



Proceeding

The 2nd International Seminar

Feed Safety for Healthy Food

AINI publication No. 01/2012

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The 2nd International Seminar “Feed Safety for Healty Food”

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



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Bogor Agricultural University

Keynote Speaker :

Director General of Animal Husbandry and Animal Health

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Prof. Abdul Razak Alimon (Malaysia)

Dr. Kevin Liu (Singapore)

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

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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 2nd International Seminar
“Feed Safety for Healty Food”

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TOXIC DOSE METHANOL EXTRACT AND RESIDUE OF *Jatropha curcas* L. MEAL ON MICE (*Mus musculus*)

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ABSTRACT

The *Jatropha curcas* meal is waste product of *Jatropha curcas* oil production. It contains high amount of nutrient (56% - 68% crude protein) which can be used as an alternative feed source. However, *Jatropha curcas* meal content high anti nutritive compound such as phorbol ester and lectin (curcin), which can be used as animal poison. The extraction method was done to get the *Jatropha* anti nutrition in high concentration. Methanol solvent was used as lipid and water extractor substance, including phorbol ester which dissolved in fat. This experiment designed in two steps with twenty five males of mice (av. 19.15 ± 3.03 g BW). Treatments in the first experiment were control (diet without *Jatropha*); R-10 (diet contained 10% residue of methanol extract of *Jatropha*); R-20 (diet contained 20% residue of methanol extract of *Jatropha*); E-5 (5% filtrate methanol extract of *Jatropha* in drinking water) and EF-5 (force drinking of 5% filtrate methanol extract of *Jatropha*). The treatment continued in the second period, same with the first experiment, except the E-5 was replaced by E-10 (10% filtrate methanol extract in drinking water). This dose (5% and 10% of filtrate) were based on total body water. The parameter observed were feed consumption, body weight, mortality, blood profile and histopathology. All data were analyzed descriptively. The results of the first experiment showed that there were decreasing of feed intake and body weight of mice fed with R-10, R-20 and E-5. Treatment with EF-5 resulted 100% mortality in the first day of treatment due to phorbol ester, which was indicated by the damage of liver and spleen cells. The result of the second experiment showed that there were decreasing of feed consumption and body weight in all treatments drastically. The treatment R-20 resulted 80% mortality on sixth days, followed by treatment E-10 on ninth days, due to the low erythrocyte and leucocyte number below the normal value. Treatment of R-10 has decreased of PCV even though they were still alive. It is concluded that dose of *Jatropha* methanol extract for mice toxicity were 20% residue in the ration or 10% filtrate in water drinking, or 5% filtrate by force drinking.

Keywords: *Jatropha curcas* meal, mice, methanol extract.

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INTRODUCTION

The *Jatropha curcas* meal is waste product of *Jatropha curcas* oil production and contains high amount of nutrient (32% - 42 % crude protein) which can be used as an alternative protein source for soybean meal replacement. Staubmann et al. (1997) reported that from *Jatropha curcas* oil production results more than 50% of meal *Jatropha curcas*. However, *Jatropha curcas* meal content high anti nutritive compound such as phorbol ester and lectin (curcin), which can be used as animal poison. Makkar et al. (1997) reported that phorbol ester and lectin (curcin) are main anti nutrition which is high toxic concentration. The extraction method was done to get the *Jatropha curcas* anti nutrition in high concentration in the filtrate. Methanol solvent was used as lipid and water extractor substance, including phorbol ester which dissolved in fat.

In Indonesia, the potential of *Jatropha curcas* plantation is very wide, from west to east Indonesia, as presented in Table 1. The most potential of seed production is in Nusa Tenggara islands while the highest plant production is in Sumbawa island.

