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Detoxification Through Fermentation By Consortium Of Aspergillus Niger And Neurospora Sitophila Towards The Degree Of Forbol Esther And Nutrition Value Of Jatropha Curcas L. For Broiler's Feed

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Abstract

Seedcake of Jatropha curcas L seed is a by product of biodiesel processing. Hard fibers and tiny raw protien is potentially useful as alternative feed for Broilers. So far, the use of Jatropha as feed is restricted due to the toxin of forbo loster. To increase the quality of seedcake, detoxification through biological fermentation was carried out. The research is aimed at achieving the followings: (1) obtaining the best product of the seed of Jatropha curcas L fermented by the consortium of Aspergillus niger ; (2) obtaining the lowest degree of anti-forbol esther nutrition as the result of the first stage research., and; (3) deciding on the quality of fermented seed of Jatropha curcas L by measuring metabolical energy. The degree of forbol esthered was measured at the laboratory of Psycho-Chemistry of Bandung Institute of Technology. The experiment employed experimental design, that is, Complete Randomization Factorial design (3 X 3). The three A-factor (dosage of consortium inoculum of Aspergillus niger and Neurospora sitophila is d1 = 2 g, d2 = 3 g, d3 = 4g) and the three B- factor (the time spent for fermentation of Aspergillus niger and Neurospora sitophila is t1 = 72 hours, t2 = 96 hours, nd t3 = 120 hours). This was repeated as much as 3 times. Data analysis was carried out by using variant analysis technique. To identify the diference between the treatments, Duncan Multiple Distance Test was employed. The research reveals that Jatropha curcas L fermented by consortium of Aspergillus niger and Neurospora sitophila with the dosage: 3 g inoculum and 96 hours of fermentation could best increase the nutrition value, that is, increasing Crude Protein as much as 16.88%, decreasing raw fat as much as 63.89%, and decreasing raw fiber as much as 14.96%. The highest decrease of forbol esther was achieved by substrate fermented by Aspergillus niger and Neurospora sitophila with the dosage of inoculum: 3 g and 3 hours of fermentation, that is, 79,69% with metabolical energy as much as 3849 kkal/kg.

Key words: Nutrition, Forbol esther, Jatropha curcas L, Fermentation, Aspergillus niger and Neurospora sitophila

Introduction

The seedcake of Jatropha curcas L seed contains crude fiber as much as 8% – 9% and Crude Protein as much as 56% - 64%. Therefore, it can be potentially used for alternative feeds (meals) for chicken, more specifically Broiler. However, its use for chicken's feed leaves some dilemmas since it contains anti-nutrition (Makkar et al., 1997).
The most toxic compound is fobol esther that can kill a cattle. Therefore, the first way to

develop an added value of seedcake of Jatropha curcas L is by detoxifying forbol esther compounds. One of the alternatives to manage organic wastes is employing the technique of fermantation using bioprocess from microorganism that can increase the quality of feed (Djunaidi et al.., 2002)

The role of microorganism in the process of fermentation is quite central. It can determine whether or not the fermentation will be successful (Said, 1987). Therefore, fermentation stipulates that the microorganism have a good quality. Molds and yeasts are good microorganisms that can be used for fermenting agriculture waste and agricultural industry waste (Ringpfeili, et al., 1987).

Molds that can be used for fermentation is Asperigillus niger and Neurospora sitophila . Asperigillus niger produces amylase enzym, protease and phitae extraceluller; meanwhile, Neurospora sitophila produces amylase enzym, protease, and lipase.

Neurospora sitophila can produce protease enzym that can change protein into a digestable amino acid, high lipase enzym can change fat or gliserida to digestable fat free-acid. In addition to this, according to Edi (1989), Neurospora sitophila , during the fermentation process can reduce aflatoxin up to 77%.

Based on the explanation above, it is important that biological detoxification for antinutrition substance of forbol esther be reduced or decreased to increase the nutrition value of seedcake of Jatropha curcas L seed through fermentation. It is expected that seedcake of Jatropha curcas L seed can be used as a composite to make feed for Broiler, through biological research by determining metabolical energy.

Literature Review

Jatropha curcas L.

According to Prihandana (2007), Jatropha curcas L fruit consits of flesh, cover seed, and core seed. The core, through pre extraction process, is a part of fruit that can produce oil for biodiesel fuel. In addition, according to Hambali (2006) Jatropha curcas L can produce (7.5 – 10) ton/ha/year depending on the quality of grain, soil fertility, and farming. To reduce antinutrition compound can be carried out by using an organic dissolver, such as cattleroleum-eter and hexas-ethanol or an organoc dissolver combined with cleansing (Aderibgbe etal, 1997; Aregheore et al., 2003; Chivandi et al., 2004; Martinez et al., 2006).

