Effectivity of *Jatropha curcas* Seed Meal Fermented with Various Moulds as Protein Source for Male Mice (*Mus musculus*)

A.S. Tjakradidjaja, Suryahadi, and R. Mahajati
Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University,
email:latj.yanuar@gmail.com

ABSTRACT

Jatropha curcas L seed meal (JCSM) is a waste product of seed processing to produce oil as biofuel. This JCSM contains high crude protein (24.3%) leading to its use as protein source for animals. However, the utilization of JCSM is limited by the presence of antinutritional factors, such as phorbolester, curcin, trypsine inhibitor, phytate, saponin, tannin and lectin. Detoxification is needed to improve its utilization. Fermentation with various moulds as biological treatment was applied to reduce phorbolester and curcin in this experiment. This experiment was carried out to evaluate the effectivity of JCSM fermented with various moulds as protein source for mice. JCSM was treated with *Aspergillus niger*, *Rhizopus oryzae*, *Rhizopus oligosporus*, *Trichoderma viride*, or *Trichoderma reesei*. Treatments applied in this experiment were R0 (control diet without JCSM), R1 (95% R0 + 5% JCSM treated with *A. niger*), R2 (95% R0 + 5% JCSM treated with R. oryzae), R3 (95% R0 + 5% JCSM treated with *R. oligosporus*), R4 (95% R0 + 5% JCSM treated with *T. viride*), and R5 (95% R0 + 5% JCSM treated with *T. reesei*). Treatments were allocated in a completely randomized design with five replications and applied to thirty five sexual matured of male mice. Variables measured were feed intake, body weight gain, feed efficiency, nutrient digestibility and mortality. The data were analyzed with descriptive analysis. The results showed that the use of fermented JCSM as protein sources reduced feed intake and feed efficiency. The mice had drastic reduction in body weight. Treatment JCSM with *A. niger* increased nutrient digestibilities such as dry matter, organic matter, protein and energy. Mice consuming JCSM treated with various moulds still had high mortality rate. The use of JCSM treated with *R. oligosporus* was better than that treated with *R. oryzae*. It is concluded that the use of fermented JCSM with various moulds at 5% in ration has not been effective as protein sources for male mice.

Key words: Jatropha curcas L., *Aspergillus niger*, *Rhizopus oryzae*, *Rhizopus oligosporus*, *Trichoderma viride*, *Trichoderma reesei*, *mice* (*Mus musculus*)

INTRODUCTION

Jatropha curcas L seed meal (JCSM) is a waste product of seed processing to produce oil as biofuel. This JCSM contains high crude protein (24.3% DM basis) leading to its use as protein source for animals (Tjakradidjaja *et al.*, 2007). JCSM also contains ether extract in high concentration (15.99% DM basis) that causes high energy content. These protein and other nutrient contents are affected by the presence of seed husk during oil extraction. Exclusion of seed husk increased significantly protein (37.56% DM basis) and lipid (35.02% DM basis) contents, and reduced crude fibre and nitrogen free extract (Tjakradidjaja *et al.*, 2007). However, the utilization of JCSM is limited by the presence of antinutritional factors, such as phorbolester, curcin, trypsin inhibitor, phytate, saponin, tannin and lectin (Makkar and Becker, 1999; Aregheore *et al.*, 2003).

JCSM had been used as protein supplements that substituted control diets (Siagian *et al.*, 2007; Wardoyo, 2007). The maximum use of JCSM was 5%. The use of greater levels of JCSM (7.5-15%) caused weakness, lost of appetite, reduction in feed intake, decrease in body weight gain, presence of yellow colour liquid in anus (rectum), and high mortality rate. These are caused by curcin and phorbolester as the main antinutrients/toxins (Stirpe *et al.*, 1976; Aderibigbe *et al.*, 1997; Evans, 1986; Brodjonegoro *et al.*, 2005). Basically, curcin has similar protein structure to that of ricin which is present in *Ricinus communis*; curcin and ricin are lectin type toxins (Aderibigbe *et al.*, 1997;

Aregheore *et al.*, 1998; Aregheore *et al.*, 2003). Curcin is capable of inactivating enzyme that synthesise proteins (Hadi, 2008). Phorbolester is an ester that dissolves in organic solvent. This analogoue of diacylglycerol (DAG) is capable of inactivating protein kinase C enzyme which changes the normal physiological function of cells (Rug *et al.*, 2006; Asaoka *et al.*, 1992; Makkar dan Becker, 1997). Concentrations of curcin and phorbolester varied among varieties of JC (Makkar and Becker, 2004; Francis *et al.*, 2006).

