In Vitro Digestibility Evaluation of Fermented Coconut Meal Using *Aspergillus Niger* NRRL 337

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ABSTRACT: Dry matter and protein digestibilities of fermented coconut meal (FCM) using *Aspergillus niger* NRRL 337 were evaluated using in vitro method. The FCM was prepared through aerobic fermentation process continued with anaerobic enzymatic process. Both fermentation and enzymatic process have increased in vitro dry matter digestibility (IVDMD) and in vitro corrected protein digestibility (IVCPD) of coconut meal. The fermentation process has increased IVCPD more effective than that of enzymatic process, whereas the enzymatic process has increased IVDMD more effective. The increase of digestibilities is correlated with the decrease of neutral detergent fibre (NDF) and the increase of corrected protein content (CPC). The highest IVDMD and IVCPD of FCM were resulted from the fermentation of coconut meal added with minerals [$(NH_4)_2SO_4$, urea, NaH_2PO_4, $MgSO_4$ and KCl] and enzymatic process at 50°C. The increase of IVDMD and IVCPD and CPC were 26.5, 66.7 and 156% respectively, whereas the decrease of NDF was 46.8%.

Key Words: Coconut Meal, Fermentation Process, *Aspergillus Niger*, *In Vitro* Digestibility

Introduction

The government of Indonesia plans to increase poultry product for the next long term program. However, the availability of poultry feedstuff containing high protein is limited. At present, it still depends on imported feed such as soybean and fish meals. Coconut meal which contain + 20% of protein (Zamora *et al.*, 1989) and produced abundantly in Indonesia as a waste from coconut oil extraction might be possible to be used as a substitute stuff. However, the use of coconut meal as poultry feedstuff is still limited because it contains 60% of fibre. The composition of the fibre is 61% of galactomannan, 26% of mannan and 13% of cellulose (Zamora et al., 1989). The fibres reduce the protein and dry matter digestibilities. Therefore, both digestibilities might be improved by addition of mannanases and cellulases or fermentation technology using mannanolytic or cellulolytic microbes. *Aspergillus niger* NRRL 337 was reported having the ability to produce mannanase (Araujo and Ward, 1990, Purwadaria et al, 1994a). Therefore, fermented coconut meal (FCM) using *A. niger* NRRL 337 is expected to be more nutritious than raw coconut meal. Before feeding trials have been applied, the technology should be developed in laboratory scale. In vitro methods can be rapidly and sensitively carried out to evaluate the digestibility of the product (Saunders et al., 1973).

This study will evaluate the in vitro dry matter digestibility (IVDMD) and true protein digestibility (IVTPD) of fermented product and also compare those results to protein and neutral detergent fibre contents.

Materials and Methods

Microbe and preparation of inoculum

A niger NRRL 337 was obtained from Northern Regional Research Laboratory, USA. Its spores prepared on the steamed rice (Purwadaria, et al., 1994b) were used as inoculum.

Fermentation process

FCM was prepared through aerobic fermentation process continued with anaerobic enzymatic process. In the first process the coconut meal was mixed with water (800 mL of water for 1 kg of coconut) and added with minerals or without minerals. The composition of minerals per dry matter added were 3.6% of $(NH_4)_2SO_4$, 2.0% of

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urea, 0.75% of NaH_2PO_4, 0.25% of $MgSO_4$ and 0.075% of KCl . After that it was steamed for 30 minutes and cooled down. Then it was inoculated with the spores (2-5 gr spores for 1 kg dry coconut meal). The incubation was carried out in plastic trays covered with the same plastic trays at room temperature (28°C) for three days. The thickness of the substrate was 1 or 2 cm. In the second process, the fermented product were compactly stored in plastic bags which then incubated at 50°C or 28°C for two days. The product was dried in the oven at 60°C.

Chemical analysis

Dry matter loss was calculated from the subtraction of dry matter of FCM from the dry matter of raw material.

Corrected protein analysis was calculated from subtraction of total nitrogen content determined according to Kjeldahl method (AOAC, 1984) with soluble nitrogen content determined according to Conway and O'Malley (1942) and then the subtraction was multiplied by 6.25. The correction was carried out to omit the addition of nitrogen content from extra nitrogen inorganics.

Neutral detergent fibre was determined according to Van Soest and Robertson (1968) to show the content of all fibres (cellulose and mannans).

