# Improvement of Nutritional Value of Cocoa Pod Husk Fermented with *Aspergillus spp.* and Two Levels of Urea and Ammonium Sulphate

Rakhmani SIW[1], Purwadaria T[2]

[1]*Indonesian Rearch Institute for Animal Production. Jl. Veteran III, Ciawi Bogor, West Java 16720, Indonesia*
[2] *Atma jaya Catholic University*
*E-mail: dyah.haryuningtyas@gmail.com*

(received 19-06-2017; revised 05-09-2017; accepted 11-09-2017)

**ABSTRAK**

Rakhmani SIW, Purwadaria T. 2017. Peningkatan nilai gizi kulit buah kakao yang difermentasi menggunakan *Aspergillus* spp. dengan dua tingkat urea dan amonium sulfat. JITV 22(3): 101-113. DOI: http://dx.doi.org/10.14334/jitv.v22i3.1670

Kulit buah kakao melimpah sebagai produk limbah perkebunan kakao dan berpotensi sebagai bahan pakan tetapi memiliki nilai gizi rendah. Untuk meningkatkan nilai gizi kulit buah kakao (CPH), telah dilakukan proses biologis melalui fermentasi substrat padat menggunakan *Aspergillus oryzae* dan *Aspergillus niger* serta penambahan dua dosis (N1 dan N2) nitrogen dalam campuran mineral. Produknya adalah Fermented Cocoa Pod Husk (FCPH).Kandungan protein meningkat dari 50 g/kg sebelum fermentasi menjadi 133,8 g/kg untuk N1 fermentasi menggunakan *A. niger* dan 150 g/kg menggunakan *A. oryzae*. Kandungan protein sejati adalah 99,8 dan 93,5 g/kg untuk perlakuan N1 dan N2 (*A. niger*); 119 dan 104,1 g/kg untuk perlakuan N1 dan N2 (*A. oryzae*). *Aspergillus niger* menunjukkan keunggulan dalam hal produksi enzim bila dibandingkan dengan *Aspergillus oryzae*. Aktivitas mannanase pada produk fermentasi *A. niger* dengan dosis N1 mencapai 2654 U / g dan *A.oryzae* adalah 1122 U/g. Bahan kering dan kecernaan protein masing-masing untuk FCPH *A. niger* adalah 47 dan 57% dan FCPH *A. oryzae* adalah 52 dan 62%. Proses fermentasi kulit buah coklat menghasilkan produk yang sangat potensial sebagai pakan alternatif dengan kadar protein lebih tinggi dan mengandung enzim mannanase.

**Kata Kunci**: Kulit Buah Coklat, *Aspergillus Niger*, *Aspergillus Oryzae*, Mannanase, Selulase

**ABSTRACT**

Rakhmani SIW, Purwadaria T. 2017. Improvement of nutritional value of cocoa pod husk fermented with *Aspergillus* Spp. and two levels of urea and ammonium sulphate. JITV 22(3): 101-113. DOI: http://dx.doi.org/10.14334/jitv.v22i3.1670

Cocoa pod husk is abundant as a waste product of cocoa plantation and potential as feed ingredient but has low nutritional value. To increase the nutritive value of cocoa pod husk (CPH), biological process through solid substrate fermentation using *Aspergillus oryzae* and *Aspergillus niger* and addition of two doses (N1 and N2) of nitrogen mixture had been done. The product is Fermented Cocoa Pod Husk (FCPH). Protein content increased from 50 g/kg before fermentation to 133.8 g. $kg^{-1}$ for N1 for *A. niger* and 150 g/kg using *A. oryzae*. True protein were 99.8 and 93.5 g/kg for N1 and N2 treatments (*A. niger*); 119 and 104.1 g/kg for N1 and N2 treatments (*A. oryzae*). *Aspergillus niger* showed a superiority in term of enzymes production when compared to *Aspergillus oryzae*. Mannanase activy in *A. niger* fermentation product with N1 dose reached up to 2654 U/g and *A.oryzae* was 1122 U/g. Dry matter and protein digestibility for *A. niger* FCPH were 47 and 57% and *A. oryzae* FCPH were 52 and 62% repectively. Fermentation processed of CPH yield a product that very potential as an alternative feed with higher in protein content and contain mannanase enzyme.

**Key Words**: Cocoa Pod Husk, *Aspergillus niger*, *Aspergillus oryzae*, Mannanase, Cellulose

## INTRODUCTION

In animal husbandry, the feed comprises the highest expense and appropriate feed quality nutrition is important. Shortage of feedstuff is also one of several factors in animal production in Indonesia, especially since most of feed materials (such as corn and soybean meal) are imported. Therefore, in addition to conventional feed ingredients, uncommon feed ingredients such as of agriculture by-product would be

advantage to solve feed insufficiency. Indonesia's cocoa crop is one of the largest estates after palm oil. Indonesian Cocoa plantation (*Theobroma cacao* L.) area currently is reached 1,745,789 hectares and is largely possessed by smallholders with a total production of 903.092 tons/year (Estate Statistic 2008-2012). It will be 695,467 tons of fresh CPH (equivalent to 893,092 dried material) by-product. Waste and by-products of cocoa fruit itself is more than half of world-wide cocoa production and estimated at 3.53 million tons (World Cocoa Foundation 2010). Indonesia, including the 20 largest cocoa producing countries and was ranked the 3rd after Ghana and Ivory Coast.

Cocoa pod husks has been investigated as an alternative feed and had been fed without toxic effects to cattle in quantities up to 7 kg per day. For dairy cows, pod meal seems to be comparable in value to corncob meal. Rations containing cocoa pod meal have a lower feed efficiency for beef cattle, but this will be compensated by the larger intake (FAO 2002). A digestion study with sheep, was carried out to determine the effect of graded dietary cocoa-pod levels between 0 and 75% in feed, the apparent digestibility of cocoa-pod by sheep was also estimated and reported that sheep digested only 23% dry matter and 51% crude protein of the pod (Smith & Adegbola 1985). Processed-CPH was used as feed material and can replace conventional feed ingredients such as corn and soybean meal and regarded as alternative animal feed (Sobamiwa & Longe 1993; Sobamiwa 1998).