Table 1. Potential of *Jatropha curcas* production in Indonesia

Region	Area (ha)	Plant Production (000)	Seed production X 10 ⁶ ton/year
West Java	3374	8435	759.15
East Java	3465	8663	779.74
Nusa Tenggara	2677	6692	60250
Sumbawa	15000	37500	3375
Kalimantan	10025	25062	2255
Sulawesi	3000	7500	675

Source : Departemen Pertanian (2008)

Permana (2007) reported that there are a different nutrient content of *Jatropha curcas* L. in some region, like in Kebumen, Lampung and East Lombok. Table 2. shows the nutrient composition of *Jatropha curcas* (seed) in some region in Indonesia.

Table 2. Chemical composition of *Jatropha curcas* from difference regions

Nutrient (%)	Lampung	Kebumen	East Lombok
Dry matter	93.19	93.24	94.10
Crude protein	42.58	37.93	32.64
Crude Fiber	20.52	12.97	6.58
Extract ether	13.82	22.38	29.62
Ash	7.31	7.01	6.78
Gross Energy(Kal/kg)	5062	4713	4915

Anti nutrient and toxic content of *Jatropha curcas* L. meal produced from Lampung, Kebumen and East Lombok were 7.39%, 6.65% and 7% for phytic acid; 0.72%, 0.70% and 0.63% for curcin and trace, 0.99 and 1.33 mg/g sample for phorbol ester, respectively (Triastuty, 2007).

Mice as animal model for evaluation toxic dose, usually uses in many studies. Toxic is defined a compound which can disturb of metabolism and healthy, even though will cause tissue damage and following with deadly animal. Lethal dose (LD) is a such

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dose of poison which given to animal (object) and will cause the animal die suddenly. LD₅₀ or LD₁₀₀ is a such dose of poison or toxic which can kill 50% or 100% of total animal population. Utilization of 40% and 50% of *Jatropha curcas* meal in mice diet caused 87% and 67% mortality in 3rd – 16th day, while in rat with 37% of *Jatropha curcas* meal in their diet caused 100% died in 3rd day (Makkar and Becker, 1997).

Phorbol ester is organic compound named 12-O-tetradecanophorbol-13-acetate (TPA) solved in water and organic solvent (polar), resistant to the high boiling temperature (>160°C) and can be extracted from *Jatropha curcas* by methanol solvent (Hecker et al., 1967; Rug et al., 2006). Curcin or lectin is phytotoxin within high toxicity, called specific non-immunoglobulin protein which can bind complex carbohydrate (Heller, 1996). Curcin have specific action to coagulate of red blood cell in all animal species (Cheeke, 1989).

Hematology status is very importance to evaluate physiological state of animal condition. Hematology status of mice has 7.7 – 12.5 (X 10⁶/mm³) of erythrocyte, 6-12.6 (X 10³/mm³) of leucocyte, 13-16 g% of hemoglobin, 41%-48% of PCV, 55% - 85% of lymphocyte and 12% -30% of netrophil (Smith and Mangkoewidjojo, 1988).

This research was to know the exact toxic dose of filtrate and residue of methanol extract *Jatropha curcas* L. meal on mice. The product can be elaborate for pesticide poison.

MATERIAL AND METHOD

Twenty five mice male (BW 19.15 ± 3.03 g), were used for up to one month. The animals were allotted into five treatments. The diet offered were mash and based on Nutrient Requirements of Laboratory Animals (1995). The treatments containing methanol extract *Jatropha curcas* L., either as residue and also filtrate. The treatments were control (diet without *Jatropha*); R-10 (diet contained 10% residue of methanol extract of *Jatropha*); R-20 (diet contained 20% residue of methanol extract of *Jatropha*); E-5 (5% filtrate methanol extract of *Jatropha* in drinking water) and EF-5 (force drinking of 5% filtrate methanol extract of *Jatropha*) for the first two weeks experiment and continued with the second period, same with the first experiment, except the E-5 was replaced with E-10 (10% filtrate methanol extract in drinking water). The dose of filtrate methanol extract in drinking water was measured from total animal body water (body water is around 70% of body weight). Composition of diet is presented in Table 3. Concentration of phorbol ester and curcin in *Jatropha curcas* L. meal were 1.33 mg/g and 0.63%, respectively (Permana et al., 2007). A one week feed control (mash) adaptation period for animals was allowed before feeding trial in the individual cages.

