PERFORMANCE OF LAYER HEN FED FERMENTED *Jatropha Curcas L.* MEAL SUPPLEMENTED WITH CELLULASE AND PHYTASE ENZYME

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Received April 28, 2012; Accepted May 30, 2012

ABSTRAK

Tujuan penelitian ini adalah mempelajari pengaruh pemberian bungkil biji jarak pagar (BBJP) yang telah difermentasi menggunakan *Rizhopus oligosporus* serta suplementasi enzim selulase dan fitase terhadap performa ayam petelur strain *ISA-Brown* unur 25-30 minggu. Penelitian ini menggunakan 200 ekor ayam petelur umur 25 minggu yang dialokasikan kedalam 5 perlakuan, masing-masing perlakuan terdiri atas 4 ulangan. Perlakuan ransum yang diberikan adalah: R0 = Ransum kontrol (tanpa BBJP), R1 = Ransum mengandung BBJP fermentasi 7,5%, R2 = Ransum mengandung BBJP fermentasi 7,5% + selulase 200 g/ton, R3 = Ransum mengandung BBJP fermentasi 7,5% + fitase 200 g/ton, R4 = Ransum mengandung BBJP 7,5% + selulase 200 g/ton + fitase 200 g/ton. Peubah yang diukur adalah konsumsi ransum, produksi telur *hen day*, produksi telur massa, bobot telur dan konversi ransum. Data dianalisis menggunakan sidik ragam (ANOVA) dan jika berbeda nyata diuji lanjut menggunakan uji jarak Duncan. Hasil penelitian menunjukkan bahwa pemberian BBJP 7,5%, baik yang tidak disuplementasi maupun yang disuplementasi enzim nyata menurunkan (P<0,05) konsumsi ransum, produksi telur hen day dan produksi telur massa, tetapi tidak mempengaruhi bobot telur. Berdasarkan analisis statistik, suplementasi enzim selulase atau fitase mampu memperbaiki konversi ransum dengan nilai sama dengan konversi ransum perlakuan kontrol.

Kata kunci: ayam petelur, fitase, Jatropha curcas, R.oligosporus, selulase

ABSTRACT

The objective of the experiment was to study the effect of feeding fermented *Jatropha curcas* L. meal (JCM) supplemented with cellulase and phytase on the performances of ISA-Brown laying hen aged 25-30 weeks. The *Jatropha curcas* meal was fermented using *Rizhopus oligosporus*. In this study 200 laying hens were used and distributed to 5 treatments and 4 replications in Completely Randomized Design. The diet treatments were: R0 = control diet (without JCM), R1; diet contained fermented JCM 7.5%, R2; diet contained fermented JCM 7.5% + celullase 200 g/ton, R3; diet contained fermented JCM 7.5% + phytase 200 g/ton and R4; diet contained fermented JCM 7.5% + cellulase 200 g/ton + phytase 200 g/ton. The parameters observed were feed consumption, hen day egg production, egg mass production, egg weight and feed conversion ratio. The results showed that feeding fermented JCM 7.5%, both enzyme supplemented as well as unsupplemented significantly decreased (P<0.05) the feed consumption, hen day egg and egg mass production. However, the treatments did not influence the egg weight. Supplementation of cellulase (R2) or phytase (R3) improved the feed conversion ratio with the value as same as the R0 diet.

Keywords : *cellulose, Jatropha curcas meal, laying hen, phytase, Rhizopus oligosporus*

INTRODUCTION

Jatropha curcas meal (JCM) is potential as poultry feed due to its rich in nutrients content. JCM with shell contains 24.71% protein (Sumiati *et al*., 2007), the seed kernels contained 31-34.5% protein. The gross energy of kernels ranged from 31.1 to 31.6 MJ/kg DM and the levels of amino acids, except lysine, were higher than that of the FAO/WHO reference of protein for a five year old

