# Proceeding

# The 2nd International Seminar

## Feed Safety for Healthy Food

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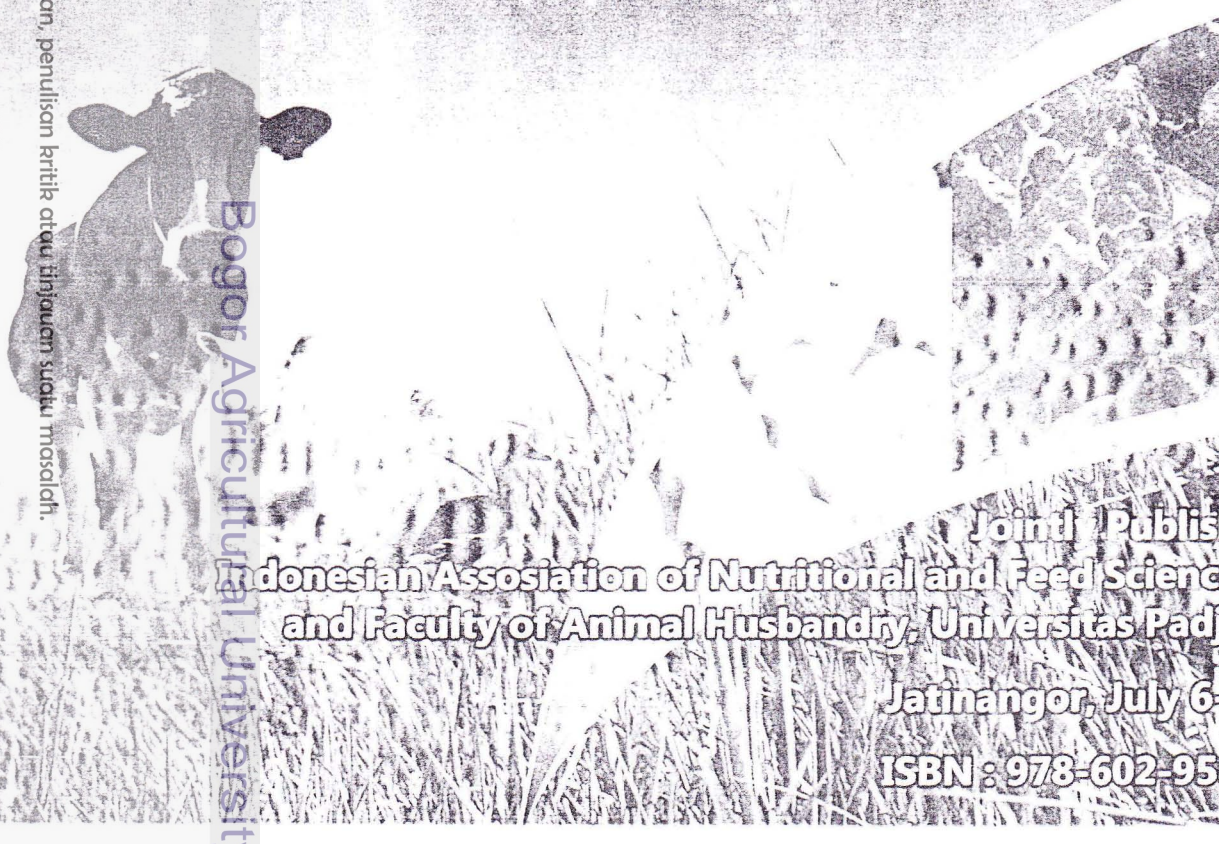

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## The 2nd International Seminar
## "Feed Safety for Healty Food"



Technical Editors :
Secretariat of The International Seminar
"Feed Safety for Healty Food"

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## The 2nd International Seminar
## "Feed Safety for Healty Food"

**Keynote Speaker :**

Director General of Animal Husbandry and Animal Health

**Main Speakers :**

Prof. Fr. Jurgen Zentek (Berlin, German)

Prof. Abdul Razak Alimon (Malaysia)

Dr. Kevin Liu (Singapore)

Prof. E. R. Ørskov, Ph D., FPAS, FRSE (Scotland)

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The 2nd International Seminar
"Feed Safety for Healty Food"



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## FOREWORD

We thank the Almighty Allah, the Most Gracious and the Most Merciful that the proceedings of the 2nd International Seminar, the 8th Biannual Meeting and 3rd Congress and Workshop of AINI with the theme "Feed Safety for Healthy Food" organized by Indonesian Association of Nutrition and Feed Science, Faculty of Animal Husbandry, Universitas Padjadjaran on 6 - 7 July 2011 have been completed.

