

## **Fermentation of *Jatropha* kernel cake (*Jatropha curcas* L.) using varieties of fungi on its chemical compositions, concentration of phorbol ester, and digestibility**

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**ABSTRACT:** This study consists of two stages. The first stage is to identify fungi that able to grow on *Jatropha* kernel cake (JKC) and the second is to determine digestibility of fermented JKC. In the first stage, the JKC is fermented using species of fungi, namely, *Aspergillus niger*, *Neurospora* sp. or *Rhizopus oligosporus*. After fermentation, the fermented JKCs (JKCF) are chemically analysed for DM, OM, CP, CF, NDF ADF and phorbol ester contents. The second stage is to determine in sacco digestibility of the JKCF when fermented using selected fungi. The in sacco degradation is conducted according to Orskov and Ryle (1990). The results show that the fermentation very significantly ( $P < 0.01$ ) increases CP and very significantly ( $P < 0.01$ ) decreases content of CF, NDF and ADF, as well as phorbol ester content of JKCF. *Rhizopus oligosporus* has the highest ability to increase CP content (26.82%), and to decrease NDF (51.04%), and ADF (55.87%). The decrease of CF is occurred best with *Neurospora* sp. (56.05%), the decrease of phorbol ester occurs from 6.54 ppm before fermentation to 0.10 ppm after fermentation with *Aspergillus niger*, 0.14 ppm with *Neurospora* sp. and 0.37 ppm with *Rhizopus oligosporus*. Based on its performances the *Rhizopus oligosporus* is the selected fungi for fermenting JKC. The degradation of DM and OM are highest when JKC and JKCF are incubated for 72 hours. Average degradation theory (DT) of DM and OM between JKC and JKCF are significantly different ( $P < 0.01$ ), much higher in JKCF than that in JKC, i.e.: 48.285 vs. 22.78% for DTDM and 61.06 vs. 55.78% for DTOM, respectively. It can be concluded that the fermentation able to increase CP content, the DTDM and DTOM, as well as, to decrease fiber fractions and phorbol ester contents.

**Key words:** (*Jatropha* kernel cake, phorbol Ester, fermentation, chemical composition, In Sacco digestibility)

### **INTRODUCTION**

One of the agricultural industry wastes that have significant potential as a source of ruminant animal feed is the *Jatropha* kernel cake (*Jatropha curcas* L.). The potential is quite promising, given the *Jatropha* production has been increasing significantly, with the non-renewable energy transition to renewable energy, then *Jatropha* is an alternative solution to the energy problem because it can grow on marginal land, does not compete with food crops, is a versatile and renewable plant.

The search result of Indonesia's National Biofuel Team (2008) reported by Siang (2009) that the prediction of critical land that has been planted with *Jatrophas* in Indonesia is 114.112 ha. scattered throughout the archipelago. *Jatropha* kernel production in Indonesia is about 3-5 tons / ha, depending on the country, climate, soil quality, and its management. If one hectare of *Jatropha* plants produce four tons of dry *Jatropha* kernels processed into crude *Jatropha* oil (CJO), it will get about three tons of *Jatropha* kernel cake (JKC), which can be used either as biogas, fertilizer and livestock feed (Indrawanto and Dibyo, 2008). *Jatropha* kernel cake has high crude protein 56.4 - 63.8% (Giibitz *et al.*, 1997). Potential of *Jatropha* kernel cake (JKC) as animal feed has constraints on its use, i.e. the existence of anti-nutritional or toxic compounds in form of phorbol esters that makes livestock at risk. Phorbol esters is also called phorbol-12 miristate-13-acetate (PMA), which can cause the body inability to absorb minerals like  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Fe^{2+}$  and  $Zn^{2+}$ . In addition, this compound also forms a complex with proteins and interacts with the

trypsin and pepsin enzymes so that it causes protein digestion in the body is not going perfectly (Makkar and Becker, 1997). Martinez *et al.* (2005) in Oktiani and Soetikno (2007) stated that the PMA was stable against heating, so that this new compound degraded by fermentation processes such as in the making of tempe (fermented soybean cake) and miso.

Inspired by some previous study results that prove that the use of *Aspergillus niger*, *Rhizopus oligosporus*, and *Neurospora sp* can reduce toxic substances in the fermented media, thereby the three types of fungi are used as isolates in the fermentation of jatropha kernel cake. Sari and Purwadaria (2004) and Murni *et al.* (2008) stated that the fermentation using fungi such as *Rhizopus oligosporus*, *Neurospora sp.* and *Aspergillus niger* could increase the nutrient value and change the physical structure of materials, preservation and reduce anti-nutrient content, and can reduce toxic compounds, increasing the durability of food and food ingredients to be better so that its economic value would be higher.