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Table 3. Nutrient composition of diets for experimental mice

Nutrient	C	R10	R20	E5	EF5
Dry matter (% of feed)	87.88	88.18	88.34	87.88	87.88
Crude protein (% DM)	22.32	20.70	21.59	22.32	22.32
Crude fiber (% DM)	1.73	4.94	7.80	1.73	1.73
Fat (% DM)	1.72	1.76	1.97	1.72	1.72
N-free extract (% DM)	71.59	58.19	54.19	71.59	71.59
Gross energy (Kal/kg)	4497	3599	3299	4497	4497

C= control diet; R10= diet contained 10% residue of methanol extract of *Jatropha*; R20 = diet contained 20% residue of methanol extract of *Jatropha*; E5 = 5% filtrate methanol extract of *Jatropha* in drinking water; EF5 (10) = force drinking of 5% filtrate methanol extract of *Jatropha*.

Data on dry matter (DM) intake was obtained from the difference between feed offered and the remainder, while nutrient intake were calculated from the DM intake times nutrient content of diet. Animals were weighed once a week and their body weight was obtained. Percentage of mortality was calculated from animal death divided by total animal times 100%. Lethal dose was defined by the concentration of toxic dose of residue and filtrate given which can cause the death animal. Blood profile (erythrocyte, PCV, hemoglobine and leucocyte) were measured as described (Sastradipradja et al., 1989). Histopathology of liver and spleen organs was observed at Histopathology Laboratory, Faculty of Veterinary and Medicine IPB. All data were analyzed descriptively.

RESULT AND DISCUSSION

Dry matter intake of mice treated with filtrate and residue methanol extract of *Jatropha curcas* meal in different levels during 2 weeks observation were around 2 – 3 g/head/d, means equal with 10% of BW. Diet containing 10% residue (R10) and 5% filtrate of methanol extract *Jatropha curcas* in drinking water (E5) caused reducing dry matter intake until 14.61% and 31.56% lower than control, respectively. Treatment of 5% filtrate methanol extract by force drinking resulted 100% death animal in a few hours after treatment. High concentration of phorbol ester in filtrate methanol extract *Jatropha curcas* as force drinking caused histamine releasing and following with hemolysis and increasing number of leucocyte which caused trans-endothelial cell migration in same organs cell. On the other hand, phorbol ester cause releasing of protease and cytokine, and also increase of NADPH oxidase activation, so that all of the reactions above cause damage some tissues and further death animal (Gunjan et al., 2007).

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Tabel 4. Dry matter and nutrient intake (g/d) of mice with different treatments at the first periode (0-2nd week observations)

Parameters	C	R10	R20	E5	EF5
Dry matter	3.01 ± 1.01	2.92 ± 0.96	3.42 ± 1.06	2.06 ± 0.76	ND
Crude protein	0.67 ± 0.20	0.53 ± 0.17	0.65 ± 0.23	0.50 ± 0.17	ND
Fat	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.02	0.04 ± 0.01	ND
Crude fiber	0.05 ± 0.01	0.13 ± 0.04	0.24 ± 0.08	0.04 ± 0.01	ND
N-free extract	2.16 ± 0.64	1.49 ± 0.49	1.64 ± 0.57	1.59 ± 0.55	ND
Energy (kal/d)	135 ± 40	92 ± 30	99 ± 34	100 ± 34	ND
Phorbol ester (mg/d)	-	0.34 ± 0.11	0.80 ± 0.28	undetected	
Curcin (ug/d)	-	115.53 ± 38	271.44 ± 95	undetected	

C= control diet; R10= diet contained 10% residue of methanol extract of *Jatropha*; R20 = diet contained 20% residue of methanol extract of *Jatropha*; E5 = 5% filtrate methanol extract of *Jatropha* in drinking water; EF5 = force drinking of 5% filtrate methanol extract of *Jatropha*. ND = no data (animal was die)