These activities were to collect variety of scientific information with the purpose to collect scientific information about feed for a healthy food, to produce a draft policy on a national feed system and to make a scientific forum for Academics, Researchers, Practitioners of animal husbandry, Health and Policy makers. Scientific papers that were presented either in oral or poster stated in the proceedings.

Thanks go to all those who have provided both moral support or material so that this seminar can be carried out and the proceeding can be issued.

Jatinangor, 5 March 2012

**Committee**

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# THE EFFECT OF CHEMICAL AND BIOLOGICAL TREATMENTS ON WEIGHT LOSS, NUTRIENTS CONTENT, TRYPSIN INHIBITOR AND LECTIN ACTIVITIES OF *Jatropha curcas* L. MEAL

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## ABSTRACT

The *Jatropha curcas* meal is by-product of *Jatropha curcas* oil production and contain high amount of nutrient (56-68% protein). The problem in using of *Jatropha curcas* meal as feed is its antinutritive content and toxic compounds such as lectin (curcin) and trypsin inhibitor. This experiment was conducted to evaluate the effectiveness of chemical and biological methods in decreasing or eliminating lectin and trypsin inhibitor activities and to know the change of nutrients content due to processing of *Jatropha curcas* meal. This research consisted of four treatments : T0 (control, untreated meal), T1 (methanol 90% extraction, with the proportion of the meal and methanol was 1:4), T2 (fermentation using *Rhizopus oryzae*) and T3 (fermentation using *Trichoderma viride*). The parameters observed were weight loss, nutrients content, trypsin inhibitor and lectin activity of the meal. The data were analyzed with descriptive analysis. The results showed that chemical and biological treatments resulted in weight loss 6.12%(T1), 20.42%(T2) and 11.04%(T3). The fermentation using *Rhizopus oryzae* was the most effective in decreasing the activity of trypsin inhibitor (67.95%). However, this treatment decreased protein content 9.39%, increased crude fiber 35.73% and it did not influence lectin activity. The fermentation using *Trichoderma viride* was the most effective in degrading lectin activity with the value of 50% compared to the untreated meal.

*Keywords : Jatropha curcas meal, Rhizopus oryzae, Trichoderma viride, methanol*

## INTRODUCTION

*Jatropha curcas* or physic nut is a drought-resistant plant which belongs to the family *Euphorbiaceae.* It could grow well on the marginal land, and at temperature of 18°C – 30°C (Haryadi, 2005). the seed kernel of the plant contains about 60% oil that can be converted into biodiesel and used as a substitute for diesel fuel. The seed cake remaining after oil extraction is an excellent source of plant nutrients (Foidl *et al.*, 2001). Its seed cake (*Jatropha curcas* meal contains high content of protein with a well-balanced of amino acid composition according to the FAO/WHO reference pattern,

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except for lysine (Martinez-Herrera *et al.*, 2006). The defatted meal has been found to contain a high amount of protein, which ranged between 50% and 62% (Makkar *et al.* 1998a). However, availability of this rich nutrients is low due to some toxic and antinutrients content in the meal. These toxic and antinutrients include curcin(lectin), tannin, trypsin inhibitors, phytate, saponin and phorbolesters (francis *et al.*, 2006). The meal has high trypsin inhibitor and lectin activities (Makkar *et al.* 1998b). The meal also contains high fiber, i.e. 32.81% (Sumiati *et al.*, 2007).

Trypsin inhibitors interfere with the physiological process of digestion through interference with the normal functioning of pancreatic proteolytic enzymes in non-ruminants, leading to severe growth depression (white *et al.*, 1989). Lectins are non enzymatic proteins or glycoproteins that bind carbohydrates and agglutinate cells and/or precipitate glycoconjugated. The lectins are toxic to young growing animals as well as mature rats (cheeke, 1989). The phorbolesters, even at very low concentration, show toxicological manisfetations in animals fed diets containing them (Goel *et al.*, 2007).