Biological processes can improve the nutritional composition and utilization of CPH and would be more advantage as feed. Fermentation process using cellulolytic fungi could be used to overcome high crude fiber content in CPH, which is as a constraint factor for direct utilization of CPH especially in poultry diet. Processing CPH had been reported such as CPH-silage (North Sumatera and Lampung provinces), and fermentation process using *Aspergillus niger* (South Sulawesi and Bali provinces) has done, but there is no detailed information on the nutritional value such as the digestibility, enzymes activities and true protein of the resulting product. It was reported an increase in protein content of 9% to 12% was achieved after fermentation with *A. niger* (Haryati & Sutikno 1994).

Fungi such as *Aspergillus* spp. produce several enzymes which is important to elevate feed digestibility. When CPH was fermented using this fungi, the final product can be fed to livestock without addition of enzymes. It was reported that minerals and inorganic nitrogen can increase cell growth and correlated on enzymes and protein productions in fermentation of cassava by-product using *Aspergillus* spp (Kompiang 1994). In this study, an experiment had been done to investigate the nutritive value of fermented CPH product by *Aspergillus oryzae* and *Aspergillus niger*. Solid substrate fermentation method was applied to steamed CPH with two levels of urea-ammonium sulphate mixtures (0,5% urea + 1% ammonium sulphate and 1% urea+ 2% ammonium sulphate). Proximate composition, cellulolytic enzymes (mannanase and cellulase) activities, dry matter and protein digestibility (*in vitro*) were also examined in the fermented product.

## MATERIALS AND METHODS

### Preparation of CPH and *Aspergillus* inoculums

Fresh CPH were obtained from Nusantara Plantation VIII-Public Company (*PTPN VIII*) at Rajamandala District- West Java, and processed at the IRIAP. The husks were cleaned, chopped into smaller pieces, solar-dried to a moisture content of ca. 10% and ground. The dried husk were then stored at room temperature until used. Fungi *Aspergillus oryzae* GS66 was previously isolated from garlic seed) whereas *A. niger* was obtained from IRIAP collection. Dried-spore inoculums of *A. oryzae* and *A. niger* were prepared by growing the fungi in in cooked-rice substrate for 3-4 days, dried at 40°C, ground and stored at 4ºC (Purwadaria et al. 1994).

### Substrates and fermentation

Three kind of treatments (C, N1, and N2) were applied. Treatment C as control, substrate+50 g rice bran/Kg. The composition of minerals mixture for 1 kg dried substrate as shown as in Table 1. Distilled water was first added to the ground dried CPH substrate to make water content of about 60% then steamed for 30 minutes. After cooling to about 40ºC the minerals and 8 g of inoculum were added. The cultures were incubated at room temperature.

**Table 1.** Mineral Content in Treatments

| Minerals/other materials | Treatments | | |
|---|---|---|---|
| | C | N1 | N2 |
| Trisuperphosphate (TSP) | 0 | 2.40 | 2.40 |
| MgSO4.7H2O | 0 | 1.25 | 1.25 |
| FeSO4. 6H2O | 0 | 0.10 | 0.10 |
| KCl | 0 | 3.80 | 3.80 |
| CaCl2.2H2O | 0 | 0.13 | 0.13 |
| (NH4)2SO4 | 0 | 10.00 | 20.00 |
| Urea | 0 | 5.00 | 10.00 |
| Rice Bran | 50 | 27.32 | 12.32 |

**Laboratory analysis**

All chemical and digestibility analysis were carried out at the Analytical Services and Feed Technology Laboratories, Indonesian Research Institute for Animal Production. Proximate analysis (Crude protein, fat, fiber, ash), Calcium and Phosphor, Energy and van Soest fiber (NDF, ADF, Lignin) were performed in both optimally fermented and unfermented CPH. Optimum fermentation period was determined based on protein content reached in particular time of incubation and was determined using modified Lowry method (Lowry et al. 1951).To obtain total protein concentration, 1 ml of 2*M* NaOH was added to 0.1 g of sample then heated for 30 min at 100ºC. After dilution ten-fold in destilled water, protein concentration was measured by a standard Lowry method using bovine serum albumin (BSA) as a standard.

***In vitro* rumen-pepsin digestibility**

Subsequently, 0.2-0.4 g of experimental samples were weight out in an 100 ml-in vitro tubes, 0.2-0.4 g samples from laboratory standards (grass hay) were added to 2 tubes and 2 tubes were used as blanks for the experiments. In each tube, 25 mL of a Rumen-McDougall buffer mixture was added under purging with $CO_2$ and caped tightly with a rubber stopper/gas-release port. Samples were incubated for 48 h in a water bath at 39°C, followed by further digestion in an acid–pepsin solution containing 6.6 g pepsin /L 0.1N hydrochloric acid (Catalog P6887-SIGMA) (25 mL of acid–pepsin solution was added to each tube) for 48 h in water bath at 39°C. All tubes were mixed by swirling (Vortex Mixer) them at 2, 4, 8, and 36 h, after adding the rumen-buffer mixture and at 2, 4, and 6 h after adding acid–pepsin. After completion of the digestion, contents were filtered into pre-weighed standard coarse fritted disk Gooch crucibles (G2) under mild vacuum, dried at 105°C for at least 12 h, weighed for determination of DM. Protein content of *in vitro* sediment was determined for protein digestibility calculation (Marais & Evenwell 1983).

**True protein analysis**

True protein was measured according Marais & Evenwell (1983) by TCA-precipitation with slightly modification. Sample was finely ground and weight out (0.5-1 g) into a 50 ml tube with caped, add 15 ml of water and incubate 10 min in a boiling water-bath, cooled and added 15 ml of 10% TCA and then shake in a medium speed using Griffin Flask Shaker for 20 min and then left for an hour at 4°C. Mixture was centrifuged at 3000 rpm for 10 min. The pellet was rinsed twice with 5 ml 1% TCA and protein content of the precipitate was determined.

**Enzymes activity**

Mannanase activity was measured using locust gum (0.5 $g.Kg^{-1}$) as substrate and followed the procedure as described by PURWADARIA et al. (2003). Enzymes was extracted from the sample (0.5 g - 1 g) with 10 ml of acetate buffer pH 5.8 and mixed for 30 min. The mannanase activity was measured as the amount of reducing sugar released from the substrate. Reducing sugar was determined by using the 3,4-dinitro salicylic acid (DNS) method of Miller (1959) and mannose was used as standard. One unit mannanase activity was defined as the amount of enzyme that required to release 1 μmole of glucose $min^{-1}ml^{-1}$ under assay condition

Cellulase assays are carried out using the carboxy methyl cellulose (CMC) as substrate. Enzymes solution was obtained by extraction of sample (0.5-1 g) in 25 ml of 0.05 M citrate buffer pH 4.8. The amount of released reducing sugar was quantified using the 3,4-dinitro salicylic acid (DNS) method of MILLER (1959) and glucose was used as standard for determining enzyme activity. One unit of enzyme activity is defined as the amount of enzyme required to release 1 μmole of glucose $min^{-1}ml^{-1}$ under assay condition (Miller 1959).

