were reported by karim (2006), Pillai *et al*. (2006), Singh and Sikka (2006) and Selle *et al*. (2007).

Table 5. The average feed conversion ratio of kampong chicken during 10 weeks of experiment (0-10 weeks of age) (g/bird)

Replication	Treatment				
	T0	T1	T2	T3	T4
1	3.29	2.76	3.64	3.92	2.81
2	2.86	4.88	3.68	2.93	2.88
3	2.59	3.30	3.01	2.87	2.54
4	2.99	3.12	3.63	3.09	3.33
Average	2.93[a]	3.51[a]	3.49[a]	3.2[a]	2.89[a]
SD	0.29	0.93	0.32	0.48	0.32

Table 6. The average final body weight of kampong chicken at 10 weeks of age (g/bird)

Replication	Treatment				
	T0	T1	T2	T3	T4
1	914.00	953.33	953.33	688.33	976.67
2	1018.00	646.67	846.67	1036.67	953.33
3	1008.33	890.00	980.00	1050.00	965.00
4	880.00	943.33	826.00	968.33	910.00
Average	955.08[a]	858.33[a]	872.34[a]	935.83[a]	951.25[a]
SD	68.6	143.82	72.27	168.84	29.10

The Effect of Treatments on Mortality Rate

There was no mortality due to the treatments found in this experiment, although feeding untreated J.*curcas* meal (T1) retarded the growth 10.13% as compared to that of the control diet (T0). These results indicated that using 5% J.*curcas* meal in the diet was not toxic to the kampong chickens, and phorbolester found in the J.*curcas* meal used in this experiment was low.

CONCLUSIONS

Feeding 5% untreated as well as fermented *Jatropha curcas* meal in the diets was safe to the kampong chickens. Supplementation of cocktail enzymes (cellulose 200 ml/ton+ phytase 1000 FTU/kg) yielded the best performances of growth and feed efficiency of kampong chickens.

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