IVDMD and IVCPD were determined after digestion with pepsin and pancreatin according to Saunders *et al.*(1973)

Aflatoxin B_1, B_2, G_1 and G_2 were determined in Research Institute for Animal Diseases, Bogor.

Statistical analysis

Statistical analysis used was completely factorial design. The fermentation process had three factors : The thickness of substrate, 1 and 2 cm, mineral addition, with and without and incubation time, 0 and three days. The enzymatic process has three factors besides thickness and mineral addition, there were also two temperature incubation, 28°C and 50°C.

Results and Discussion

Dry matter loss

Dry matter loss increased along the fermentation process (Figure 1) due to the activity of *A. niger* in degrading carbohydrate into CO_2, H_2O and energy. The highest loss per day was reached after 2 days incubation where the mold grew very active. The dry matter loss of 2 cm thickness and mineral addition tended to be higher than that of 1 cm thickness and without mineral addition. It could be concluded that the mineral addition stimulated growth more than the one without mineral. The same result that more mineral produced more cells was also obtained from cassava fermentation (Kompiang et al, 1994). The air penetration from 1 cm thickness was to much, this thickness is good only if the growth was expected to be fast such as in spore production (Purwadaria, 1994). The dry matter loss of enzymatic process was not reported in this paper. It was observed in another experiment that the dry matter loss of enzymatic process was negligible.

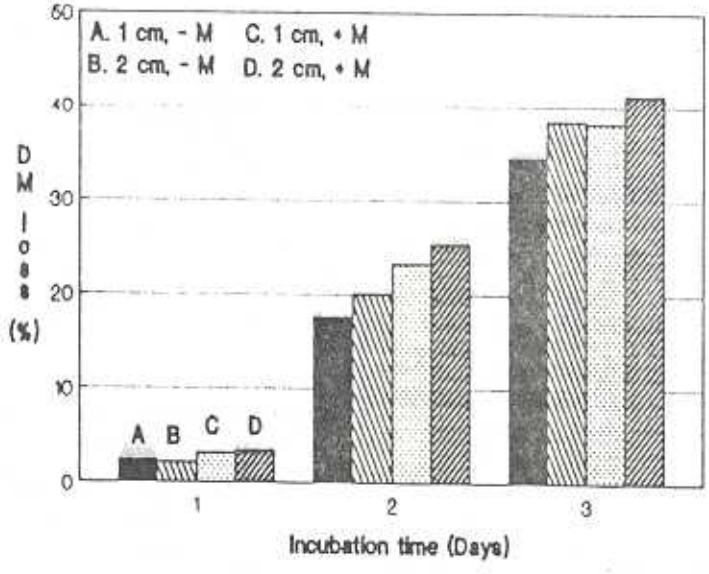

Figure 1. Dry matter losses along fermentation of coconut meal using *A. niger* NRRL 337.

In vitro dry matter digestibility and Neutral Detergent Fibre

IVDMD values and NDF contents of FCM after aerobic fermentation and anaerobic enzimatic were presented in Table 1 and 2, respectively. NDF of FCM after aerobic fermentation was significantly different with that of raw material (0 hour incubation time). The difference occurred due to the mold activity which produced fibre degrading enzymes such as : mannanase and cellulases. The ability of *A niger* NRRL 337 producing mannanase was reported by Araujo and Ward, 1990, whereas Enari (1983) reported that complex cellulases expecially ß-glucosidases were produced by *Aspergilli*. Fibre degradation was continued in the enzymatic process. In this stage the degradation was not accompanied by anabolism due to the inhibition of cell growth. Therefore, the decrease of NDF from aerobic fermentation was 24.9%, whereas that from anaerobic enzymatic was 29.1 %. The decrease after both process from the raw material was 46.7%.

The activity of the enzymes hydrolyzing fibres into shorter molecules should increase IVDMD of an aerobic fermentation product. However, the IVDMD after the first process was not different, the significant difference only occurred after enzymatic result. The increase of IVDMD after second process with raw material was 26.5%. The result was not similar to the result found in FCM using *Eupenicillium javanicum* (Haryati et al., 1994). In this process IVDMD increased in the first and second process. The different may be due to the different of degradation mechanism. *E javanicum* which is more mannanolytic than *A niger* NRRL337 (Purwadaria et al., 1994a) directly degraded fibres into digested molecules in aerobic fermentation, while *A niger* only degraded well after enzymatic process.