*Rhizopus oligosporus* is isolate that is isolated from tempe yeast, can suppress the growth of toxigenic fungi, including *Aspergillus flavus* that can produce aflatoxin (Kusumaningtyas *et al.*, 2005). Sabrina *et al.* (1988) suggested that the fermentation process using *Rhizopus oligosporus* increased protease enzyme activity so that most of the proteins contained in the substrate were reorganized into amino acids and used by microbes for growth. Sari and Purwadaria (2004) suggested that *Aspergillus niger*, one type of fungi that is able to convert starch into protein by the addition of inorganic nitrogen materials, capable of reducing toxin and able to live with high fat content, does not produce mycotoxins that are not harmful for consumption.

*Neurospora sitophila* and *Neurospora crassa* are fungi that commonly grows on oncom (fermented cake made from soybean sediment) which give the color black and orange on oncom so that in the making of oncom normally use *Neurospora sp* fungi. Mappiratu (1990) stated that *Neurospora sp.* fungi used in the making of oncom contained provitamin A (carotenoid) content of 12% which gave a color orange to red. Rusdi (1992) stated that the *Neurospora sp.* has its advantages as an isolate of fermentation because it could reduce the toxic aflatoxin as much as 77%, so that it enabled to reduce and even to eliminate the toxin binding to the fatty acid, because *Neurospora sp.* produced a variety of enzymes such as lipase and protease enzymes that were active during the fermentation process and played an important role in the decomposition of starch into sugar, decomposition of the cell wall materials, and of fat, and the formation of a little alcohol and a variety of esters that were aromatic dan smell good (Siswono, 2002). Van Veen *et al.* (1961) in Oktiani and Soetikno (2007) stated that *Neurospora sp* (*N. sitophila* and *N. crassa*) could reduce aflatoxin in peanut cake as much as 50-70% during the fermentation so that Oncom could be consumed by humans.

The objectives of the study were describe the type of fungus that can improve the chemical composition, and lower *neutral detergent fiber* (NDF), *acid detergent fiber* (ADF) and the *phorbol ester* content in jatropha kernel cake, and to analyze the digestibility of dry and organic matters of fermented jatropha kernel cake by using the technique *in sacco*.

## MATERIALS AND METHODS

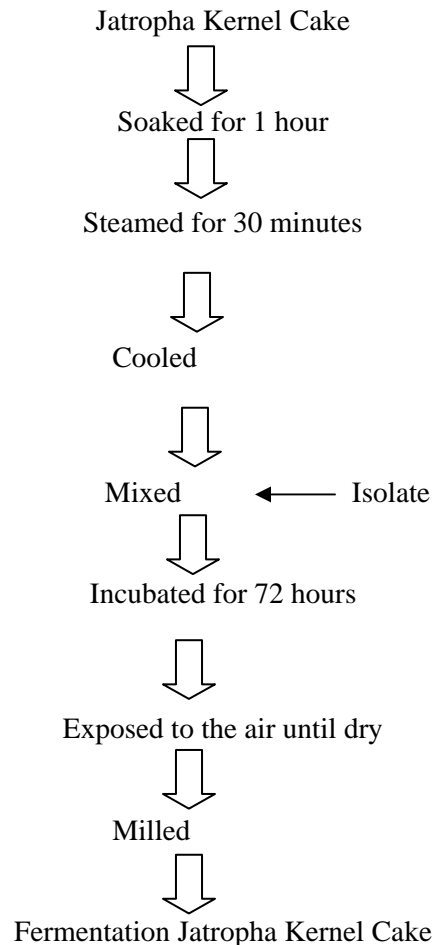
### *Stage I. Chemical Composition of Jatropha Kernel Cake and Content of Phorbol ester*

The study is performed in Nutrition Laboratory, Department of Nutrition and Feed Science, Faculty of Animal Science, and the study of *phorbol ester* content is performed in Laboratory of Integrated Study and Testing (LPPT) of Gadjah Mada University.

**Materials.** Materials and tools used include: jatropha kernel cake, plastic bags in two-kilogram sizes, isolates of *Aspergillus niger*, *Rhizopus oligosporus*, and *Neurospora sp.*, a pair of scales of 5 kg-capacity, electric scales, a set of fermentation equipment and a set of proximate analysis tools.