**Statistical analysis**

Data were subjected to a one-way analysis of variance (ANOVA).

## RESULTS AND DISCUSSION

Growth of *A. niger* and *A. oryzae* on CPH substrate during fermentation period is presented in Table 2. In the first 24 hours mycelium growth was undetected. Mycelia formation was detected in the 2nd day of incubation of both *Aspergillus niger* and *A. oryzae* along with young spores formation scattered for about 10% that can be observed from the substrate surface. For treatment using lower inorganic nitrogen addition (N1), mycelia formation was tend to be slower than higher inorganic nitrogen treatment (N2).

Mycelia became thick and fungal hyphae had been penetrated and bind the substrate was clear in the third day of incubation and a distinction spores color for both species were detected. It was hard to report the difference between inorganic treatments in term of mycelia formation in this stage and in the next day beyond fermentation period. Mycelia thickness was remained in the fourth day and older spore formation

**Table 2.** Visual observation of fungi growth on CPH substrate during fermentation periods

| Incubation Period (day) | *Aspergillus niger* | | *Aspergillus oryzae* | |
|---|---|---|---|---|
| | N1 | N2 | N1 | N2 |
| 1 | * | * | * | * |
| 2 | ++ | ++ | ++ | ++ |
| 3 | +++ | ++++ | +++ | ++++ |
| 4 | ++++ | ++++ | ++++ | ++++ |
| 5 | *** | *** | *** | *** |

*Mycelia growth undetected, raised in temperature was observed
++ Thin covering mycelia, tiny spores
+++ Penetration of mycelia
++++ mycelia covering all over substrate
***spores covering all over substrate surface

almost completely found in both species. In the 5th day, spores were found all over the substrate surface.

In every process of fermentation, dry matter loss of the substrate is inevitable. Dry matter loss of CPH during incubation is presented in Figure 1. In the process, substrate was added water up to 60% water content. It was reported that water holding between 60-65% was suitable for solid substrate fermentation (Chalall 1985; Singhania et al. 2006). In this experiment we found out that the maximum capacity of water holding for CPH was in the ratio of CPH-water 100 : 120. The water content was 600 g/kg, however, when it was higher than that ratio, water was not held perfectly and was flowing out from the substrate. It was reported that 70% moisture level in the substrate prevents oxygen penetration and facilitates the contamination, whereas the low moisture level inhibits the growth, enzyme activity and accessibility to nutrients (Mekala et al. 2008). Dry matter loss profiles for both *A. niger* and *A. oryzae* were similar, all below 300 g/kg, and for control group (without addition of minerals), DM loss was detected below 150 g/kg. The moisture level of culture medium affects the physiology of the microorganism.

It was a significant increased ($P<0.05$) in crude protein content of fermented CPH when compared with the control group. The highest protein content in the fermentation product of *A. niger* was shown in the 4th day of incubation (Figure 2). Protein content in N1 treatment was 133.8 g/kg and N2 treatment 131.9 g/kg statistically not significantly different When CPH was fermented using *A. oryzae*, the highest protein content was detected at the 3rd day of incubation (154 g/kg for N1 and 148.8 g/kg for N2). The CP in the *A. oryzae* FCPH statistically not significantly different between N1 and N2. It had been reported that fungal fermentations on several agro-byproduct such as cassava by-product, coffee husk, corn bran and rice bran have also reported similar increases in protein content (Leifa et al. 2001; Iyayi & Aderolu 2004). Solid-state fermentation of CPH using *Rhizopus stolonifer* LAU was reported an increase in CPH protein up to 95% (Lateef et al. 2008). The increased in protein content of CPH fermented by *A. niger* and *A. oryzae* could be due to bioconversion of some of soluble carbohydrates in the substrate into mycelia protein or single cell protein (SCP) by the growing fungus (Iyayi 2004). It was also reported that the growth and sporulation of the fungus *Aspergillus niger* is influenced by the level of nitrogen (from ammonium sulfate and urea) in the culture medium (Swe et al. 2009). However, in this experiment, in the higher of substrate-nitrogen level (N2) CP was not significantly different as of lower nitrogen level (N1). It showed that nitrogen level that supposed to be used by fungi for growing not necessarily high but in some point it will be efficient. Nitrogen level of N1 treatment showed efficiency in using inorganic fertilizer for fermentation process. It will open an opportunity for using more CPH fermentation product as non-ruminant feed ingredient without worried to urea toxic effect.