child on a dry matter basis (Martinez –Herrera *et al*., 2006). The availability of this rich nutrients is limited by toxins and antinutrients contained in the meal. These toxic and antinutrients included curcin (lectin), tannin, trypsin inhibitors, phytate, saponin and phorbolesters (Francis *et al*., 2006). According to Saetae and Suntornsuk (2011), main toxic compound of JCM was phorbolesters and its anti-nutritional factors were trypsin inhibitors, phytic acid, lectin and saponin. Goel *et al. (*2007) reported that interaction of phorbol ester with protein kinase C (PKC) affected activities of several enzymes, biosynthesis of protein, DNA, polyamines, cell differentiation processes, and gene expression. Lin *et al*. (2003) reported that the curcin had a powerful inhibitory action upon protein synthesis in reticulocyte lysate. Singh (2008) reported that phytic acid was an anti-nutritional constituent of plant derived feeds. As a reactive anion, it formed a wide variety of insoluble salts with minerals including phosphorus, calcium, zinc, magnesium and copper. Phytic acid was also known to form complexes with protein and proteolytic enzymes (pepsin and trypsin). To optimize JCM utilization as poultry feed, the detoxification of the meal toxins and antinutrients is needed.

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 minutes. Extraction with ethanol followed by treatment with 0.07%$NaHCO_3$ considerably decreased lectin activity. The extraction treatment also decreased the phorbolester content by 97.9% in seeds (Martinez-Herrera *et al*., 2006). Sumiati *et al*. (2011a) reported that fermentation of JCM using *Rhizopus oligosporus* was the best method to detoxify the toxins, but the meal still contained high fiber and phytic acid. Poultry can not digest fiber, especially cellulose, even the fiber could interfere other nutrients contained in the feed. 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 feeding fermented *Jatropha curcas* meal using *Rhizopus oligosphorus* supplemented with cellulase and phytase on the performances of laying hen age 25-30 weeks old.

MATERIALS AND METHODS

This experiment used *Jatropha curcas* meal fermented by the method of Sumiati *et al*. (2009). The culture that usually used to ferment soybean and to make a food called *tempe* in Indonesia was used as source of *Rhizopus oligosphorus*. The JCM was added with plain water to make the moisture of the meal 60%. Then the moist meal was autoclaved at 121^{o}C during 30 minutes. The autoclaved JCM was allowed to be cooled before inoculated with the fungi. The cake was then inoculated with the tempe fungi 7g/kg JCM. The inoculated meal was incubated at room temperature during 3 days. The growth of the fungi was terminated by oven drying the meal at 60°C during 24 hours. The dry and ground meal was used in the formulation of the experimental diets. The phorbolesters of untreated and fermented JCM was analysed at Animal Research Institute, Ciawi, Bogor, Indonesia.

A Completely Randomized Design using 5 treatments and 4 replications (10 birds for each) was used in this experiment. Two hundred of Isa-Brown laying hens aged 25 weeks were used in this study and were reared up to 30 weeks of age. The hens were distributed into 100 battery cages with the size of each cage was 45 cm x 45 cm x 45 cm (2 hens per cage). The diet treatments were : R0 = control diet (without JCM), R1 = diet contained fermented JCM 7.5%, R2 = diet contained fermented JCM 7.5% + celullase 200 g/ton, R3 = diet contained fermented JCM 7.5% + phytase 200 g/ton and R4 = diet contained fermented JCM 7.5% + cellulase 200 g/ton + phytase 200 g/ton. The diets were isocaloric and iso protein according to Leeson and Summers (2005). The composition and nutrients content of the diets are presented in Table 1.

The parameters observed were feed consumption (g/day/hen), hen day egg production (%), egg mass production (g/hen), feed conversion ratio (feed consumption/egg mass production) and egg weight (g/egg). The data were analyzed using analyses of variance and the significant data were further analysed using Duncan's Multiple Range Test (Mattjik and Sumertajaya, 2000).

RESULTS AND DISCUSSION

The data of laying hens performances fed fermented JCM during 5 weeks are presented in