Properly detoxified *Jatropha curcas* meal is needed to make it available for the poultry feed. Some methods could be used to eliminate the antinutriens contained in the *Jatropha curcas* meal, such as physical, chemical, and biological. The objective of this research was to study the effectiveness of JCM methanol extraction, fermentation of JCM using *Rhizopus oryzae* and *Trichoderma viride* in reducing the antinutrients content in the JCM and to study the effect of these treatments on the nutrients content of the JCM.

## MATERIALS DAN METHODS

This research consisted of four treatments : T0= control, untreated meal, T1= methanol 90% extraction, with the proportion of the meal and methanol was 1:4, T2= fermentation using *Rhizopus oryzae* , T3= fermentation using *Trichoderma viride*. The parameters observed were weight loss, nutrients content, trypsin inhibitor and lectin activity of the meal.

### *Jatropha curcas* Meal and Fungi

Jatropha curcas meal(JCM) were obtained from Indocement Cibinong, Bogor, Indonesia. Metanol 90 % was used to extract the JCM, *Rhizopus oryzae* and *Trichoderma viride* were used to ferment the JCM.

### Methanol Extraction of *Jatropha curcas* Meal

The extraction of JCM using methanol 90% was done according to modified method of Aregheore *et al.* (2003). 2 kg of JCM was mixed with 8 litre of methanol 90% (1:4 ratio/w:v). The mixture was shaked in the shakerbath during 24 hours, then the residue and filtrate were separated. The residu was further shaked during another 24 hours to maximize the JCM fat extraction. The extracted JCM was dried at 60°C during 24 hours, the JCM was ready to be analysed.

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**Jatropha curcas Fermentation Using *Rhizopus oryzae***

This fermentation was conducted according to modified method of Rotib (1990). 493 ml of distilled water was added to 300 g of JCM to reach the water content of JCM about 66% (optimum water content for fungi growth). The mixture was autoclaved at 121 °C for 15 min. The autoclaved meal was allowed to be cooled before inoculating with the fungi. The meal was then inoculated with 14 g of *Rhizopus oryzae* starter. The inoculated substrate was wraped using plastic and incubated at room temperature for 4 days. After 24 hours incubation, the whole plastic wrap was sticked using a small needle. The growth of the fungi was terminated by oven drying the substrate at 60°C for 48 hours.

***Jatropha curcas* Fermentation Using *Trichoderma viride***

This fermentation was conducted according to modified method of Rotib (1990). 150 g of *Jatropha curcas* meal was mixed with distilled water to make the water content of the meal approximately 57%. The mixture was autoclaved at 121 °C for 15 min. The autoclaved meal was allowed to be cooled before inoculating with the fungi. The meal was then inoculated with *Trichoderma viride* culture diluted in 8 ml distilled water. The inoculated substrate was wraped using plastic and incubated at room temperature for 4 days. After 24 hours incubation, the whole plastic wrap was sticked using a small needle . The growth of the fungi was terminated by oven drying the substrate at 60°C for 48 hours.

**Nutrients and Antinutrients Analyses**

Nutrients content were analysed according to method of AOAC (1990). Lectin activity was analysed using the method after Aderibigbe *et al.* (1997). trypsin inhibitor activity was analysed using the method of Smith *et al.* (1980). The data obtained were analysed descriptively.

## RESULTS AND DISCUSSION

**The Effect of Treatments on Weight Loss of *Jatropha curcas* meal**

The chemical as well as biological treatments decreased the weight of *Jatropha curcas* meal (Table 1). The fermentation using *R. oryzae* (T2) resulted in highest weight loss of the meal with the value of 20.42%. The decreasing of the meal weight could be due to *Jatropha curcas* meal nutrients used by the fungi during their growth. The weight loss of the meal yielded by methanol extraction treatment (T1) was 6.12% and that of due to *Rhizopus oryzae* fermentation was 11.04%.