The dry matter loss mineral addition was significantly ($P< 0.05$) increased IVDMD and decrease NDF of both processes than that of without mineral addition. The mineral addition resulted in better cell growth and enzyme activities. The same result did discovered by Haryati et al (1994). The treatment of thickness was not affect on IVDMD and NDF of aerobic fermentation, but only affected on the enzymatic process. The 2 cm thickness did not influence the cell growth, but in the enzymatic process it had more materials or was carried out in bigger bags which was better for the degradation activities.

Enzymatic incubation at 50°C also increased IVDMD and decreased NDF significantly. Optimal temperature for mannanase produced by *Phellinus abietis* was 45°C (Zouckova et al.,1977) and enzyme activity for molds included *A niger* NRRL 337 determined at 50°C (Araujo and Ward, 1990). The optimal temperature resulted optimal activities.

Table 1. In vitro dry matter digestibility (IVDMD) values of fermented coconut meal (%DM).

Incubation time (days)	Enzymatic temperature (°C)	1 cm thickness		2 cm thickness	
		-M	+M	-M	+M
Aerobic fermentation:					
0	--	49.9^{r*}	53.6^{p}	49.9^{r}	53.6^{p}
3	--	50.1^{r}	53.8^{p}	51.2^{q}	52.7^{pq}
Anaerobic enzymatic:					
2	28°	55.4^{d}	55.7^{cd}	56.2^{bcd}	59.5^{bcd}
2	50°	62.1^{abc}	62.6^{ab}	60.4^{bcd}	67.8^{a}

[a] Different characters in the same process are significantly different ($P< 0.05$).

Table 2. Nitrogen detergent fibre content of fermented coconut meal (%DM).

Incubation time (days)	Enzymatic temperature (°C)	1 cm thickness		2 cm thickness	
		-M	+M	-M	+M
Aerobic fermentation:					
0	--	68.8^{s*}	69.0^{s}	68.8^{s}	69.0^{s}
3	--	51.8^{p}	58.4^{q}	52.8^{p}	60.3^{q}
Anaerobic enzymatic:					
2	28°	47.1^{d}	46.5^{d}	43.6^{c}	44.8^{c}
2	50°	37.4^{ab}	38.1^{b}	38.3^{b}	36.7^{a}

[a] Different characters in the same process are significantly different (P< 0.05).

Table 3. In vitro correction protein digestibility (IVCPD) values of fermented coconut meal (%DM)

Incubation time (days)	Enzymatic temperature (°C)	1 cm thickness		2 cm thickness	
		-M	+M	-M	+M
Aerobic fermentation:					
0	--	37.8^{s*}	40.8^{r}	37.8^{s}	40.8^{r}
3	--	57.5^{q}	61.5^{p}	57.2^{q}	61.4^{p}
Anaerobic enzymatic:					
2	28°	57.3^{d}	62.9^{c}	65.1^{bc}	64.5^{bc}
2	50°	63.0^{c}	68.0^{a}	66.2^{ab}	68.0^{a}

[a] Different characters in the same process are significantly different (P< 0.05).

In vitro true protein digestibility (IVCPD) values and correction protein contents (CPC) of fermented coconut meal (FCM)

IVCPD and CPC of FCM after aerobic fermentation and anaerobic enzymatic are presented in Table 3 and 4 respectively. Aerobic fermentation which did not affect for IVDMD increased both IVCPD and CPC significantly (P< 0.05). The result occurred due to both IVCPD and CPC directly depended on cell growth, while IVDMD was more affected by enzyme activities. Therefore, if the cell growth in aerobic fermentation are good, both values will be increased. The increase of IVCPD and CPC after that process was 50.7% and 118% respectively. Both values were higher than those resulted from anaerobic enzymatic namely: 10.5% and 17.5%. Besides protein formation from cell growth, the increase of CPC was also undirectly affected by dry matter losses of carbohydrate and degradation of fat. The increase of CPC than affected the increase of IVCPD. The similar result was also discovered in the fermentation of cassava leaves (Darma et al., 1994).