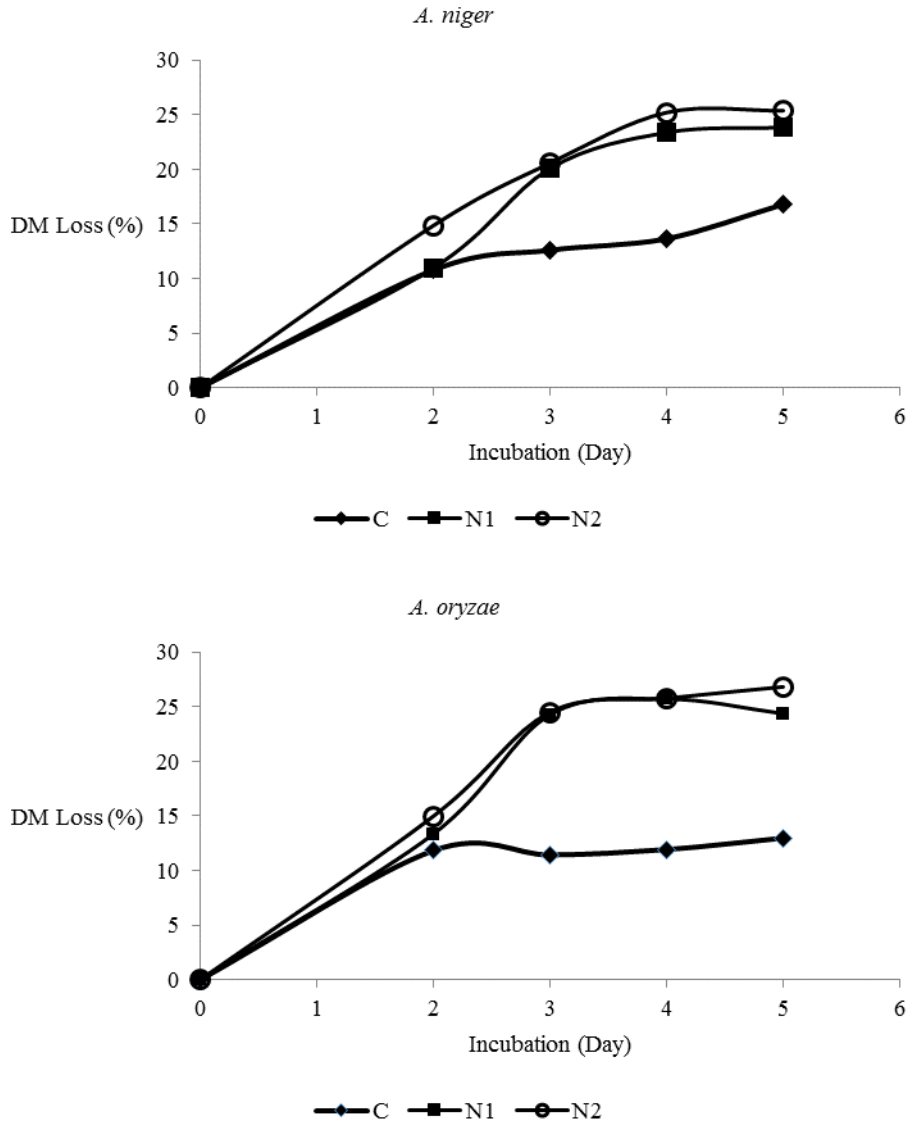


**Figure 1.** Dry matter loss on CPH substrate fermented with *A. niger* and *A. oryzae.*

Total protein analysis with KJELDAHL digestion measures crude protein, where the total nitrogen content of sample is multiplied by 6.25 to express the results on a protein equivalent basis. The total amount of nitrogen in feed/feed ingredient however, comes from both protein and non-protein nitrogen sources. True protein reflects only the nitrogen associated with protein and does not include the nitrogen from non-protein sources. The true protein was lower than crude protein content and shown in Figure 3. The true protein content in control treatment was between 33.7 and 51.11 g/kg, whereas in fermentation product were 99.8 g/kg and 93.5 g/kg for N1 and N2 treatments respectively (*A. niger*) and 119.0 g/kg and 104.1 g/kg both for N1 and N2 treatments (*A. oryzae*) respectively. The true protein content of fermentation products of *A. oryzae* significantly higher than *A. niger* in both nitrogen levels. It is important to measure the true protein instead of crude protein due to inorganic nitrogen that was added to boost the growth of fungi in the beginning of fermentation will give inaccurate in crude protein calculation. In this experiment, the non-protein fraction in fermented CPH was calculated between 23 and 30%. Residual inorganic nitrogen is included in this fraction. Inorganic nitrogen such as from ammonium sulfate or urea will affect negatively especially in non-ruminant animals, but ruminant can tolerate urea and is used by rumen microbe for protein synthesis.

Proximate analysis of fermented product is presented in Table 3. Control treatment was ground CPH, which was not added any mineral mixture and directly fermented using *A. niger* (AN) and *A. oryzae*

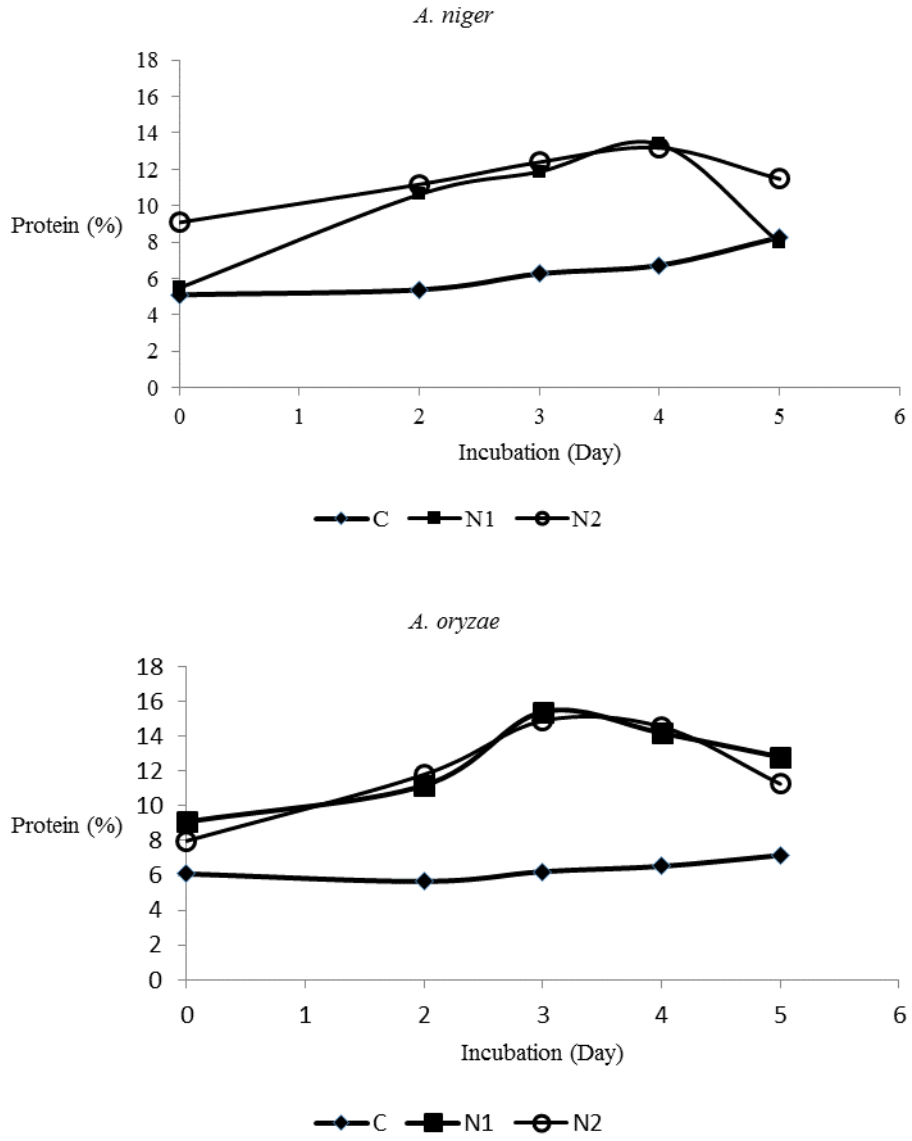


**Figure 2**. Protein profile of fermentation product (Statistic analysis was done for 3rd and 4th day incubation: the CP was not significantly different between N1 and N2 but significantly higher in *A. oryzae* than *A. niger*).

