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 contained 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 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 convert the toxic jatropha curcas meal to high quality of meal as poultry feed. However, the fiber and phytate contained in the meal were still high. This experiment was conducted to study the effects of using fermented *Jatropha curcas* meal supplemented with cellulase and phytase in the kampong chicken diets on the growth and mortality rate. The experiment used 200 kampong chickens and were reared from day old chicks up to 10 weeks of age. This experiment using completely randomized design with 5 treatment diets and 4 replications, each replication used 10 birds. The experimental diets were: T0 (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 data were analysed using analyses of variance. The results showed that there was no significant different 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 final body weight approximately 10.13% compared to 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 of the control (T0) diet. The values of the final body weight of the 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 values of the feed conversion ratio of the T0, T1, T2, T3 and T4 were 2.93, 3.51, 3.49, 3.20, and 2.89, respectively. The values of the feed consumption per bird during 10 weeks of the T0, T1, T2, T3, and T4 were 2567.53 g, 2663.76 g, 2752.32 g, 2685.05g, and 2520.5 g, respectively. There was no mortality observed for all treatments.

Keywords: 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 contained 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 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 convert the toxic jatropha curcas meal to high quality of meal as poultry feed. However, the fiber and phytic acid contained in the meal were still high. Poultry can not digest fiber, especially cellulose, even the fiber could interfere another nutrients coantained 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 supplemented 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 Faculty of Animal Science, Bogor Agricultural University (Table 1).

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

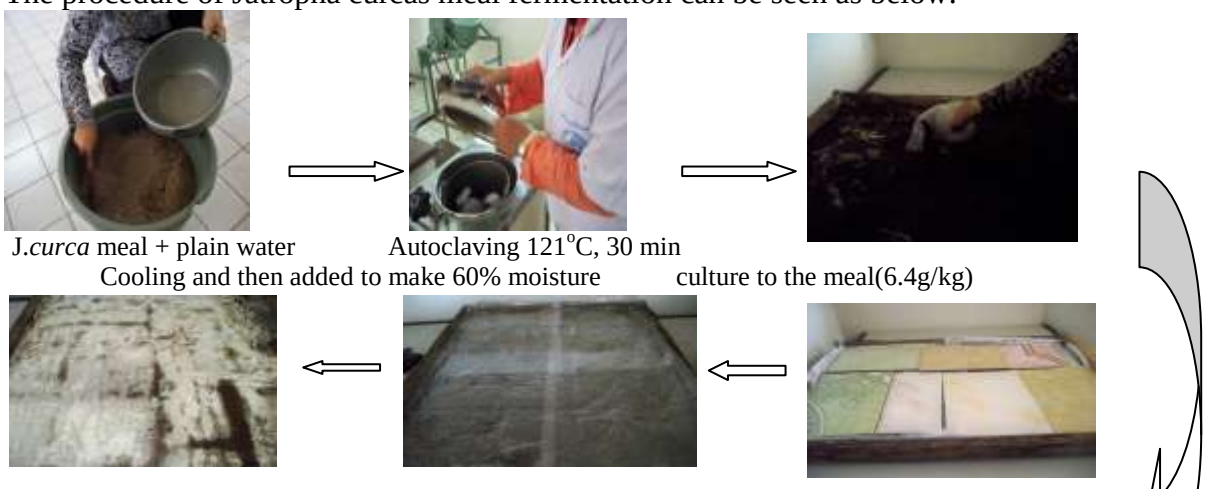
Component	Untreated J.curcas	Fermented J.curcas
Dry matter, %	84.99	94.01
Ash, %	5.63	5.95
CP, %	24.71	22.39
EE, %	5.8	0.39
CF, %	32.58	44.22
NFE, %	16.27	21.06
Ca, %	1.00	0.68
P, %	0.99	0.35
GE, kcal/kg	3893	3984
Pytic acid, %**	10.18	7.45

*The nutrients were analyzed at Laboratory of Feed Science and Technology, Faculty of Animal Science, Bogor Agricultural University, ** Phytic acid was analyzed at 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 as below:



J.*curca* meal + plain water

Autoclaving 121^{o}C, 30 min

Cooling and then added to make 60% moisture culture to the meal(6.4g/kg)

3rd day fermentation
The meal was ready
for poultry feed

2nd day fermentation
(uncovered)

1st day fermentation
(the meal was wrapped o be dried
with plastics inside, and covered)

Feeding Trial Using Kampong Chickens

This experiment used 200 kampong chickens which were reared from day old chicks up to 10 weeks of age. This experiment using completely randomized design with 5 treatment diets and 4 replications, each replication used 10 birds. The experimental diets were: T0 (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). Composition of experimental diets is presented in Table 2.