In general, dry matter and nutrients intake of mice in the second period (2nd – 4th weeks) showed decreasing and become lower than in the first period. The longer animal consume phorbol ester and curcin, the more toxic they can get. In this research, mice consumed 0.34 (R10) and 0.80 mg/g (R20) phorbol ester, while curcin were consumed around 115 and 271 ug/d. Aregheore et al (2003) reported that the safety dose of phorbol ester in the diet is 0.014 mg/g. Rat fed with 16% of *Jatropha curcas* meal, contained 0.13 mg/g phorbol ester caused decreasing of feed consumption and body weight. Sumiati et al (2007) reported that 5% (with 2.33 mg curcin), 10% (with 1.72 mg curcin) and 15% (with 0.42 mg curcin) of *Jatropha curcas* meal in the broiler ration had LD₅₀ in the 28th, 14th and 7th day, respectively. Lin et al. (2003) reported that curcin can bind glucoprotein of cell which caused death cell. Curcin also can resist protein synthesis in the ribosome.

 Table 5. Dry matter and nutrient intake (g/d) of mice with different treatments at the second period (2nd – 4th week observations)

Parameters	C	R10	R20	E10
Dry matter	2.04 ± 0.92	2.32 ± 1.45	2.07 ± 1.85	1.79 ± 0.73
Crude protein	0.45 ± 0.14	0.48 ± 0.31	0.45 ± 0.39	0.40 ± 0.16
Fat	0.04 ± 0.01	0.04 ± 0.02	0.04 ± 0.02	0.03 ± 0.01
Crude fiber	0.04 ± 0.01	0.13 ± 0.04	0.16 ± 0.04	0.03 ± 0.01
N-free extract	1.45 ± 0.46	1.35 ± 0.87	1.12 ± 0.99	1.28 ± 0.52
Energy (kal/d)	91 ± 28	83 ± 53	68 ± 6	80 ± 32
Phorbol ester (mg/d)	-	0.30 ± 0.19	0.55 ± 0.49	undetected
Curcin (ug/d)	-	104 ± 58	186 ± 116	undetected

C= control diet; R10= diet contained 10% residue of methanol extract of *Jatropha*; R20 = diet contained 20% residue of methanol extract of *Jatropha*; E10 = 10% filtrate methanol extract of *Jatropha* in drinking water.

Performance of mice fed by residue and filtrate methanol extract *Jatropha curcas* during 4 weeks showed that there were losing of body weight dramatically. Average daily gain in R20 (both in first and second period) were the worst compare to other treatments. In the first two weeks experiment, there were no mortality except in

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force drinking treatment. In the second round of feeding toxicity showed that R20 and E10 had 80% mortalities in 6th and 9th day feeding trial, while the deadly in the control was happened by accident at day 12th caused by trapped of the cage.

Table 6. Performance of mice with different treatments at the first periode (0-2nd week observations)

Parameters	C	R10	R20	E5	EF5
Initial BW (g)	19.81 ± 2.37	21.07 ± 2.24	19.36 ± 3.61	19.10 ± 2.85	17.01 ± 3.87
Final BW (g)	21.37 ± 3.03	20.38 ± 2.88	17.14 ± 2.14	17.31 ± 2.15	-
ADG (g/d)	0.11 ± 1.25	-0.05 ± 0.23	-0.16 ± 0.14	-0.13 ± 0.14	-
Mortality (%)	0	0	0	0	100

C= control diet; R10= diet contained 10% residue of methanol extract of *Jatropha*; R20 = diet contained 20% residue of methanol extract of *Jatropha*; E5 = 5% filtrate methanol extract of *Jatropha* in drinking water; EF5 = force drinking of 5% filtrate methanol extract of *Jatropha*.