Detoxification Seedcake of Jatropha curcas L seed

According to Makkar et al.. (1997), detoxification of seedcake of Jatropha curcas L seed has occured differently , either through physical, chemical, or biological process. Phisycal detoxification is done through heating. Chemical detoxification is done by purifying 92% methanol as much as 4 times followed by heating by reducing forbol esther up to 0.09 mg/g which can not be tolerated by cattle.

Forbol Esther

The highest concentration of forbol esther is contained by Jatropha curcas L. it is relatively stable from heating and can maintain its stability up to 30 minutes at high temperature, 160 degree C. However, the concentration of phorbolesther can be reduced by chemical treatment (that is, adding NaOH and NaOCL).

Fermentation

At fermentation process, a microba needs to take amount of energy to grow and to develop. This can be gained through changing substance of food in substrate. A chemical change in substrate is caused by enzym activity which is produced by the microba. The change covers complex molecules such as carbohydrate, protein that changes into more simple and digestable molecules (Winarno, 1983).

Fermentation can improve characteristics of basic substances, such as enhancing digestibilty , reducing toxin compounds, and creating preferable smells (Prescott and Dunn, 1959; Shurtleff and Aoyagi, 1979).

Aspergillus niger

This mold is aerobical in nature. The mold colony that has produced spora commonly has black colour, with phH more or less 2.8 – 8.8 and humidity 80% - 90%. It belongs to

mesofilic microba which can optimally grow at 35 degree C – 37 degree C.

The mold has a good amilolitical and proteolitical ability that can produce extracelluler phitase enzym (Coneely, 1992). The product of fermentation can be used as resource for potential energy (Abun, 2003). When mold is growing, it is directly related to substance of food which is available in substrate, simple molecules surrounded by hipha can be directly absorped; meanwhile, more complex molecules should be splitted before they can be absorped into cells by producing several extraceluller enzym.

Neurospora sitophilla

Neurospora sitophilla can produce several intraceluller enzyms. Some enzyms, then, is exresed through cell walls that can serve as extraceluller out side cell (Pelczar and Chan, 1986). The enzyms are celullase, xinase, amylase, pectinase, and proteae (Irawadi, 1991).

Crude Protein

Crude Protein is an organic substance containing carbon, hydrogen, nitrogen, oxigen, sulphure, and phosphore. In our body, protein repairs net , builds essential enzyms for normal body condition, and particular hormones.

Enzymes are essential for normal function of body, and certain hormones. Protein is essential in life because this substance constitutes active protoplasm in overall cell of life. Protein is built up from molecular unity which is intertwined each other termed amino acid. In the course of fermentation, the content of protein increases it is caused by increasing protein from the microbe body on its growth.

Hard Fiber

Crude fiber constitutes prime component that contains much energy for sea-shell, so that some portions of fraction of crude fiber are used as energy resources for the growth of sea-shell. As a consequence there is reduction of contain of crude fiber on the substratum (Cain, 1980).

Crude Fat

Crude fat is a group of substances which is not dissolved into water but it is dissolved into ether, chloroform, and benzene. Over a period of metabolism, fat results 2. 25 much bigger energy than carbohydrate (Anggorodi, 1979). According to Shurtleff and Aoyagi (1979), the shift happened along fermentation can occur on the fat of substrate , neutral fat gets hydrolyzed to be immune fat acid and used to the growth of sea-shell so that fat content in the substrate will reduce.

Methodology

Fermentation of Seedcake of Jatropha curcas L seed

The first step, seedcake of Jatropha curcas L seed, is sterilized using autoclave on the temperature 121^0C with pressure 1 atm for 15 minutes. After being sterilized the temperature is lowered up to 30-35^0C. Then, it is inoculated by mixture inoculums 2, 3, and 4. Then, it is incubated in the fermentation room on the temperature 30^0C with durations: 72 hours, 96 hours, and 120 hours. This activity was carried out as much as three times.

The second step, with experimental nature is experimental design applying complete randomization design (RAL). To see the impact of treatment toward variable observed, it is analyzed based on the method of variance types which is continued by the test of Duncan multiple spaces.

The test was carried out on a room temperature with current rate 1mL/minute and wave length 280 nm. The content of forbol esther can be seen by comparing width of sample area with width of forbol di-acetate standard area which has been known their concentration.

The third method, the cattle used in the experiment is Broiler final stock strain Cobb with the age of 35 days and the number of 15 chickens, with pre average weight 1. 23 kg/chicken and variant coefficient 2. 65%.

Result and Discussion

The Content of Crude Protein of Seedcake of Jatropha curcas L seed which is fermented by consortium Aspergillus niger and Neurospora sitophila

According to Sulaiman (1988), the content of protein resulted from fermentation will proportionally increase in line with length of fermentation up to certain limit of time, then let it lower.