(AO) inoculums respectively. Growth of fungi in control treatment was detected at the 2nd day of fermentation but for the next day onward the growth was not optimal. The slightly growth of fungi in control batch could explained on increased of crude protein (CP) content of control treatment. The CP content and other data of fermented products from the 3rd and 4th day of incubation period using *A. niger* and *A. oryzae* as fermentation agents were subjected to statistical analysis. Crude protein (CP) content of fermented CPH in N1 treatment was increased up to 140 and 205% when fermented with *A. niger* and *A. oryzae* respectively. However, another treatment (N2) was shown a lower CP increases up to 63 and 187% for *A. niger* and *A. oryzae* FCPH.

Crude fat was decrease significantly for N2 treatment (34 and 35% for *A. niger* and *A. oryzae* fermented CPH) and was higher than in N1 treatment. Neutral Detergent Fiber (NDF) and ADF also decrease 13% for *A.niger* FCPH - N1 treatment, 26% for *A. oryzae* FCPH-N1 treatment, 29% for *A. niger* FCPH N2-treatment and 27% AO FCPH N2 treatment. Crude fiber (CF) also decreased 42% and 55% for *A.niger* FCPH N1 and *A. oryzae* FCPH N1 respectively, and a lower value of decreased was detected on N2 treatment

that is 36 and 46% for *A. niger* FCPH and *A. oryzae* FCPH respectively.

Rumen-pepsin dry matter digestibility is presented in Figure 4. It is clearly shown that fermented product of CPH have higher DM digestibility when compared to control. And *A. oryzae* FCPH both N1 and N2 treatments showed a higher DMD value than *A. niger* FCPH. The same pattern is also shown for rumen-pepsin protein digestibility (Figure 5). Rumen-pepsin protein digestibility of *A. oryzae* FCPH N1 treatment was closed to the protein digestibility of King grass that was run at the same time as a comparison.

Cocoa pod husks utilization for poultry is constrained by the high content of fiber, including lignin (14% w/w) and non-starch polysaccharides (NSPs) such as hemicellulose (110 g/kg), cellulose (350 g/kg) and pectin (60 g/kg) (Alemawor et al. 2009) and need enzyme fortification to increase digestibility. Fermentation using *Aspergillus niger* showed a superiority in term of mannanase and cellulase enzymes production (Figures 6 and 7) when compared to *Aspergillus oryzae*. Mannanase activy in *A. niger* FCPH N1 treatment reached up to 2654 U/gDM and *A. oryzae* FCPH N1 was only 1122 U/gDM. Cellulase activity was lower than mannanase, for AN FCPH N1 treatment reached 1255 U/gDM. Fermentation process increased nutrients composition and digestibility of CPH