Table 1. Composition and Nutrients Content of Treatment Diets

Ingredient	Treatment Diets				
	R0	R1	R2	R3	R4
	..... (%).....				
Yellow corn	53	52	52	52	52
Corn gluten meal	4	4	4	4	4
Rice bran	4	0	0	0	0
Soybean meal	19	15.8	15.8	15.8	15.8
Fermented JCM	0	7.5	7.5	7.5	7.5
Fish meal	6	6	6	6	6
Crude Palm Oil	4.2	5	5	5	5
CaCO3	8.5	8.5	8.5	8.5	8.5
DCP	0.5	0.57	0.57	0.57	0.57
Salt	0.2	0.2	0.2	0.2	0.2
Premix **	0.5	0.3	0.3	0.3	0.3
DL-methionine	0.1	0.13	0.13	0.13	0.13
Total	100	100	100	100	100
Celulase (g/ton)***	0	0	200	0	200
Phytase (g/ton) ***	0	0	0	200	200
Nutrients content (based on calculation):*					
Metabolizable Energy (kcal/kg)	2902.40	2904.59	2904.59	2904.59	2904.59
Crude protein (%)	19.02	19.09	19.09	19.09	19.09
Crude fat (%)	6.21	6.94	6.94	6.94	6.94
Crude fiber (%)	1.89	3.85	3.85	3.85	3.85
Calcium (%)	3.85	3.93	3.93	3.93	3.93
Phosporus avl (%)	0.48	0.47	0.47	0.47	0.47
Lysine (%)	0.99	0.94	0.94	0.94	0.94
Methionine (%)	0.41	0.43	0.43	0.43	0.43

*Nutrients content of ingredients according to Leeson and Summers (2005): **1 kg premix contain Vit A 4000000, Vit D3 800000 IU, Vit E 4500 mg, Vit K3 450 mg, Vit B1 450 mg, Vit B2 1350 mg, Vit B6 480 mg, Vit B12 6 mg, Ca-d-pantothenate 270 mg, folic acid 7200 mg, choline chloride 28000 mg, Dl-methionine 28000 mg, L-lysine 50000 mg, Fe 8500 mg, Cu 700 mg, Mn 18500 mg, Zn 14000 mg, Co 50 mg, Iod 70 mg, Se 35 mg

*** DSM Nutrition product (2010)

Table 2. Feeding fermented JCM 7.5% in both enzyme supplemented as well as unsupplemented during 5 weeks were significantly decreased ($P<0.05$) the feed consumption, hen day egg production and egg mass production. However, feeding the meal did not influence the egg weight. The feed conversion ratio of laying hens significantly increased ($P<0.05$) by feeding JCM 7.5% without enzyme supplementation (R1). These results indicated that phorbolesters contained in the JCM still interfered the utilization of the nutrients by the hens.

Feeding JCM in this experiment decreased feed consumption of the laying hens and thus lowered nutrients consumption. Sumiati *et al*. (2011a) reported that decreasing feed

Table 2. The Performances of ISA-Brown Laying Hens Aged 25-30 Weeks

Parameters	Treatment Diets									
	R0		R1		R2		R3		R4	
Feed consumption (g/hen/day)	99.10±	2.34[a]	86.43±	11.52[b]	84.91±	5.76[b]	84.94±	7.44[b]	84.39±	8.38[b]
Hen day egg production (%)	64.05±	7.56[a]	28.33±	13.43[c]	43.37±	5.33[b]	35.11±	6.68[bc]	28.91±	9.67[c]
Egg mass production (g/hen)	1508.40±192.44[a]		659.69±320.17[c]		1042.40±134.55[b]		804.06±137.30[bc]		620.98±284.27[c]	
Egg weight (g/egg)	55.40±	1.00	54.27±	1.80	55.53±	1.16	53.30±	2.06	53.38±	1.31
Feed conversion ratio	3.24±	0.94[a]	8.67±	5.31[b]	5.88±	1.30[ab]	5.14±	0.63[ab]	5.70±	1.46[ab]

Mean values within the same row with different superscripts are significantly different (P<0.05)

Table 3. The Consumption of Metabolizable Energy and Protein of ISA-Brown Laying Hens Aged 25-30 Weeks

Parameters	Treatment Diets				
	R0	R1	R2	R3	R4
ME consumption (kcal/hen/day)	287.64±6.78[a]	251.05±33.46[b]	246.63±16.73[b]	246.72±21.60[b]	245.12±24.35[b]
Protein consumption (g/hen/day)	19.03±0.45[a]	16.50± 2.20[b]	16.21± 1.10[b]	16.22± 1.42[b]	16.11± 1.60[b]

Mean values within the same row with different superscripts are significantly different (P<0.05)

consumption of broiler chickens fed JCM due to liver damage to detoxify the toxin such as phorbolester. The energy and protein consumption of laying hens in current study are presented in Table 3.