Detoxification is needed to reduce phorbolester and curcin and to improve its utilization. Fermentation with various moulds is one biological method that can be applied. It is expected that the enzyme activities present in various moulds and production of ethanol can degrade the toxins from JCSM during fermentation. The previous result indicates that fermentation with various moulds reduced protein and lipid concentrations, and increased ash and crude fibre contents although curcin and phorbolester contents were not detected (Tjakradidjaja *et al.*, 2007). The fermented products of JCSM need to be evaluated as protein source in ration for animals. Therefore, the present experiment is an evaluation of the effectivity of JCSM fermented with various moulds as protein source for mice (*Mus musculus*).

MATERIALS AND METHODS

Materials

This experiment used 35 heads of male mice (*M. musculus*) aged at about 28 days with average body weight was 22.77 $\pm$ 4.95 g/head. The mice were kept in individual cages (36 cm x 28 cm x 12 cm) for 7 weeks (28 days). Each individual cage was provided with feed through and drinking bottle, rice husks were used to cover the basis of each individual cage. Moulds used for fermenting JCSM were *Aspergillus niger*, *Rhizopus oligosporus*, *Rhizopus oryzae*, *Trichoderma viride*, and *Trichoderma reesei*. Comercial broiler starter (BR 1 CP 511, PT Charoen Pokphand) was used as a control diet.

Treatments

The control diet was a commercial diet, and about 5% of control diet was substituted with fermented JCSM. All diets were given in pelleted form. Treatment diets (6 treatments) were:

R0 : Control ration
R1 : 95% R0 + 5% unfermented JCSM
R2 : 95% R0 + 5% JCSM fermented with *A. niger*
R3 : 95% R0 + 5% JCSM fermented with *R. oryzae*
R4 : 95% R0 + 5% JCSM fermented with *R. oligosporus*
R5 : 95% R0 + 5% JCSM fermented with *T. viride*
R6 : 95% R0 + 5% JCSM fermented with *T. reesei*

Experimental Design and Data Analysis

Treatments were allocated in a completely randomized design with five replications; each replication consisted of 1 male mice. Variables measured in this experiment were feed and nutrient intakes, nutrient digestibility, body weight gain, feed efficiency, and mortality rate. The data were analysed with descriptive analysis. The descriptive analysis was conducted due to high mortality rates of mice during experiment (Steel and Torrie, 1981).

Procedures

Medium and Inoculum Preparation

Bean sprout extract medium was used as basal medium for growing the moulds. The medium was prepared following the method of Lestari (2006). Bean sprout (250 g) was mixed with distilled water (1000 ml) and boiled by keeping the volume at 1000 ml. The mixture was then filtered with muslin cloth. The filtrate (100 ml) was then mixed with Bacto agar (2 g) and boiled (100°C) until the medium become clear. The medium was then divided into 10 tubes (3ml/tube). The tube was covered with cotton and aluminum foil, and was sterilized with autoclaving (121°C 15 min). The tubes were then cooled.

Inoculum was prepared by adding sterillised distilled water (10 ml) into a tube containing culture stock of each mould. Each tube was then homogenised with sterilised glass loope. This mixture was then inoculated on to bean sprout extract medium. The cultures were then incubated for 3 days at 30°C (Lestari, 2006).

JCSM Fermentation with Various Moulds

JCSM was put into a plastic bag which was added with water up to 60% moisture. This mixture was then homogenised and autoclaved (121°C 15 min). Each incubated cultures were diluted with sterill water (6 ml) and homogenised. The sterille JCSM was then cooled at room temperature. The cooled JCSM was then placed and spread in a plastic tray. This JCSM was then inoculated with each mould inoculate (3 ml inoculum/50 g sterille JCSM) and covered with plastic wrap. Holes were made in plastic wrap as fermentation was conducted in aerobic condition at room temperature (30°C) for 6 days.