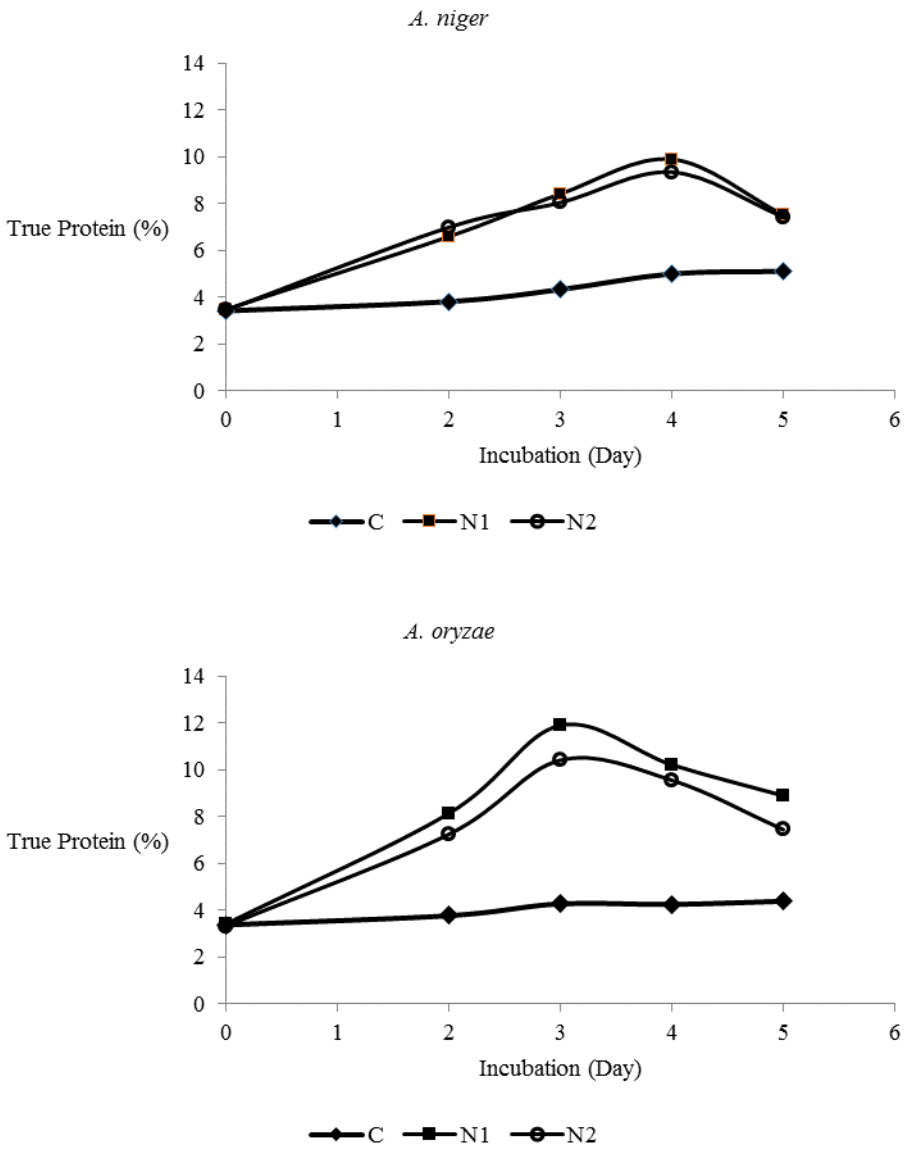


**Figure 3.** True Protein content of *A. niger* and *A. oryzae* fermentation product.

**Table 3.** Proximate analysis of fermented CPH at specific day of incubation (unit in g/Kg except it is stated)

| Composition | Control | | | | | | N1 | | | | N2 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | D0 | | D3 | | D4 | | D0 | | D4 | D3 | D0 | | D4 | D3 |
| | *A.nig* | *A.ory* | *A.nig* | *A.ory* | *A.nig* | *A.ory* | *A.nig* | *A.ory* | *A.nig* | *A.ory* | *A.nig* | *A.ory* | *A.nig* | *A.ory* |
| CP | 58.2 | 51.9 | 88.0 | 56.4 | 92.9 | 62.7 | 58.2 | 51.7 | 139.4 | 157.9 | 58.2 | 54.2 | 95.1 | 155.4 |
| Fat | 11.2 | 11.2 | 7.6 | 7.6 | 7.3 | 7.3 | 9.2 | 9.2 | 7.8 | 8.6 | 11.2 | 11.4 | 7.4 | 7.4 |
| Ash | 99.3 | 99.3 | 98.4 | 98.4 | 102.9 | 102.9 | 112.9 | 112.9 | 104.4 | 104.4 | 114.5 | 115.7 | 119.3 | 119.3 |
| CF | 518.4 | 519.0 | 517.6 | 553.7 | 543.2 | 542.9 | 517.8 | 526.7 | 302.0 | 234.6 | 513.3 | 542.1 | 328.0 | 289.8 |
| GE (Kkal/kg) | 3,870 | 3,857 | 3,844 | 3,832 | 3,848 | 3,838 | 3,849 | 3,837 | 3,805 | 3,795 | 3,756 | 3,618 | 3,770 | 3,770 |
| NDF | 652.0 | 652.0 | 651.3 | 641.3 | 666.2 | 643.2 | 650.0 | 650.2 | 565.8 | 482.5 | 667.8 | 653.7 | 473.2 | 476.8 |
| ADF | 621.3 | 621.3 | 631.8 | 631.6 | 641.5 | 641.0 | 611.1 | 612.0 | 482.3 | 497.7 | 622.8 | 623.4 | 416.5 | 416.5 |
| Ca | 3.6 | 3.8 | 4.8 | 4.8 | 4.5 | 4.6 | 7.2 | 7.7 | 4.9 | 5.0 | 6.6 | 6.5 | 6.5 | 6.5 |
| P | 1.8 | 1.8 | 1.8 | 1.8 | 1.6 | 1.6 | 2.3 | 2.2 | 1.9 | 1.9 | 1.8 | 1.8 | 2.3 | 2.3 |

A.nig= *Aspergillus niger*; A. ory=*Aspergillus oryzae*; CP= crude protein; CF= crude fiber; GE= Gross energy; NDF= neutral detergent fiber; ADF= acid detergent fiber