**Methods.** Jatropha kernel cake is mixed with water until it reaches the water content in accordance with the needs of each fungus *Aspergillus niger* (WC = 55%), *Rhizopus oligosporus* (WC = 65%) and

*Neurosphora sp.* (WC = 60%). The materials are mixed until homogeneous and then steamed for 30 minutes and cooled. Furthermore, it is inoculated with spores according to the treatment. Subsequently incubated in plastic bags of 20x30 cm in size with a thickness of 2 inches, stored at room temperature (29-30°C) for 3 days. Then the fermentation results are harvested, dried and then milled and ready for use. Procedure of fermenting of jatropha kernel cake (Figure 1), is as follows:



**Figure 1.** Fermentation of Jatropha Kernel Cake.

### **Observed Variables**

**Chemical Composition.** The chemical composition is analyzed proximately following the AOAC procedures (2005). The content of the fiber fraction is to follow the procedures Goering and Van Soest (1970), which includes the contents of *neutral detergent fiber* (NDF) and *acid detergent fiber* (ADF).

**Phorbolster Content.** Phorbolster content is prepared by following procedures of Makkar *et al.* (2007) and then analyzed using high-performance liquid chromatography (HPLC) according to Wyss *et al.* (1999) in laboratory of integrated study and testing (LPPT) of GMU. HPLC conditions are: column (C18); Efluent, A = 1.75 ml *o-phosphoric acid* (85%) / liter of H<sub>2</sub>O; B (CH<sub>3</sub>CN) where A:B = (20:80); Flow (1.3 ml/min) and  $\lambda$  (235 nm). The final result uses the formula = (conc.alat (ppm) x add end (ml)) / sample weight.

**Data Analysis.** This study uses Complete Random Design (CRD) consisting of four treatments and three replications. Data are processed according procedures of Steel and Torrie (1989). Treatments that

show significant differences are continued process using *Duncan's new multiple range test* (DMRT).

### ***Stage 2. Dry Matter and Organic Matter Digestibility In Sacco***

**Materials.** This study uses two fistulated cows of Onggole hybrid, jatropha kernel cake, plastic bags of 2-kg in size, *Rhizopus aligosporus* isolate (selected fungus), a pair of scales of 5-kg capacity, electric scales, a set of fermentation equipments.

**Methods.** The provided rations are raja (king) grasses and concentrates at the main living level. The making of nylon bags is pressed and filled with feed sample to be tested. Nylon bag linked by nylon yarn with the weighting tool (675 g) is incubated into the rumen. The sample is dried first in an oven at temperature 55°C for 24 hours, then pulverized uniformly to achieve the homogeneous particle size of 3 mm. Approximately 5 grams (dry matter) of each sample is inserted into the nylon bags of dimension 11x6 cm with pore size 48 µm (Soejono et al., 1997). The bag side containing the sample is closed tightly and tied with rubber bracelets. The bags were incubated into the rumen of Onggole hybrid (PO) female cattle that has been fistulated. The incubation times are 2, 4, 6, 8, 16, 24, 48, 60 and 72 hours, at every point of incubation is repeated four times. The bags are taken in accordance with the incubation times, the water soluble material in each sample is obtained by the washing on the washing machine with a water flow without incubation. Feed residues after incubation are dried in an oven at temperature 55°C until they reach constant weight, then each residue is weighed to calculate the loss of DM and continued with OM the analysis.

### ***Observed Variables***

**Degradation of Dry Matter and Organic Matter.** The dry matter and organic matter contents of the remaining material after incubation are analyzed. DM measurements are carried out with drying the sample in an oven at 110°C for 24 hours (constant weight), incinerating in a furnace at temperature 550°C for 24 hours and leaving in the furnace until it reaches the lowest temperature (24 hours) to determine the ash content. The raw data obtained from the incubation process is the digestibility level of dry and organic matters of samples at a certain incubation time. The data is then processed based on an exponential relationship according to Orskov and Ryle (1990) with the equations  $P = a + b(1 - e^{-ct})$ , with the hypothesis that the rate of OM digestibility (kd) is constant with time. Where P (the losing material at time t), a (rapidly soluble fraction), b (insoluble fraction but potential to be fermented), c (degradation rate) and Lt (lag time, the time that microbes take to form colonies) can be estimated, i.e.,  $TD = a + (b \times c/c + kp)$ , where the lag phase (L) is calculated with the model  $P = a$  for  $t < t_0$ ,  $p = a + b(1 - e^{-c(t-t_0)})$  for  $a > t_0$  (Dhanoa, 1988), where the intersection of beginning time on the curve  $a + b$  is the asymptote of the curve and reflects the degraded material if it has been determined, c is a degradation level constant and P is a loss because of degradation at a certain time (t).

### ***Statistical Analysis***

Variance of DM and OM Digestibilities is analyzed by one-tailed complete random design. Treatments that show significant or highly significant effects are followed by *Duncan's multiple range test* (DMRT) according to Steel and Torrie, 1980.