Feeding JCM 7.5% significantly decreased (P<0.05) metabolizable energy(ME) and protein consumption of the laying hens compared to the control diet (R0). The consumption of ME decreased about 12.7 - 14.78%, from 287.64 kcal/hen/day (R0) to 245.12 kcal/hen/day (R4). The protein consumption decreased about 13.29 - 15.3%, from 19.03 g/hen/day (R0) to 16.11 g/hen/day (R4). These nutrients consumption were lower than those of Leeson and Summers recommendation (2005), the laying hens of age 18-32 weeks old need 20 g crude protein/hen/day and 260 kcal ME/hen/day. This lower consumption had implication to the lower egg production of the hens. Leeson and Summers (2005) reported that decreasing in energy and protein intake resulted in decreasing egg production. According to Leeson and Summers (2001), protein consumed from the diets were utilized for production of an egg, maintenance of body protein for one day, growth per day, and feather growth per day.

According to Goel *et al*. (2007), the phorbolesters, even at very low concentrations, shows toxicological manifestations in animals fed

diets containing them. This toxicity limits the use of many nutritive plants and agricultural by-products containing phorbolesters to be used as animal feed. The untreated JCM used in this experiment contained 24.33 µg/g phorbolesters and the fermented meal contained 15.28 µg/g phorbolesters. Aregheore (2003) reported that concentration of 0.13 mg/g phorbolesters present in the *Jatropha curcas* has a significant adverse effect on food intake and growth rate of rats. The reduced food intake, loss in body weight, and low protein efficiency ratio (PER) may have resulted from higher concentrations of phorbolesters in the test diets compared to the control. Besides the phorbolesters, the decrease of egg production could be due to curcin contained in the JCM. Sumiati *et al*. (2011b) reported that JCM used in this experiment contained 6.25 mg/ml curcin (lectin activity). Lin *et al*. (2003) reported that curcin has a high RNA *N*-glycosidase activity and its inhibit protein synthesis.

Supplementation of cellulase 200 g/ton to the diet contained fermented JCM 7.5% (R2) significantly increased ($P<0.05$) hen day egg production and egg mass production compared to the unsuplemented diet (R1). These finding indicated that cellulase added hydrolyzed the cellulose contained in the diet, although the egg production did not reach as much as the control diet. It could be too low concentration of the enzyme to hydrolyze high crude fiber in the diet. The crude fiber content of the control diet was 1.89% and that of the R2 diet was 3.85%. However, it was interesting finding that supplemented cellulase 200 g/ton in the diet (R2) yielded the hen day egg and egg mass production as much as the control diet (R0) at 30 weeks of age (Figure 1 and Figure 2).

The feed efficiency of the laying hens fed the diets supplemented with cellulase 200 g/ton (R2), phytase 200 g/ton (R3) as well as the enzyme mixture (R4) were statistically the same as the control diet (R0). These results indicated that enzymes supplementation could improve the feed efficiency of the laying hens. Traylor *et al.* (2001) reported that phytase supplementation was effective in improving the availability of Calcium and Phosphorus. Phytate binds nutrients in addition to P, and the addition of phytase to feed caused the release of these nutrients and allows their absorption by the bird. Nutrients affected by phytates include minerals and protein (Ravindran *et al.*, 1995; Selle *et al*., 2000), and phytase had been shown to affect the release of energy (Scott *et al*., 2001).

CONCLUSION

Feeding *Rhizopus oligosporus* fermented *Jatropha curcas* meal 7.5% decreased feed consumption and egg production of ISA-brown laying hen age 25-30 weeks. Supplementation of cellulase 200 g/ton (R2) or phytase 200 g/ton (R3) improved feed efficiency of the laying hens with the value as same as the control (R0) diet (without JCM). Supplementation cellulase 200 g/ton (R2)