**The Effect of Treatments on Nutrients Content of *Jatropha curcas* Meal**

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Chemical as well as biological treatments increased dry matter and ash content compared to the untreated meal (Table 2). The increasing of dry matter was range from 3.13% (T1) to 7.51% (T2), and that of ash from 5.65% (T3) -6.87% (T1). Extraction of JCM using methanol 90%(T1) and fermentation of JCM using *Trichoderma viride* (T3) increased protein content 27% and 14%, respectively, while fermentation of JCM using *R.oryzae* decreased protein content 9.4%. Increasing of crude protein of methanol extracted JCM was due to decreasing nitrogen free extract (NFE) leached during exctraction. Aregheore *et al.* (2003) reported that crude protein increasing because of loosing of NFE fraction during the treatment. Decreasing of protein in fermented JCM using *R.oryzae* (T2) could be the usage of protein by the fungi. Wang *et al.* (1979) reported that fermentation process could decrease the substrate protein because of proteolitic process or it increased the protein due to fungi protein synthesis from dry matter degradation.

Chemical and biological treatments also decreased the ether extract of the JCM. The highest decreasing was obtained by the *Rhizopus oryzae* fermentation treatment (T2), i.e. 93.81% compared to that of the untreated meal. *R. Oryzae* secreted lypase enzyme to degrade triacylglicerol become glicerols and fatty acids. Tillman *et al.* (1989) reported that fat could be hydrolysed by the lypase to yield mono-and diglicerides and free fatty acids. Decreasing ether extract content of extracted methanol JCM (T1) because of methanol action in dissolved the fat fraction, and hopefully the phorbolesters would be eliminated from the meal, because this toxin is part of the meal fat. The phorbolesters are moderately polar, and methanol has major affinity for them (Martinez-Herrera *et al.*, 2006).

Chemical and biological treatments elevated the crude fiber content of the JCM and the highest increasing was obtained by the *Trichoderma viride* fermentation treatment (T3). The increasing of crude fiber content of the JCM was from 20.4% (T1) to 37.5 % (T3) compared to the untreated meal. It could be due to lower cellulase enzyme activity of the fungi because of high content of lignin in the *Trichoderma viride* fermented JCM (Van Soest analyses, Table 3). The lignin inhibit the fungi to degrade the cellulose of this JCM. Volk (2004) reported that *T. viride* could degrade the cellulose, but not the lignin.

**Effect of the Treatments on Trypsin Inhibitor Activity of *Jatropha curcas* Meal**

The treatments eliminated trypsin inhibitors activities (TIA) 33.05% (T1), 62.9% (T2), and 32.3% (T3) (Table 2). The *Rhizopus oryzae* fermentation treatment resulted in highest decreasing of TIA. The trypsin inhibitors were easily inactivated by moist heating at 121oC for 25 minutes (Martinez-Herrera et al., 2006). Trypsin inhibitors were heat-labile (Aderibigbe *et al.*, 1997). Beside the heating factor, the *Rhyzopus oryzae* secreted proteases to breakdown the trypsin inhibitors complex.

**Effect of the Treatments on Lectin Activity of *Jatropha curcas* Meal**

Lectin is generally considered to be another toxic factor in Jatropha curcas meal (Panigrahi *et al.*, 1984). Lectin in the body caused blood agglutination (Makkar and Becker, 1997). Lectin activity is a minimum amount of the sample required to show the agglutination. This reseach showed that lectin activity occured at 30 minutes

observation (Table 4). The fermented JCM using *T.viride* (T3) needed highest amount of the meal (50 mg/ml) to agglutinate the blood. It means that the lectin activities of that meal was the lowest. This treatment was effective to reduce lectin activity of the JCM. The lectin activity of this meal (Indonesian JCM) was lower than that of the defated Jatropha curcas from Mexico observed by Martinez-Herrera *et al.* (2006), i.e. 0.35 to 1.46 mg/ml).