## **RESULTS AND DISCUSSION**

### ***Chemical Composition of Jatropha Kernel Cake and Content of Phorbolster***

The analysis results of chemical composition and concentration of jatropha kernel cake *phorbolster* unfermented and fermented with various types of fungi are presented in Table 1 below:

**Table 1.** Chemical Composition and Content of *Phorbolester* Jatropha Kernel Cake unfermented and Fermented

	Chemical Composition and				Content of Phorbol Ester	
	DM, %	CP, %	CF, %	NDF, %	ADF, %	Phorbol Ester, ppm
JKCUF	96,01	23,41 <sup>a</sup>	18,86 <sup>a</sup>	31,75 <sup>a</sup>	20,63 <sup>a</sup>	6,54 <sup>a</sup>
JKCFAn	83,80	29,96 <sup>b</sup>	10,91 <sup>b</sup>	22,08 <sup>b</sup>	15,10 <sup>b</sup>	0,14 <sup>b</sup>
JKCFNsp	83,54	24,09 <sup>c</sup>	11,67 <sup>c</sup>	23,95 <sup>c</sup>	16,59 <sup>c</sup>	0,10 <sup>c</sup>
JKCFRo	84,13	43,03 <sup>d</sup>	13,78 <sup>d</sup>	16,59 <sup>d</sup>	11,13 <sup>d</sup>	0,37 <sup>d</sup>

DM = Dry matter, CP = Crude protein, CF = Crude fibre, NDF = Neutral detergent fibre

ADF = Acid detergent fibre, JKCUF = Jatropha Kernel Cake unfermented;

JKCFAn= Jatropha kernel cake fermented with *Aspergillus niger*;

JKCFN.sp= Jatropha kernel cake fermented with *Neurospora sp.*

JKCFRo= Jatropha kernel cake fermented with *Rhizopus oligosporus*

<sup>a, b, c, d</sup> : Different superscripts in the same column show highly significant differences (P <0.01)

In Table 1 it appears there is a change of chemical composition, especially the content of CP, CF, NDF and ADF. The changes in crude protein content fermented with fungi increase respectively, i.e., jatropha kernel cake fermentation with *Rhizopus oligosporus* (JKCFRo) from 23.41% to 43.03% (45.60%), jatropha kernel cake fermentation with *Aspergillus niger* (JKCFAn) from 23.41% to 29.96% (21.86%), and jatropha kernel cake fermentation with *Neurospora sp.* (JKCFN.sp) from 23.41% to 24.09% (2.82%).

The statistical analysis shows highly significant effect (P <0.01) on crude protein content. The further test results show a highly significant difference (P <0.01) between treatment with Jatropha Kernel Cake unfermented (JKCUF) and with JKCFRo; highly significant (P <0.01) between with JKCFN.sp and with JKCUF; highly significant (P <0.01) between with JKCFAn and with JKCUF. The increase of crude protein fermented with various types of fungi as a whole caused by the protease enzyme that can dissolve complex proteins to become simpler ones, and fungi have the ability to convert starch into protein by the addition of inorganic nitrogen through the fermentation. Winarno *et al.* (1980) stated that the fermentation could cause changes in properties of food materials as a result of breaking the food substance caused by microbial enzyme activity.

The highest increase of crude protein is seen in the fermentation with *Rhizopus oligosporus*, this is due to population growth of fungi is more fertile than that of the other two types of fungi, the high growth in jatropha kernel cake contributes biomass protein on the media used. The same was suggested by Sari and Purwadaria (2004) and Padang (2009) that fermentation increased the protein content caused by changes in inorganic nitrogen into cell protein during the growth of microbes, the more fertile the growth of fungi is the higher protein contents will be, caused by microbial populations that have been dead and is one of the contributions of single cell protein for the media because most of the fungal cell is a protein.

The crude fiber content (CF) decreases, namely, in jatropha kernel cake fermented with *Aspergillus niger* it decreases from 18.86% to 10.91% (42.15%), *Neurospora sp.* CF decreases from 18.86% to 11.67% (38.12%) and *Rhizopus oligosporus* from 18.86% to 13.78% (26.94%). Neutral detergent fiber (NDF) on the jatropha kernel cake fermented with *Rhizopus oligosporus* decreases from 31.75% to 19.72% (37.89%), with *Aspergillus niger* from 31.75% to 22.08% (30.46 %), and with *Neurospora sp.* from 31.75% to 23.95% (24.57%), and acid detergent fiber (ADF) also decreases respectively, with *Rhizopus oligosporus* from 20.63% to 11.13% (46.05%), with *Aspergillus niger* from 20.63% to 15.1% (26.81%), and with *Neurospora sp.* from 20.63% to 16.59% (19.58%).