Table 2. Composition of experimental diets

Ingredient	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	1000	1000
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	18.2
EE, %	5.6	5.15	5.43	5.43	5.4
CF, %	3.81	4.77	5.65	5.65	5.65
Ca, %	0.91	0.91	0.91	0.91	0.91
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)] Ingredient nutrients composition based on Leeson and Summers (2005)

The experimental diets were fed to 2 weeks old up to 10 weeks old of chicks in order to minimize the mortality. During two weeks (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

Effect of treatments on feed consumption

The average of feed consumption of kampong chickens in this experiment is presented at Table 3.

Feeding diets contained J.curcas (T0, T1, T2, T3, T4) did not affect feed consumption. It showed that feeding 5% untreated as well as fermented J.curcas did not influence on its acceptance, and thus it indicated that J.*curcas* used in this experiment was low phorbolesters variety. 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.

Table 3. Average of feed consumption of kampong chicken during 10 weeks (g/bird)

Replication	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[a]	2663.76[a]	2752.32[a]	2685.05[a]	2520.50[a]
SD	186.26	160.67	21.47	193.58	211.45

Effect of treatments on body weight gain

Table 4. Average of body weight gain of kampong chicken during 10 weeks (g/bird)

Replication	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[a]	823.88[a]	837.99[a]	901.18[a]	916.75[a]
SD	70.26	143.05	71.53	169.90	29.25

Statistically, there was no significant different between treatments in body weight gain of kampong chickens. However, feeding 5% untreated J.*curcas* meal (T1) impaired the growth with the value of 10.39% compared to the control (T0). Feeding fermented J.curcas meal supplemented with cellulose (T2) tended to increase the growth with the value of 1.7% compared to untreated J.curcas meal diet (T1). Supplementation of fermented J.curcas with phytase (T3) increase body

weight gain with the value of 9.38%, and supplementation of cellulase + phytase to fermented J.curcas diet elevated the growth with the value of 11.27% (T4). The body weight gain of T4 was similar to the control (T0/without J.curcas meal in the diet).

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

Phytase supplementation in 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 + phytase in fermented J.*curcas* meal diet (T4) gave more body weight gain compared to single enzyme supplementation (T2 and T3).

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) reduce feed efficiency with the value of 19.8% compare to the control (T0/without J.*curcas* meal in the diet). Supplementation of cellulase seemed not effective in increasing feed efficiency. However, phytase supplementation in the diet (T3) increased feed efficiency with the value of 8.83%. While, supplementation cellulase + phytase in the diet (T4) yielded the highest feed efficiency with the value of 17.66%. These results showed that supplementation of enzyme cocktail (cellulose + phytase) gave higher effect on feed efficiency compared to single enzyme supplementation.

Table 5. Average of feed conversion ratio of kampong chicken during 10 weeks (g/bird)

Replication	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

Effect of treatments on final body weight

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

Replication	T0	T1	T2	T3	T4
1	914.00	953.33	836.67	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

Feeding 5% untreated J.*curcas* meal (T1) decrease final body weight with the value of 10.135% compared to the control (T0/without J.*curcas* in the diet). Otherwise, supplemented enzymes to diets contained fermented J.*curcas* meal tended to raise final body weight of kampong chicken. Supplementation cellulase + phytase in the diet (T4) yielded final body weight similar to the control diet (T0). This data indicated that phytase was effective to degrade phytic acid contained in the meal. There are several studies which indicate 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).

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 with the value 10.13% compared to control (T0). These results indicated that using 5% J.*curcas* meal in the diet was not toxic to kampong chickens, and phorbolester contained in the J.*curcas* meal used in this experiment was low (low phorbolester variety).

CONCLUSION

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

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