The cell growth was also correlated with mineral addition. This treatment affected significantly (P<0.05) IVCPD and CPC of both processes. The same result was also obtained from dry matter loss, IVDND and NDF. It can be concluded that mineral addition directly affected the cell growth or CPC and indirectly affect the formation of enzymes which responsible for both processes. The treatment of thickness only

significantly ($P<0.05$) affected CPC of first process and IVCPD of second process. The two cm thickness was better than one cm thickness. If this result is correlated with IVDMD, NDF and dry matter loss, the thickness of 2 cm could be better carried out for big scale production. The ability for thicker substrate will reduce the area of processes.

The treatment of enzymatic incubation at 50°C also significantly ($P<0.05$) increased IVCPD and CPC. It was also interacted with mineral addition. Therefore this treatment which is suitable for enzyme activities is important for FCM production.

Table 4. Correction protein content (CPC) of fermented coconut meal (%DM).

Incubation time (days)	Enzymatic temperature (°C)	1 cm thickness		2 cm thickness	
		-M	+M	-M	+M
Aerobic fermentation:					
0	--	10.6^{t}	11.0^{t}	10.6^{t}	11.0^{s}
3	--	20.1^{s}	23.4^{q}	21.2^{r}	24.0^{p}
Anaerobic enzymatic:					
2	28°	21.2^{c}	23.8^{c}	22.9^{d}	24.6^{b}
2	50°	23.2^{cd}	23.2^{cd}	23.4^{cd}	28.2^{a}

[a] Different characters in the same process are significantly different.

Table 5. The nutritive value analysis of fermented coconut meal and raw materials

Analysis (% DM)	FCM[a]	Raw material[b]
CPC	28.2	11.0
NDF	36.7	69.0
Fat	6.5	18.0
Ca	0.05	0.05
P	0.75	0.69
IVDMD	67.8	53.6
IVCPD	68.0	40.8
Mycotoxins:		
aflatoxin B_1	ND	ND
aflatoxin B_2	ND	ND
aflatoxin G_1	ND	ND
aflatoxin G_2	ND	ND

[a] FCM is a product from aerobic fermentation of 2 cm thickness with mineral addition followed by anaerobic enzymatic at 50°C.

[b] Raw material is a substrate mixed with minerals from 0 hr fermentation and anaerobic enzymatic.

[c] ND is not detected

The nutritive value comparison of fermented coconut meal and raw material.

Overall analyses of FCM and raw material are presented in Table 5. It was already discussed that aerobic fermentation followed by anaerobic enzymatic increased IVDMD,IVCPD and CPC and decreased NDF. The process was also reduce fat content. Eventhough in this experiment the fat content was not determined for every sample, since fat content caused rancidity, this result is also important. The high fat content of raw coconut meal due to poor fat extraction also reduced the protein content of raw coconut meal, therefore the ability of *A niger* to degrade fat would also increase the protein content of FCM. It was reported by Zamora et al. (1989), the fat content of coconut meal used in their experiment 10% or lower than that we used, the protein content was 20% or higher than that we used. The content of Ca was not affected, while the content of P was increased 8.7%. Although the increase only occurred due to dry matter loss, the avaibility of those minerals might be increased. It was reported that *A niger* produced phytase which degrade phytat (complex compound) into inositol and phosphoric acid (available phosphate) (Wang et al., 1980).

The use of fermentation technology to improve law quality feed should also concern the possibility of hazardous contaminants. The suitable microenvironment for *A. niger* NRRL337, will also be suitable for mycotoxin molds such as *A. flavus* , therefore the aflatoxin was also determined. The FCM product was free from aflatoxin. The possibility of product containing bongkrek acid or toxoflavin had not been evaluated. Since the last toxins were caused by bacteria (*Pseudomonas cocovenenans*), which produced slime, the contaminants would be possibly noticed. The hazardous contaminants was also avoided by high spore inoculation.

The possibility of the use of FCM for poultry after in vitro evaluation was high. Zamora et al. (1989) reported that broilers fed by coconut meal treated with mannanases was better than untreated one. In this experiment the enzymes produced directly in the substrate.

Conclusion

Aerobic fermentation followed by anaerobic enzymatic increased the nutritive value of coconut meal.

The best result obtained from the treatment of 2 cm thickness, mineral addition and enzymatic incubation at 50°C.

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