Diet Pelleting, Diet and Fecal Analysis and Body Weight Measurement

Fermented JCSM was dried under the sun which was then dried in an oven (60°C 24 h) and ground. Commercial diet was ground; the ground commercial diet (95%) was mixed with each of dried ground fermented JCSM (5%). Each mixture was homogenised using a mixer, and was then placed in pelleting machine. The pellet diets were then dried.

The pelleted diet were given to the animals based on the treatment applied. Diets and water were given *ad libitum*. The amount of diets given and the residual diets from the through or from the base cage were collected and recorded every week. Fecal collection was carried out for a week. The animals were kept for 7 weeks consisting of 1 week of preliminary period and 6 weeks of experimental period. Diet and fecal samples were dried under the sun and in an oven (60°C 24 h) which were then analysed with proximate analysis for nutrient contents.

Body weights were determined with a scale at the beginning of experiment and every weeks until the end of experiment. Body weight gain (g/day) was calculated by substracting body weight at a week with body weight at a week before which was then divided by a period of measurement.

RESULTS AND DISCUSSION

Nutrient Content of Treatment Diets

Results of proximate analysis of treatment diets showed variations in nutrient content among the diets (Table 1). The commercial diet (R0) contained 91.98% DM, and replacing R0 with 5% untreated JCSM slightly reduced DM content (R1). Reductions in DM contents were also observed in R5 and R6 (*Trichoderma* spp.), but increases in DM contents were found in R2 and R3 (*A. niger* and *R. oligosporus*). The highest DM content was in R4 (*R. oryzae*). Differences in DM contents were due to addition of water before fermentation process and due to heat and drying treatment during pelleting.

Variations in ash contents caused variations in OM contents. Ash and OM contents in R0 were 6.20% and 93.80%. The ash content was reduced in R1, R3, R4, R5 and R6 causing increases in its OM contents. Reverse results were observed in R2. There were no significant variations in crude protein contents among treatment diets. Ether extract contents in all diets containing JCSM (R2-R6) were lower than that of control diet (R0). On the other hand, there were significant increases in crude fibre contents in all JCSM containing diets compared to that of control diet (R0). Variations were found in nitrogen free extract (NFE), Ca, P and gross energy contents among treatment diets.

Variations in nutrient contents among treatment diets were affected by nutrient contents of JCSM fermented with various moulds (Fardiaz, 1989; Winarno, 2002; Tjakradidjaja *et al.*, 2007). Reductions in ether extract may indicate that there were degradations of lipid by the enzyme produced by the moulds, especially by *Rhizopus spp.* (Nuraida *et al.*, 2000; Salleh, 1993). Reductions in ether extract may also indicate the reductions in phorbolester contents as this antinutrient is an ester compound. On the other hand, the increases in crude fibre contents may demonstrate that not all the moulds degrade crude fibre for their growth (Rahma, 1996; Yuniah, 1996). However, all nutrient contents in all treatment diets were still in the ranges that are recommended by Smith and Mangkoewidjojo (1988) for mice, except for crude fibre contents.

Feed and Nutrient Intakes

Due to high mortality rates occurred in this experiment, data in feed and nutrient intakes are presented according to the number of mice and the time of mice life or the time of experimental period (Table 2). Feed intake for R0 was 3.91 g/head/d. Replacing control diet (R0) with untreated (R1) and fermented JCSM (R2-R6) reduced feed intake. The same trends were also found for intakes of DM and other nutrients. Mice consuming R4 (95%R0 + 5% *R.*

oligosporus fermented JCSM), R5 (95%R0+5% *T. viride* fermented JCSM) and R2 (95%R0 + 5% *A. niger* fermented JCSM) tended to have greater feed and nutrient intakes.