## CONCLUSION

Methanol 90% extraction of *Jatropha curcas* meal increased protein content 27%, but it decreased NFE and gross energy of the meal. The fermentation using *Rhizopus oryzae* was the most effective in decreasing the activity of trypsin inhibitor (67.95%) and crude fat of Jatropha curcas meal (93.81%), and hopefully it could decrease the phorbolesters of the meal. However, this treatment decreased protein content 9.39%, increased crude fiber 35.73% and it did not influence the lectin activity. The fermentation using *Trichoderma viride* was the most effective in degrading lectin activity with the value of 50% compared to the untreated meal, but it increased the lignin content in the highest value.

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Table 1. Weight Loss of Untreated and Treated *Jatropha curcas* Meal

| Treatment | Before treatment weight (g) | After treatment weight (g) | % Weight loss |
|---|---|---|---|
| T0 | 1000 | 1000 | 0 |
| T1 | 1000 | 968,18 | 6.12 |
| T2 | 1000 | 855,56 | 20.42 |
| T3 | 1000 | 936 | 11.04 |

T0= control, untreated JCM, T1=methanol 90% extraction, with the proportion of the JCM and methanol was 1:4, T2= fermentation JCM using *Rhizopus oryzae*), T3= fermentation JCM using *Trichoderma viride*

Table 2. Nutrients Content and Trypsin Inhibitor Activity (TIA) of Untreated and Treated *Jatropha curcas* Meal

| Component | T0 | T1 | T2 | T3 |
|---|---|---|---|---|
| | ----------(%)---------- | | | |
| Dry matter | 87.44 | 90.18 | 94.01 | 92.00 |
| Ash | 5.63 | 6.87 | 5.95 | 5.65 |
| Organic matter | 94.37 | 93.13 | 94.05 | 94.35 |
| Crude protein | 24.71 | 31.41 | 22.39 | 28.18 |
| Ether extract | 5.8 | 4.29 | 0.39 | 1.35 |
| Crude fiber | 32.58 | 39.23 | 44.22 | 44.80 |
| NFE | 19.41 | 10.22 | 21.06 | 12.02 |
| Ca | 1.00 | 0.71 | 0.68 | 0.99 |
| P | 0.99 | 0.89 | 0.35 | 0.55 |
| Gross Energy (kcal/kg ) | 3893 | 3852 | 3948 | 3952 |
| TIA*(mg/g fat free sample) | 23.75 | 15.9 | 7.612 | 16.075 |

T0= control, untreated JCM, T1=methanol 90% extraction, with the proportion of the JCM and methanol was 1:4, T2= fermentation JCM using *Rhizopus oryzae*), T3= fermentation JCM using *Trichoderma viride*; * TIA = trypsin inhibitor activity

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Table 3. Van Soest Analyses of Untreated and Treated *Jatropha curcas* Meal

| Component | T0 | T1 | T2 | T3 |
|---|---|---|---|---|
| NDF(%) | 76,22 | 76,67 | 68,99 | 75,11 |
| ADF(%) | 56,11 | 42,31 | 42,04 | 63,59 |
| Hemicellulose (%) | 20,11 | 34,36 | 26,95 | 11,52 |
| Cellulose (%) | 20,60 | 16,22 | 14,38 | 12,28 |
| Lignin (%) | 35,08 | 25,80 | 27,44 | 51,01 |
| Silica (%) | 0,40 | 0,23 | 0,18 | 0,23 |

T0= control, untreated JCM, T1=methanol 90% extraction, with the proportion of the JCM and methanol was 1:4, T2= fermentation JCM using *Rhizopus oryzae*), T3= fermentation JCM using *Trichoderma viride* ; NDF (Neutral Detergen Fiber), ADF (Acid Detergen Fiber).

Table 4. Lectin Activity (mg/ml) of Untreated and Treated *Jatropha curcas* Meal

| Observation Time (min) | T0 | T1 | T2 | T3 |
|---|---|---|---|---|
| 0 | - | - | - | - |
| 30 | 25 | 25 | 25 | 50 |
| 60 | 6,25 | 12,5 | 6,25 | 12,5 |
| 90 | ND | ND | ND | ND |
| 120 | ND | ND | ND | ND |

T0= control, untreated JCM, T1=methanol 90% extraction, with the proportion of the JCM and methanol was 1:4, T2= fermentation JCM using *Rhizopus oryzae*), T3= fermentation JCM using *Trichoderma viride* ;ND = not detected