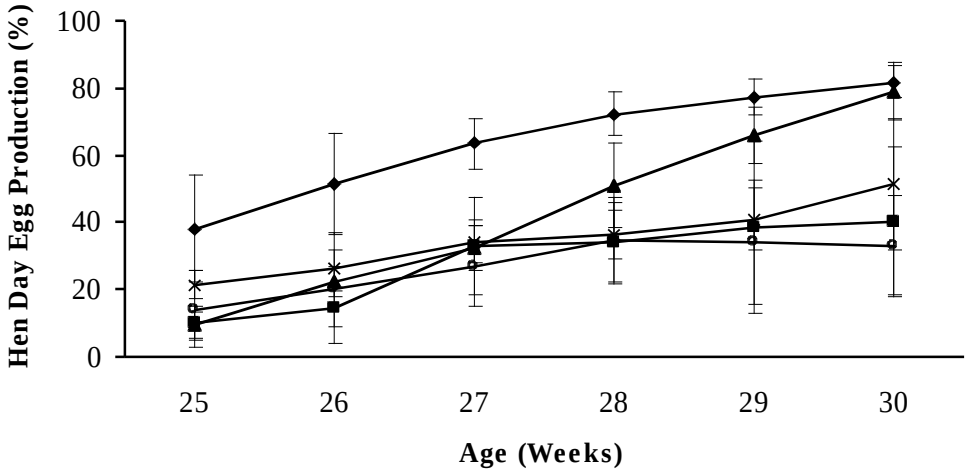

Figure 1. Hen day Egg Production of Laying Hens During 5 Weeks Experiment. (◆) = control diet (without JCM). (■) = diet contained fermented JCM 7.5%. (▲) = diet contained fermented JCM 7.5% + celullase 200 g/ton. (**x**) = diet contained fermented JCM 7.5% + phytase 200 g/ton and (o) = diet contained fermented JCM 7.5% + cellulase 200 g/ton + phytase 200 g/ton

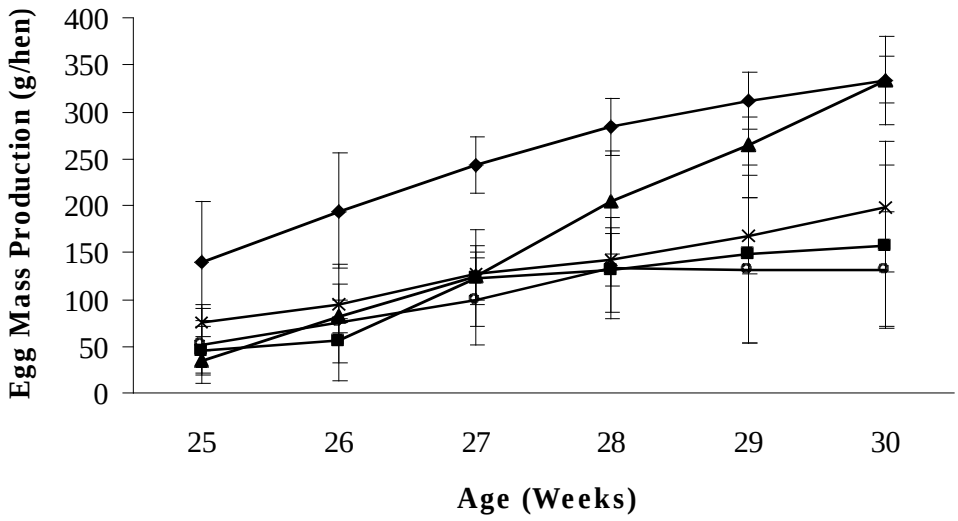

Figure 2. Egg Mass Production of laying Hens During 5 Weeks Experiment. (◆) = control diet (without JCM). (■) = diet contained fermented JCM 7.5%. (▲) = diet contained fermented JCM 7.5% + celullase 200 g/ton. (**x**) = diet contained fermented JCM 7.5% + phytase 200 g/ton and (o) = diet contained fermented JCM 7.5% + cellulase 200 g/ton + phytase 200 g/ton.

improved the egg production compared to the unsupplemented diet (R1) and yielded hen day egg and egg mass production as much as the control diet (R0) at 30 weeks of age

ACKNOWLEDGMENTS

Authors gratefully acknowledge the Hibah Bersaing Project of Higher Education. KEMENDIKBUD. Indonesia for funding this research and to PT INDOCEMENT for giving *Jatropha curcas* meal.

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