Results in feed intakes were comparable to those obtained by Siagian *et al.* (2007) (1.68-3.04 g/head/day), but were lower than that recommended by Smith and Mangkoewidjojo (1988) (7g/head/day) and Tjakradidjaja *et al.* (2009) (4.26 g/head/day). The results also confirmed the other experimental results in which using untreated and treated JCSM reduced feed and nutrient intakes (Siagian *et al.*, 2007; Wardoyo, 2007; Tjakradidjaja *et al.*, 2009). Low feed intakes in untreated JCSM diets were due to the presence of curcin and phorbolester as antinutrients and crude fibre (Becker and Makkar, 1998; Aregheore *et al.*, 2003; Wardoyo, 2007). Fermentations JCSM with various moulds have not yet produced significant effects in increasing feed and nutrient intakes although fermentation with *R. oligosporus* showed a better result. This could be due to differences in enzyme activity among the moulds (Shurtleff and Aoyagi, 1971; Sutopo, 1987; Hardjo *et al.*, 1989), the nutrient content of JCSM fermented with various moulds (Table 1, Tjakradidjaja *et al.*, 2007), and nutrient utilization in the alimentary tract of mice (Makkar and Becker, 1999).

Nutrient Digestability

Digestibilities of dry matter, organic matter, crude protein and energy of R0, repectively, were 91.68, 94.60, 92.30 and 93.62%. Using untreated JCSM in R1 tended to reduce DM digestibility, but slightly increase other nutrient digestability. This may be due to high crude fibre content and the presence of antinutrients/toxins. Treatment JCSM with *A. niger* increased all nutrient digestibilities. This was because of crude fibre content was low with high crude protein and ether extract contents in R2.

Tabel 1. Nutrient content of treatment diets

Nutrient contents	Treatment diets[1]						
	R0	R1	R2	R3	R4	R5	R6
Dry matter (%)	91.98	90.08	92.24	92.92	95.42	88.90	87.68
Ash (%DM)	6.20	5.63	6.50	6.11	5.31	4.68	5.86
Organic matter (%DM)	93.80	94.37	93.50	93.89	94.69	95.32	94.14
Crude protein (%DM)	24.53	24.40	25.80	24.21	24.67	24.51	25.01
Ether extract (%DM)	8.39	7.13	7.94	6.52	6.46	7.21	6.91
Crude fibre (%DM)	2.34	5.06	4.37	4.72	5.43	4.45	5.66
Nitrogen free extract (%DM)	58.55	57.78	55.39	58.43	58.13	59.15	56.56
Ca (%DM)	0.04	0.04	0.05	0.05	0.05	0.06	0.08
P (%DM)	0.10	0.09	0.11	0.08	0.08	0.08	0.05
Gross energy (cal/gDM)	4408.57	4601.47	4185.82	4100.30	4211.91	4466.82	4639.60

Note: [1]R0 : control diet; R1 : R0 (95%) + untreated JCSM (5%); R2 : R0 (95%) + *A. niger* fermented JCSM (5%); R3 : R0 (95%) + *R. oryzae* fermented JCSM (5%); R4 : R0 (95%) + *R. oligosporus* fermented JCSM (5%); R5 : R0 (95%) + *T. viride* fermented JCSM (5%); R6 : R0 (95%) + *T. reseei* fermented JCSM (5%).

Table 2. Feed and nutrition intakes

Intakes (g/head/day)	Treatment diets[1]						
	R0	R1	R2	R3	R4	R5	R6
Fresh (feed)	3.91±0.70	1.48±0.29	2.03±0.30	1.60±1.08	2.62±0.95	2.31±1.08	1.8±0.54
Dry matter	3.60±0.64	1.34±0.26	1.87±0.28	1.49±1.01	2.50±0.91	2.05±0.96	1.64±0.47
Organic matter	3.38±0.60	1.26±0.24	1.75±0.26	1.40±0.94	2.37±0.86	1.96±0.91	1.54±0.45
Crude protein	0.88±0.02	0.33±0.06	0.48±0.07	0.36±0.24	0.62±0.22	0.50±0.23	0.41±0.12
Ether extract	0.30±0.05	0.10±0.02	0.15±0.02	0.10±0.07	0.16±0.06	0.15±0.07	0.11±0.03
Crude fibre	0.08±0.02	0.07±0.01	0.08±0.01	0.07±0.05	0.14±0.05	0.09±0.04	0.09±0.03
Nitrogen free extract	2.11±0.38	0.77±0.15	1.04±0.16	0.87±0.59	1.46±0.53	1.21±0.57	0.93±0.27
Energy (cal/head/day)	158.72±28.40	61.46±11.92	78.36±11.75	60.94±41.26	105.40±38.29	91.62±42.80	75.94±21.94
n (head)	5	1	1	1	1	3	1
Weeks	6	5	6	6	6	3	6