Table-1: The Test of Duncan Double Space Partaining with the Impact of Inoculums Dosage Treatment toward the Contain of Crude Protein by the Consortium of Sea-Shell Aspergillus niger and Neurospora sitophila

Treatment	Average (%)	Significance 0.05
d1 (2g)	18.44	A
d2 (3g)	19.21	B
d3 (4g)	19.67	B

Description: the letter which is different from the way of column shows real difference on the space $\alpha<0.05$

Table-2: The Test of Duncan Multiple Duble Space Concerning with the Impact of Fermentation Length toward the Contain of Crude Protein by Consortium of Sea-Shell Aspergillus niger and Neurospora sitophila

Treatment	Average (%)	Significance 0.05
w1 (72 hours)	17,94	A
w2 (96 hours)	19,64	B
w3 (120 hours)	19,74	B

Description: the letter which is different from the way of column shows real difference on the space $\alpha<0.05$

Based on table 1 and 2, the best treatment bringing about the highest increase of crude protein on seedcake of Jatropa curcas L's seed which was fermented by Aspergillus niger and Neurospora sitophila is inoculums dosage 3 g and fermentation length 96 hours before fermentation 17. 00% after fermentation 19. 87% so that the increase of the contain of crude protein is 16. 88%.

The Contain of Crude Fat of Seed Bungkil Jatropha curcas L which is fermented by consortium Aspergillus niger and Neurospora sitophila

Table-3: The Test of Duncan Multiple Space dealing with the Interaction of Consortium Inoculums Dosage and Fermentation Length toward the Contain of Crude Protein by Consortium of Sea-Shell Aspergillus niger and Neurospora sitophila

Length of fermentation (hours)	Dosage of Consortium Inoculum ($X10^7$)		
	d1 (2g)	d2 (3g)	d3 (4g)
W1 (72 hours)	2.53 b B	2.48 b B	1.46 a A
W2 (96 hours)	1.39 a A	2.05 a B	5.01 b C
W3 (120 hours)	5. 86 c C	4.62 c A	5.15 B

Description: - Small letter which is different toward vertical line indicates significant differences ($\alpha<0.05$).

- Capital letter which is different toward horizontal line indicates significant differences ($\alpha<0.05$).

Based on table 3, treatment bringing about reduction of the best crude fat for efficiency is consortium inoculums dosage 2 g and the length of fermentation 96 hours. It is caused by

different rate of sea-shell growth between Neurospora sitophila and Aspergillus niger. Sea-shell Neurospora sitophila is faster to grow on the substrate . It causes realignment of crude fat, but the growth is not supported by supply of nutrition in the substrate which is lower. It is in accordance with Tanuwidjaja's view (1975) that the higher the population is, the faster the sporulation is. So that some of the energy is not used to multiply the cell.

The best treatment resulting reduction of the highest crude fat on seedcake of Jatropha curcas L seed fermented by Aspergillus niger and Neurospora sitophila is inoculum dosage 2 g and the length of fermentation 96 hours before fermentation 3.85% after fermentation 1.39% so that the reduction of crude fat content is 63.89%.

The Content of Crude Fat of seedcake Jatropha curcas L seed fermented by Neurospora sitophila

According to Setiyatwan (2007) the more the dosage of sea-shell inoculums is used, the more concise the time required to reduce the content of crude fiber. The reduction of crude fiber is in line with the growth of molt mycelia. According to Cain (1980) the reduction is happened because sea-shell requires energy resource for its growth, so that the majority of crude fibers is reformed and become energy resource.

Table-4: The Test of Duncan Duble Space Concerning with the Impact of Treatmentt of Inoculums Dosage toward the Content of Crude Fiber by Sea-Shell Consortium Aspergillus niger and Neurospora sitophila

Treatment	Average (%)	Signification 0.05
d3 (4 g)	20.50	A
d2 (3 g)	21.15	B
d1 (2 g)	21.56	B

Description: The letter which is different toward column indicates significant difference with the level of $\alpha<0.05$.

Based on table 4, the best treatment bringing about the reduction of the highest crude fiber on seedcake of Jatropha curcas L seed fermented by Aspergillus niger and Neurospora sitophila is inoculums dosage 4 g and the length of fermentation 96 hours before fermentation 24.66% after fermentation 20.97% so that the reduction of crude fiber is 14.96%.

The Test of Substantial Content of Anti-nutrition Forbol Esther

The analysis using HPLC together with the column of highly working liquid chromatography (KCKT) shows the existence of compound of forbol esther on seedcake of Jatropha curcas L seed. The compound of forbol esther is detected in the length of retention 3.9-4 minutes. By using comparer forbol 12-13- di-acetate so that it can be known the content of forbol esther on each fermentation treatment.