The Statistical analysis shows very significant effects (P<0.01) on CF, NDF and ADF contents. Further test results on CF content show highly significant differences (P<0.01) between JKCFAn and JKCUF treatments; highly significant (P<0.01) between JKCFN.sp and JKCUF; highly significant (P<0.01) between JKCFRo and JKCUF. Similarly, differences of NDF contents are highly significant (P <0.01) between JKCFAn and JKCUF; highly significant difference (P<0.01) between JKCFN.sp and JKCUF and differences are highly significant (P <0.01) between JKCFRo and JKCUF. There are highly

significant differences ( $P < 0.01$ ) in ADF contents between JKCFA.n and JKCUF; a highly significant difference ( $P < 0.01$ ) between JKCFN.sp and JKCUF; and highly significant ( $P < 0.01$ ) between JKCFRo and JKCUF. The decrease of crude fiber components is caused by the presence of cellulase and hemicellulase enzymes contained in the fungi that can loose (reorganize) fiber fractions contained in the fermented media. CF decrease is highest in jatropha kernel cake fermented with *Aspergillus niger* and the lowest is in that one fermented with *Rhizopus oligosporus*. The decrease is lower in jatropha kernel cake fermented with *Rhizopus oligosporus* caused by the fertile growth in the fermented media causes other than to be able to loosen the fiber fractions, on the walls of mycelium fungus itself contains high crude fibers, so that it is the contribution of crude fibers to the media itself. Similarly submitted by Purwadaria (2004) that the fermentation process that showed the fertile growth of fungi there is actually the decrease of crude fiber content lower than that of the less fertile fungal growth, it is caused by the spore cell walls contain more crude fiber, so that it can also increase the crude fiber content of the fermented material.

*Phorbol ester* content with fermented is lower than that unfermented. It decreases through the treatment of fermentation, with *Neurospora sp.* it decreases from 6.54 ppm to 0.10 ppm (97.86%), with *Aspergillus niger* from 6.54 ppm to 0.14 ppm (98.47%), and with *Rhizopus oligosporus* it decreases to 0.37 ppm (94.34 %).

The results of statistical analysis show the effects of treatments are highly significant ( $P < 0.01$ ) on *phorbol ester* content. The further test results in *phorbol ester* contents show a highly significant ( $P < 0.01$ ) difference between JKCUF and JKCFA.n treatments; highly significant ( $P < 0.01$ ) between JKCFN.sp and JKCUF; highly significant ( $P < 0.01$ ) between JKCFRo and JKCUF. Similarly, the difference in the contents of *phorbol ester* is highly significant ( $P < 0.01$ ) between JKCFA.n and JKCUF; highly significant ( $P < 0.01$ ) difference between JKCFN.sp and JKCUF and; highly significant ( $P < 0.01$ ) difference between JKCFRo and JKCUF. There are highly significant ( $P < 0.01$ ) differences in ADF content between JKCFA.n and JKCUF; highly significant ( $P < 0.01$ ) between JKCUF and JKCFN.sp; and highly significant ( $P < 0.01$ ) between JKCFRo and JKCUF.

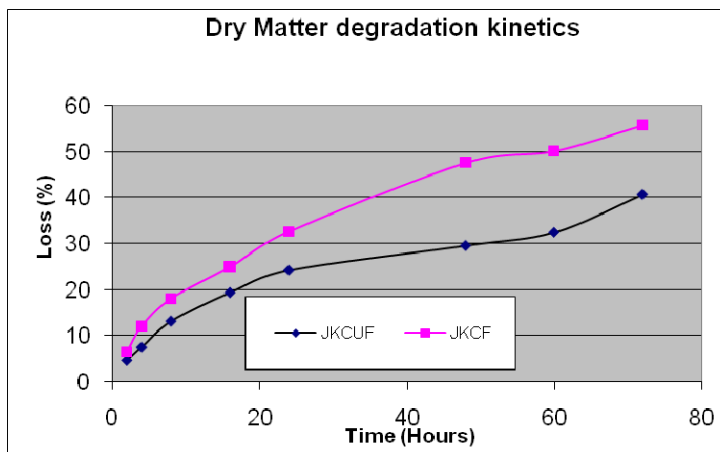
*Phorbol Ester* content decreases are caused by the three fungi used in the fermentation process contains enzymes (esterase enzymes) that can dissolve esters binding to *phorbol*, so that the *phorbol Ester* contents with a low concentration could eventually be used as the arranging materials of concentrate. The same was suggested by Sari and Purwadaria (2004) that the fermentation process could eliminate the toxic substances contained in a material. Mappiratu (2009) stated that in *phorbol Ester* molecule there are two forms of the ester, namely, the ester formed from acetic acid and myristic acid, both of these esters can be fractionated by mineral acids and *lipase* as well as *esterase* enzymes. Esters formed from myristic acid  $\text{CH}_3(\text{CH}_2)_{11}\text{CH}_2\text{-COOH}$  are relatively easily broken down either by the lipase and esterase enzymes. Factors that determine the ability of the enzymes to hydrolyze esters of myristic acid is the nature of the lipase or esterase specificity towards fatty acid substrates. Lipase which has a very specific nature towards myristic acid will hydrolyze with a faster reaction rate compared with lipase or esterase which has the nature of low specificity towards myristic acid (Mappiratu, 2009).