Note: [1] R0 : control diet; R1 : R0 (95%) + untreated JCSM (5%); R2 : R0 (95%) + *A. niger* fermented JCSM (5%); R3 : R0 (95%) + *R. oryzae* fermented JCSM (5%); R4 : R0 (95%) + *R. oligosporus* fermented JCSM (5%); R5 : R0 (95%) + *T. viride* fermented JCSM (5%); R6 : R0 (95%) + *T. reseei* fermented JCSM (5%).

Fermenting JCSM with *Rhizopus* spp. (R3 and R4) and *Trichoderma* spp. (R5 and R6) did not produce similar effects to that of *A. niger*. The use of JCSM treated with *R. oligosporus* was slightly better than that treated with *R. oryzae* in nutrient digestibility. This could be due to differences in type and activity of the enzyme secreted by the two species of *Rhizopus* (Shurtleff and Aoyagi, 1971; Nuraida *et al.*, 2000; Salleh, 1993). No significant differences in nutrient digestibilities of JCSM treated with both *Trichoderma* spp. Feed intake data (Table 2) indicate that mice given R4 and R5 tended to be greater than those of R3 and R6. This means that higher feed intakes may cause less feed retention in the gastrointestinal tract and less feed contact with digestion enzyme; this made feed were less digested and reduced nutrient availability in gastrointestinal tract (McDonald *et al.*, 2002).

Body Weight Gain and Feed Efficiency

With DM intake was 3.60 g/head/day, mice consuming R0 had the highest body weight gain and feed efficiency. All diets containing untreated and treated JCSM caused negative body weight gain and feed efficiency (Table 4). However, treatment JCSM with *A. niger* produced the lowest body weight lost with the highest feed efficiency among treatment JCSM with various moulds. These negative effects were due to low feed intakes in relation to high content of crude fibre, the presence of antinutrients/toxins and low nutrient digestibility and availability (Fajariah, 2007; Asaoka *et al.*, 1992, Wardoyo, 2007; McDonald *et al.*, 2002). These results showed that treatment JCSM with various moulds has not yet improved performance of mice.

Table 3. Nutrient digestability

Nutrient digestibility (%)	Treatement diets[1]						
	R0	R1	R2	R3	R4	R5	R6
Dry matter	91.68±1.24	89.56±1.44	93.34±1.70	70.72±45.84	89.04±2.19	80.53±22.85	82.28±5.90
Organic matter	94.60±0.80	95.38±0.64	97.20±0.71	87.60±19.41	94.07±1.19	92.50± 8.81	90.78±3.07
Crude protein	92.30±1.14	93.57±0.88	95.92±1.04	80.19±31.01	91.63±1.68	89.16±12.72	88.04±3.98
Energy	93.62±0.95	94.61±0.74	96.36±0.93	82.79±26.95	92.31±1.54	90.60±11.03	89.81±3.40
n (head)	5	1	1	1	1	3	1
weeks	6	5	6	6	6	3	6

Note: [1]R0 : control diet; R1 : R0 (95%) + untreated JCSM (5%); R2 : R0 (95%) + *A. niger* fermented JCSM (5%); R3 : R0 (95%) + *R. oryzae* fermented JCSM (5%); R4 : R0 (95%) + *R. oligosporus* fermented JCSM (5%); R5 : R0 (95%) + *T. viride* fermented JCSM (5%); R6 : R0 (95%) + *T. reseei* fermented JCSM (5%).