From the result of analysis of seedcake Jatropha curcas L seed fermented by consortium of sea-shell Aspergillus niger and Neurospora sitophila with the dosage of inoculums 3 g and the length of fermentation 96 hours, it provides real impact ($\alpha< 0.05$) with lower content of forbol esther. Reduction has occurred on forbol esther with the percentage of 79. 69%, from the previous state of fermentation 7.19µg/g become 1.46µg/g.

It is because compound of forbol esther is degraded by enzymes produced by consortium of sea-shell Aspergillus niger and Neurospora sitophila . Besides producing enzyme of cellulose to disentangle crude fiber, Aspergillus niger also produces enzyme of lipase namely enzyme disentangling bond of esther on fat become glycerol and fat acid. It is in accordance with Winarno's thought (1986) that sea-shell producing lipase and having been used commercially is Aspergillus niger. Neurospora sitophila results enzyme of lipase which is strong and able to disentangle fat become glycerol and fat acid. Edi (1989) proposed Neurospora sitophila during fermentation enables to reduce apha toxin 77%. In accordance with Rusdi's view (1992) that is fermentation can cause shift of nature of meal as the consequence of detangling the content of nutrition available on it.

To know the benefit of fermented product, biological test was carried out to the chicken

through determining metabolism energy. The potency of nutrition fact on the fermented product can be established by chemical analysis, proximate analysis. The more chicken consumes substance of meal, the higher metabolism energy of fermented product is. That is an indicator of how high the quality of processed product.

The measurement toward Metabolism Energy of Seedcake of Jatropha curcas L seed

Table-5: The list of metabolism energy (kkal/kg) seedcake of Jatropha curcas L seed

Test	EM Unfermented feed (kkal/kg)	EM Fermented feed (kkal/kg)
1	3579,9	4051,9
2	3891,8	3878,2
3	3563,3	4130,8
4	4163,1	3252,4
5	4065,1	3901,7
6	3728,3	3690,6
7	4112,2	4126,9
8	3705,3	3681,1
9	3776,9	3796,0
10	3979,7	4094,1
11	3911,9	3710,8
12	3869,2	3751,9
13	3865,8	3809,8
14	4186,5	4184,2
15	3886,2	3674,3
Total	58285,4	57734,8
Average	**3885,7**	**3849,0**

Based on table 5, the value of metabolism energy of seedcake Jatropha curcas L seed without process of fermentation is 3885.7 kkal/kg while the fermented one is 3849 kkal/kg. From the value of metabolical energy from the experiment, the conversion from bruto energy of feed to metabolism energy occured. For unfermented feed, the energy converts into 78.27 % while for fermented one, the energy converts into 77. 53%.

If it is seen from conversion of energy, whether fermented product or unfermented one, both of them have high efficiency of using energy over 70%. It is in line with Wahju's opinion (1997) that stated material is categorized as energy resources when it has efficiency of using energy over 70%. It reveals that seedcake of Jatropha curcas L seed belongs to a high categoryof energy resource.

Aspergillus niger produces cellulase enzymes that convert crude fiber into glucose which is a source of energy and for growth, causing the cell into many cells and is itself a protein, thus decrease crude fiber. Neuspora sitophila produce lipase enzyme, the increase in value occurs because of decreased protein content of crude lipid which turns into fatty acids for growth. Although crude fiber and crude lipid is broken down by cellulase enzymes that produced by Aspergillus niger and lipase enzyme that produced by Neurospora sitophila, phorbol ester is still present in Jathropa curcas L. seed result of fermentation, so there is a difference of metabolic energy utilization efficiency between of fermented feedstuffs and non-fermented feedstuffs.

Forbol esther is still available on seedcake of Jatropha curcas L seed which has been fermented. So that there is some differences on the efficiency of using metabolism energy from fermented raw substance for feed and non-

fermented one. It is caused by compound of forbol esther in the raw substance of feed.

Conclusion

Consortium of Aspergillus niger and Neurospora sitophila with the treatment of dosage of inoculums 3 g, the length of fermentation 96 hours can increase the highest crude protein in level 16.88%; treatment of dosage of inoculums 2 g and length of fermentation 96 hours can reduce the highest crude fat in level 63.89% and treatment of dosage of inoculums 4 g, the length of fermentation 96 hours can reduce the highest crude fiber in level 14.96%. The reduction of content of forbol esther in the result of HPLC method test, seedcake Jatropha curcas L seed which has not been fermented, the content of forbol esther is 7.19µg/g, after fermentation is 1.46µg/g. the reduction is 79.69%

The value of metabolism energy on detoxificated product through fermentation by consortium Aspergillus niger and Neurospora sitophila on the dosage of inoculums 3g and the length of fermentation 96 hours can be gained the content of metabolism energy in level of 3849kkal/kg.

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