### ***Degradation of Dry Matter and Organic Matters of Jatropha Kernel Cake Unfermented and Fermented***

Degradation kinetics of dry and organic matters of jatropha kernel cake fermented and fermented in the time of incubation 2, 4, 8, 16, 24, 48, 60 and 72 hours are shown in Figure 2. There is a cumulative increase in the degradation kinetics of dry and organic matters is accompanied by decreasing degradation speed in line with the length of incubation time. The degradation level of dry matter of jatropha kernel cake unfermented with a level of loss in accordance with the incubation period of 2, 4, 8, 16, 24, 48, 60 and 72 hours respectively, are 4.67, 7.58, 13.19, 19.43, 24.29, 29.59, 32.51 and 40.72%, respectively. While the degradation level of jatropha kernel cake fermented loses dry matter in accordance with the incubation period of 2, 4, 8, 16, 24, 48, 60 and 72 hours, are 6.53, 12.10, 18.10, 24, 96, 32.67, 47.56, 50.18, 55.70%, respectively. Increasing the length of incubation time gives more time for microbes to

utilize the material in a nylon bag so that it is hopeful the digestibility level will be higher.

The loss level of dry matter of jatropha kernel cake fermented is higher than that of dry matter of jatropha kernel cake unfermented, and there is a cumulative increase in line with the length of incubation period. The degradation level of dry matter in both jatropha kernel cake unfermented and that fermented reaches the optimal point in the length of incubation time 72-hours. The following is the graph of dry matter degradation kinetics of jatropha kernel cake fermented and unfermented fermentation treatments (Figure 2)



**Figure 2.** Degradation of dry matter of unfermented or fermented jatropha kernel cake

JKCUF = Jatropha kernel cake unfermented

JKCF = Jatropha kernel cake fermented

The degradation level of jatropha kernel cake without fermentation seems to require a longer time for rumen microbes to degrade the feed components. The degradation levels of dry matter shown in both jatropha kernel cake with and without fermentation have low degradation levels in comparison with the degradation level of soybean meal (60.96% for the duration of incubation 48 hours), as reported by Chumpawadee et al. (2005). This is due both to jatropha kernel cake with and without fermentation have fat (protected) contents, which have barriers to degrade dry matters contained therein. The degradation levels of organic matters in jatropha kernel cake without fermentation for the duration of incubation 2, 4, 8, 16, 24, 48, 60 and 72 hours lack the organic matters: 4.49, 5.29, 14.56, 18.77, 24.65, 29.30, 32.47, 40.68%, respectively. While the degradation levels of organic matters of jatropha kernel cake with fermentation for the duration of incubation 2, 4, 8, 16, 24, 48, 60 and 72 hours, lack the organic matters: 6.15, 9.36, 19.19, 24.45, 29.60, 36.75, 43.50 and 45.38%, respectively.

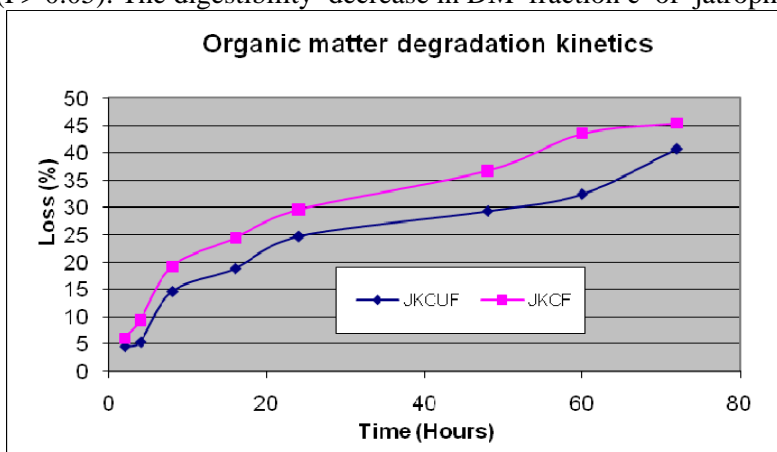
The degradation levels of OM both in jatropha kernel cake unfermented and fermented treatments are highest in the duration of incubation 72 hours, 40.68 and 45.38%, respectively. The high degradation levels of OM in jatropha kernel cake indicate that with fermentation it will make easier for rumen microbes to degrade the organic matters of feed.