Table 4. Body weight gain and feed efficiency

Variables	Treatment diets[1]						
	R0	R1	R2	R3	R4	R5	R6
Dry matter intake g/head/day)	3.60±0.64	1.34±0.26	1.87±0.28	1.49±1.01	2.50±0.91	2.05±0.96	1.64±0.47
Body weight gain (g/head/day)	0.19±0.19	-0.47±0.17	-0.43±0.21	-0.45±0.30	-0.44±0.45	-0.48±0.29	-0.54±0.26
Feed efficiency (%)	4.68±4.27	-35.58±12.25	-22.58±8.93	-38.16±31.65	-32.00±52.64	-25.10±13.00	-35.26±16.55
n (head)	5	1	1	1	1	3	1
weeks	6	5	6	6	6	3	6

[1] R0 : control diet; R1 : R0 (95%) + untreated JCSM (5%); R2 : R0 (95%) + *A. niger* fermented JCSM (5%); R3 : R0 (95%) + *R. oryzoe* fermented JCSM (5%); R4 : R0 (95%) + *R. oligosporus* fermented JCSM (5%); R5 : R0 (95%) + *T. viride* fermented JCSM (5%); R6 : R0 (95%) + *T. reseei* fermented JCSM (5%)

Table 5. Mortality rates

Treatment diets[1]	Mortality per week (%)						Total (%)
	1	2	3	4	5	6	
R0	0	0	0	0	0	0	0
R1	0	0	40	40	20	0	100
R2	0	0	40	20	20	20	100
R3	40	20	20	0	0	0	80
R4	20	0	0	60	0	0	80
R5	0	40	60	0	0	0	100
R6	20	40	20	0	0	0	80

[1] R0 : control diet; R1 : R0 (95%) + untreated JCSM (5%); R2 : R0 (95%) + *A. niger* fermented JCSM (5%); R3 : R0 (95%) + *R. oryzae* fermented JCSM (5%); R4 : R0 (95%) + *R. oligosporus* fermented JCSM (5%); R5 : R0 (95%) + *T. viride* fermented JCSM (5%); R6 : R0 (95%) + *T. reseei* fermented JCSM (5%).

Mortality Rate

Mortality rate was zero for R0 (Table 5). Given untreated and treated JCSM caused high mortality rates. 100% mortality rates were found in mice consuming R1 (untreated JCSM), R2 (*A. niger* fermented JCSM), and R5 (*T. viride* fermented JCSM). A lower mortality rate (80%) was found in mice eating R3 (*R. oryzae* fermented JCSM), R4 (*R. oligosporus* fermented JCSM) and R6 (*T. reseei* fermented JCSM). For mice consuming R1 and R2, high mortality rates occurred at the 3rd week up to the 5th and 6th weeks. Given R3, R4, and R6 caused death at the 1st week,death occurred at the 2nd week for mice fed with R5. These differences in mortality rate and the occurrence of death among mice may indicate differences among treatment diets producing negative effects on mice, and mice had different response or tolerance to the presence of curcin or phorbolester. These antinutrients/toxins reduced feed intake and nutrient digestions in gastrointestinal tract, and the metabolites released damaged other organs such as intestinal organ, liver, renal, and lungs (Adam, 1974; Makkar and Becker, 1998[a]; Makkar and Becker, 1998[b]). The death mice showed the same characteristics as those found by Wardoyo (2007), Fachrudin (2007) and Fajariah (2007).

Since the use of JCSM fermented with various moulds at 5% has not yet produced positive effects on mice performance, it is necessary to carry out other treatments such as combined fermentation with two or more species of mould, or combined treatment between physical, chemical or biological treatments. Such experiment had been done by Hadriyanah (2008) in which JCSM treated with methanol or 4% NaOH combined with drying and autoclaving (121°C 15 min 15 psi). The use of this treated JCSM at 5% in a diet has produced good results in body weight gain of mice. Therefore, it is warrant to carry out combined treatments to detoxify antinutrients.

CONCLUSIONS

The use of fermented JCSM with various moulds at 5% in ration has not been effective as protein sources for male mice. Combined treatments should be applied to improve JCSM utilization.

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