Table 7. Performance of mice with different treatments at the second periode (2nd - 4th week observations)

Parameters	C	R10	R20	E10
Initial BW (g)	23.49 ± 3.03	20.38 ± 2.88	17.14 ± 2.14	17.31 ± 2.15
Final BW (g)	19.25 ± 0.77	16.75 ± 3.36	14.71 ± 1.83	12.88 ± 1.66
ADG (g/d)	-0.30 ± 0.04	-0.25 ± 0.05	-0.47 ± 0.29	-0.45 ± 0.32
Mortality (%)	60*	0	80	80

C= control diet; R10= diet contained 10% residue of methanol extract of *Jatropha*; R20 = diet contained 20% residue of methanol extract of *Jatropha*; E10 = 10 % filtrate methanol extract of *Jatropha* in drinking water; * (by accident at day 12th).

Blood profile is presented in Table 8. Result showed that diet containing residue and filtrate methanol extract of *Jatropha curcas* were lower than control treatment and normal condition of living mice. The worst hematology status was happened in treatment E10, which is the animal has no immunity anymore ($0.70 \times 10^3/\text{mm}^3$). It is said that dose of 10% filtrate methanol extract *Jatropha curcas* in the drinking water and 5 % filtrate by force drinking, to become toxic dose for mice. The toxic dose of residue methanol extract *Jatropha curcas* is 20% in diet, which cause damage of liver and spleen organs.

Table 8. Blood profile of mice with different treatments at the second periode (2nd - 4th week observations)

Parameters	C	R10	R20	E10	Normal*
Erythrocyte ($10^6/\text{mm}^3$)	10.39	7.44	7.29	3.65	7 - 12
Hemoglobine (g %)	13.40	9.60	12.00	11.00	13 - 16
Pack Cell Volume (%)	39.50	23.25	28.00	33.50	41 - 48
Leucocyte ($10^3/\text{mm}^3$)	8.05	4.35	6.40	0.70	6 - 12

C= control diet; R10= diet contained 10% residue of methanol extract of *Jatropha*; R20 = diet contained 20% residue of methanol extract of *Jatropha*; E10 = 10 % filtrate methanol extract of *Jatropha* in drinking water; * Smith and Mangkoewidjojo (1988)

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Histopathology profile of liver and spleen mice treated by residue and filtrate methanol extract of *Jatropha curcas* showed there were degenerative epithelium liver cell and tubuli spleen. Liver is special organ that can metabolize all nutrients which are absorbed by intestine, while spleen is organ which can excrete all metabolites excess. Those two organs are very important to the body, so that if those organs are damage, it will make disorder metabolism and at the end, the animal will die. Both toxics, phorbol ester and curcin in such dose intake in this research caused some oedema and infiltration of lymphocyte cell into liver and spleen which is the main causing of death animal.

Table 1. Histopathology profile of mice liver and spleen with different treatments

Treatments	Liver	Spleen
R20	-Wide sinusoids - lymphocyte cell Infiltration multifocus -Necrosis	- Oedema -Degenerative epithelium tubuli
E5	- Degenerative liver cell - lymphocyte cell Infiltration -Sinusoid oedema	-Oedema - Degenerative and fatty in tubuli -Dilatation in spleen tubuli
E10	- Degenerative liver cell -Sinusoid widely -Oedema	- Degenerative epithelium tubuli - Oedema

R20 = diet contained 20% residue of methanol extract of *Jatropha*; E5 (10) = 5 % (10%) filtrate methanol extract of *Jatropha* in drinking water;

CONCLUSION

It was concluded that toxic dose of *Jatropha curcas* L, from heavy to light dose, were 5% (of total body water) filtrate methanol extract by force drinking which caused 100% death, followed by 10 % filtrate in drinking water, 20% of residue in the diet and 10% of residue in the diet, respectively.

Death animal was happened because of high concentration of phorbol ester and curcin in the filtrate of methanol extract of *Jatropha curcas* L., and such amount in residue. The histopathology profile showed that there were many degenerative and infiltration in liver and spleen cells, followed by decreasing of number blood profile.

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