Averaging values of several degradation characteristics of fermented and unfermented jatropha kernel cakes can be seen in Table 2.

The results show that fraction a of JKCF DM (2.65%) are significantly ( $P < 0.01$ ) higher than that without fermentation (0.88%); as well as the fraction a of the OM with fermentation (4.32%) are significantly ( $P < 0.01$ ) higher than that without fermentation (3.96%). This shows that the height of fraction a in both the DM and OM indicates that protein content after fermentation is much higher than that without fermentation.

Fraction b in fermented DM (53.06%) is significantly ( $P < 0.01$ ) higher than that unfermented (39.84%), the fraction b in fermented OM (41.06%) is significantly different ( $P < 0.01$ ) than that

unfermented (36.72%). This means that the degradation potential in both dry matter and organic matter of jatropha kernel cake fermented is higher than that unfermented. Fraction c in both DM and OM shows no significant effect ( $P > 0.05$ ). The digestibility decrease in DM fraction c of jatropha kernel cake



**Figure 3.** Degradation of organic matters of unfermented and fermented jatropha kernel cake

JKCUF = Jatropha kernel cake unfermented

JKCF = Jatropha kernel cake fermented

**Table 2.** The average value of degradation of fraction a, b, c and TD dry matter and organic matter of jatropha kernel cake treated with and without fermentation (%)

Parameter	Treatment	
	JKCUF	JKCF
DM Degradation		
a,%	0,88 <sup>a</sup>	2,65 <sup>b</sup>
b,%	39,84 <sup>a</sup>	53,06 <sup>b</sup>
c,%	0,03 <sup>a</sup>	0,04 <sup>a</sup>
TD	22,78 <sup>a</sup>	48,28 <sup>b</sup>
OM Degradation		
a, %	3,96 <sup>a</sup>	4,32 <sup>b</sup>
b, %	36,72 <sup>a</sup>	41,06 <sup>b</sup>
c,%	0,03 <sup>a</sup>	0,04 <sup>a</sup>
TD	55,78 <sup>a</sup>	61,06 <sup>b</sup>

<sup>a,b</sup>: Different superscript on the same row shows highly significant difference ( $P < 0.01$ ), <sup>a,b</sup> JKCUF: Jatropha kernel cake unfermented; JKCF : Jatropha kernel cake fermented TD : Theoretical degradation

unfermented and with fermented makes these matters to be degraded slowly in the rumen, so that the contained-protein is hopefully to be *bypass*-protein source for increasing protein supply of postrumen (small intestine). Similarly, Rusdi *et al.* (2009) submitted that the feed materials that have a high protein content but low level of degradation in the rumen it is hopefully to be *bypass*-protein source in livestock postrumen.

Fraction TD in both DM and OM show significant ( $P < 0.01$ ) effects. The value of TD in fermented DM (48.28%) is significantly ( $P < 0.01$ ) higher than that without fermentation (22.78%). The value of TD in fermented OM (61.06%) is significantly ( $P < 0.01$ ) higher than that without fermentation (55.78%). The height of TD value in both DM and OM is caused by the value of a (easily degraded fraction) in both DM and OM are high, resulting in value of the theoretical degradation (TD) is also high.



## CONCLUSIONS

Based on the results and discussion, we can draw some conclusions:

Fermentation can raise content of crude protein and lower contents of crude fiber, NDF and ADF and of *phorbolester* in jatropha kernel cake. *Rhizopus oligosporus* has the highest percentage to raise the crude protein content of jatropha kernel cake. There is a decrease in *phorbolester* contents of jatropha kernel cake from before fermentation (6.54 ppm) to 0.1 ppm (98.47%) after fermentation with *Aspergillus niger*; *Neurospora sp.* 0.14 ppm (97.86%) and *Rhizopus oligosporus* 0.37 ppm (94.34%).

The level of dry matter and organic matter digestibility at both jatropha kernel cake without and with fermentation and fermented is the highest in the duration of incubation 72 hours. Fraction a of DM in jatropha kernel cake fermented (2.65%) is significantly higher than that unfermented (0.88%); as well as fraction a of OM fermented (4.32%) is significantly higher than that unfermented (3.96%). Fraction b in fermented DM (53.06%) is higher than that unfermented (39.84%), fraction b in fermented OM (41.06%) higher than that unfermented (36.72%). Fraction TD in both fermented DM and OM (48.28%) are higher than that unfermentation (22.78%). The value of TD in fermented OM (61.06%) is higher than that unfermented (55.78%).

## LITERATURE CITED

- AOAC. 1990. Official Methods of Analysis. 15th edition. Association of Official Analytical Chemists. Washington, DC.
- Chumpawadee, S., S Kritapon., V., Thevin, and P. Virote. 2005. In sacco degradation-characteristics of protein feed sources in Brahman-Thai Native Crossbred Steers. *Walailak J.Sci & Tech* 2005: 2(2): 219-229.
- Dhanao, M.S. 1988. On the analysis of dacron bag data for low degradability feeds. *Grass and Forage Science*, 43:441-444.
- Goering, H. K. and P. J. Van Soest. 1970. Forage Fiber Analysis (apparatus, reagent, procedures and some applications). *Agric. Handbook* 379, ARS, USDA, Washington D.C.
- Giibitz, G., M; M. Mittelbach, dan M. Trabi. 1997. Biofuels and industrial products from *Jatropha curcas*. Developed from the symposium "Jatropha 97" Managua, Nicaragua.
- Indrawanto, C. dan Dibyo, P. 2008. Jarak Pagar (*Jatropha curcas* L). Konferensi Jarak Pagar Internasional. Pusat Penelitian dan Pengembangan Perkebunan, Badan Penelitian dan Pengembangan Pertanian. *Bulletin* Volume 3, Nomor 8, Agustus 2008.
- Makkar, H. P. S., P. Sidduraju and K. Becker. 1999. *Methods in molecular biology in Plant Secondary Metabolites*. Vol. 393. Humana Press Inc., Totowa, New Jersey.
- Mappiratu, G. S. Hutomo dan Nurhaeni, 1990. Produksi Bahan Pewarna Provitamin A pada Medium Tongkol Jagung dengan Kapang Oncom Merah, Laporan penelitian, Universitas Tadulako Palu.
- Mappiratu 2009. Identifikasi Kadar Phorbol ester. Fakultas MIFA Universitas Tadulako, Palu.
- Murni R., Suparjo, Akmal, dan B. L. Ginting. 2008. *Buku Ajar Teknologi Pemanfaatan Limbah untuk Pakan*. Laboratorium Makanan Ternak Fakultas Peternakan, Universitas Jambi
- Oktiani, M. Dan L. Soetikno. 2007. Fermentasi Bungkil *Jatropha curcas* oleh *Rhizopus oligosporus* untuk Pakan Ternak. Pusat Perpustakaan, Institut Teknologi Bandung, Bandung.
- Ørskov, E.R and M. Ryle. 1990. *Energy Nutrition in Ruminants*. Elsevier Applied Science. London.
- Rusdi, D.U. 1992. Fermentasi Konsentrat Campuran Bungkil Biji Kapuk dan Onggok serta Aplikasi Efeknya Terhadap Pertumbuhan Ayam Broiler. Disertasi. Universitas Padjadjaran, Bandung.
- Rusdi; Arief.R.; Agus, 2009. Pengaruh Pengeringan Daun Turi (*Sesbania grandiflora*) Terhadap Degradasi Bahan Kering dan Protein Dalam Rumen. Laporan Hasil Penelitian, Fakultas Pertanian, Universitas Tadulako, Palu.
- Sari, L., and T. Purwadaria. 2004. Pengkajian Nilai Gizi Fermentasi Mutan *Aspergillus niger* pada Substrat Bungkil kelapa dan Bungkil Inti Sawit. Pusat Penelitian Bioteknologi LIPI. Balai Penelitian Ternak. Ciawi, Bogor.
- Sabrina, Harnentis, Y. Heryandi dan T. Aisjah. 1998. Biokonversi kulit ubi kayu dengan *Rhizopus oligosporus* sebagai pakan ternak. *Jurnal Peternakan dan Lingkungan*. Vol. 07 No. 1.
- Siang, C., C. 2009. Development of a New Oil Crop For Biofuel. New And Renewable Energy Group Strategy and Industry Research Unit. The Institute Of Energy Economics (IEEJ), Japan.

- Siswono, 2002. Oncom, Menutup Kekurangan Energi dan Protein. file:///D:/NEURO%202/fullnews.cgi.htm. Diakses tanggal 28 November 2009.
- Soejono, M., B. P. Widyobroto, R. Utomo dan A. Agus. 1997. Standarisasi Pengukuran Degradasi Insacco untuk Perbaikan Sistem Evaluasi Protein pada Ternak Ruminansia di Indonesia. Departemen Pendidikan Nasional. Direktorat Jenderal Pendidikan Tinggi Direktorat Pembinaan Penelitian dan Pengabdian pada Masyarakat.
- Steel, R. G. D. dan J. H. Torrie. 1989. Prinsip dan Prosedur Statistika. Suatu Pendekatan Biometrik. Penerbit PT Gramedia, Jakarta.
- Winarno, F. G., S. Fardiaz, dan D. Fardiaz. 1980. Pengantar Teknologi Pangan. Penerbit PT. Gramedia. Jakarta.