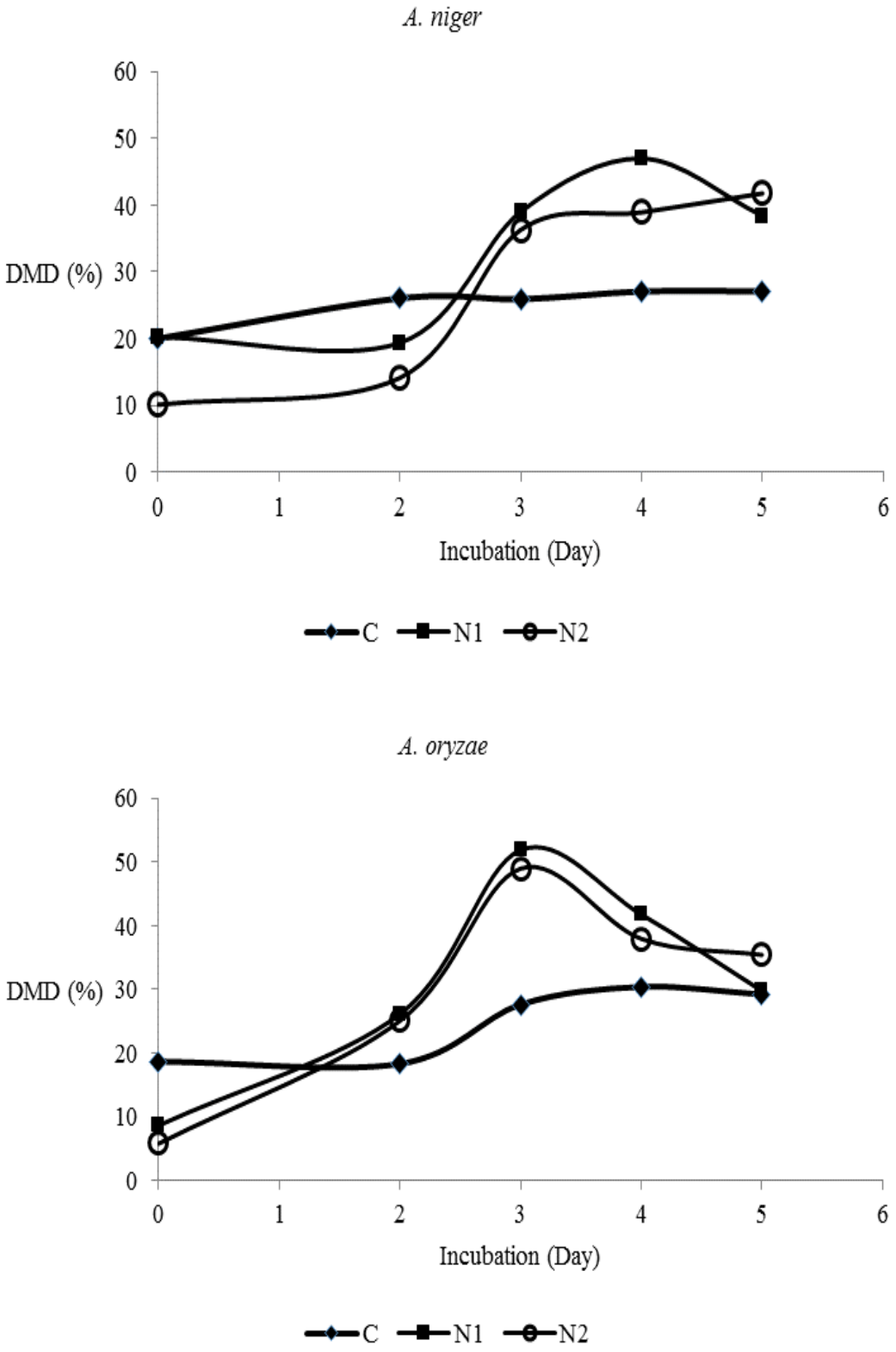


**Figure 4.** Dry matter digestibility of FCPH after rumen-pepsin *in vitro*.

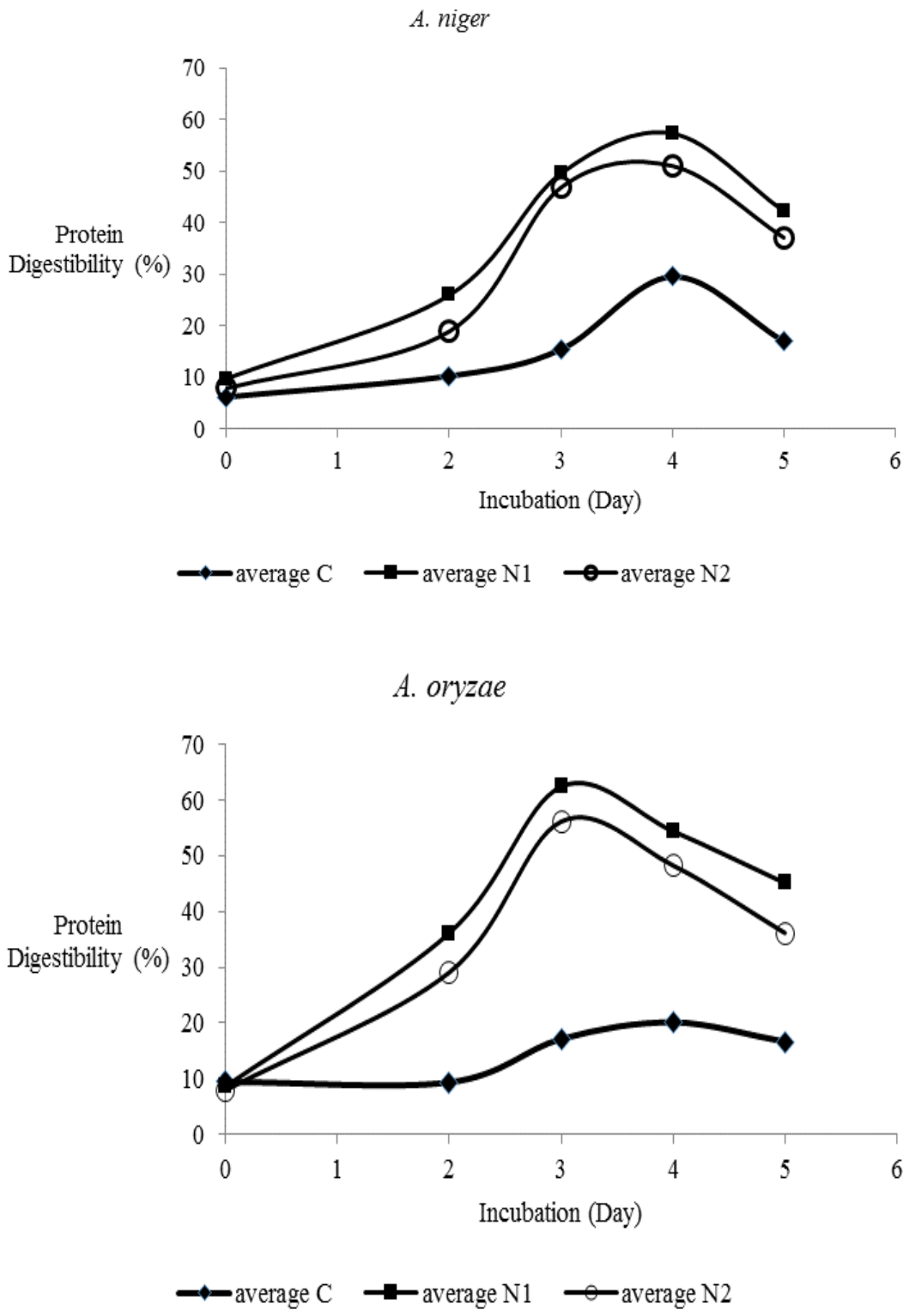


**Figure 5.** Protein digestibility *in vitro* rumen-pepsin of FCPH.

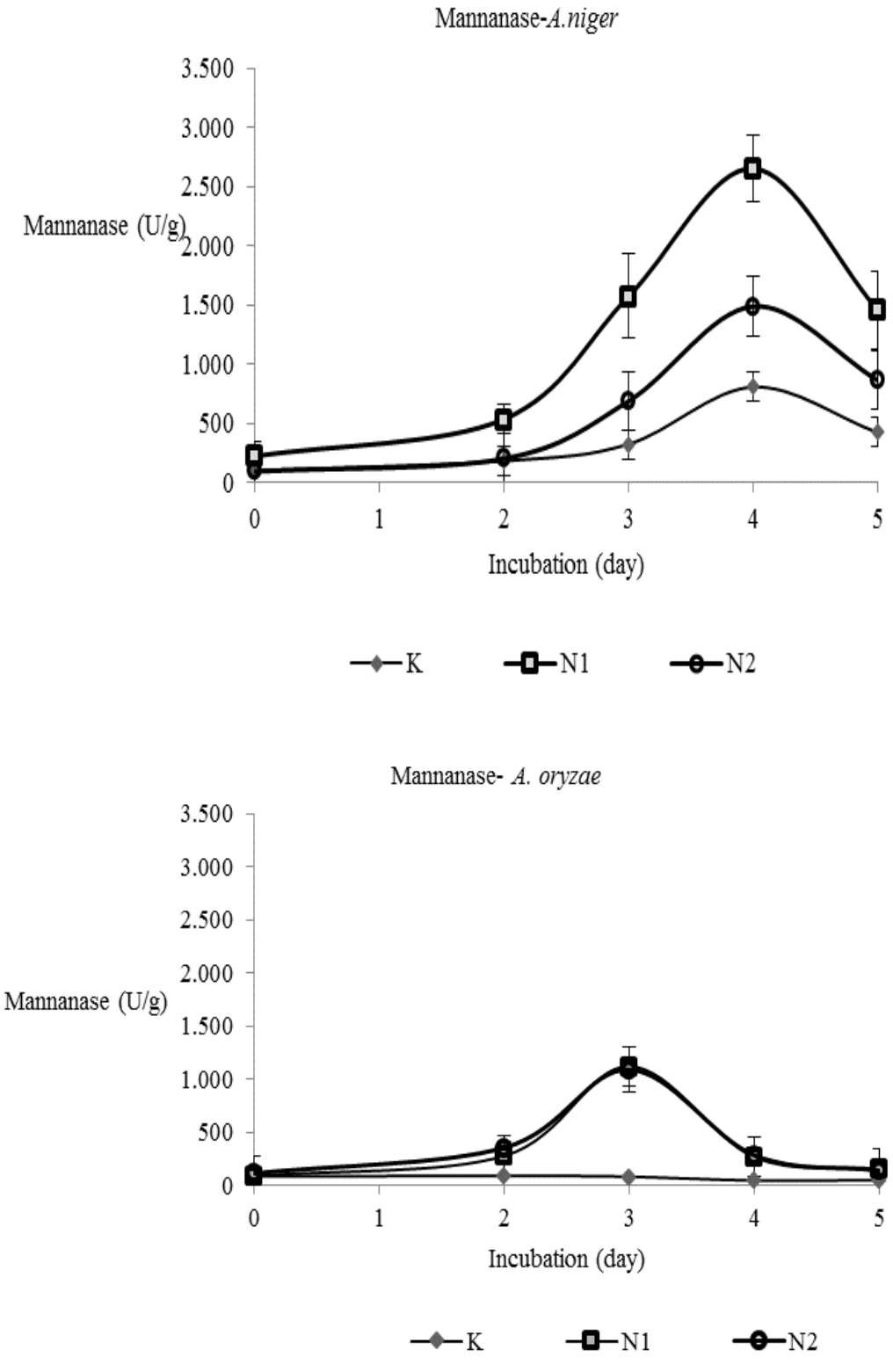


**Figure 6.** Mannanase activity in fermentation products.

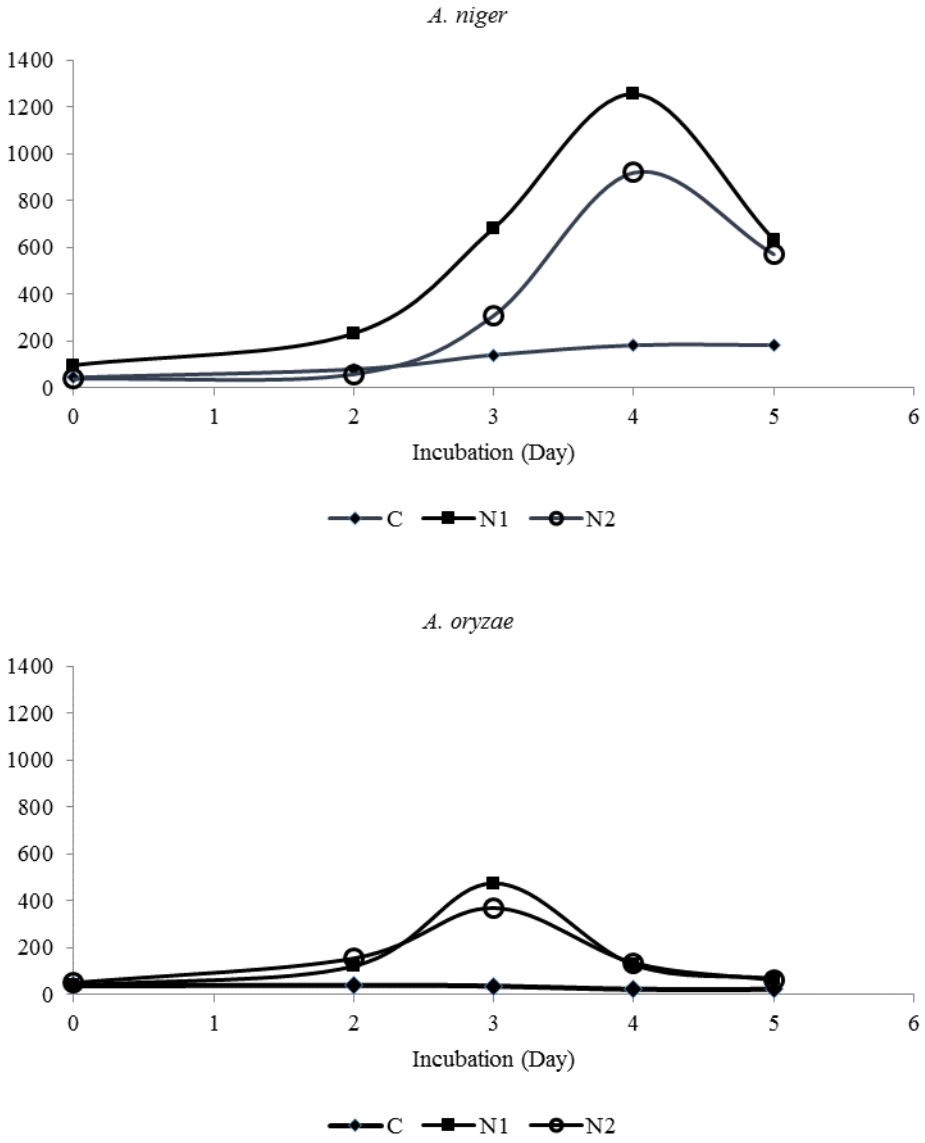


**Figure 7**. Cellulase activity in fermentation products.

## CONCLUSION

The Nutritive value cocoa pod husk can be improved through fermentation using *A. niger* and *A. oryzae*. It was an increased in protein content and decreased in fiber fractions (crude fiber, NDF and ADF). Mannanase and cellulase content also contributed for increasing in digestibility. Fermentation product of CPH is very potential as an alternative feed for mitigation in feed shortage.

## ACKNOWLEDGEMENT

This study was funded by the Indonesian government (APBN 2011). The authors thank all the technicians (Mrs. Emma Ludia and Mrs, Emi Sujatmika) who helped in preparation of samples and doing laboratory analyses. We also express our appreciation to Romaulina Yohana Septiani (AKA Bogor Polytechnic) who took part in this research activity.

## REFERENCES


Alemawor F, Dzogbefia VB, Emmanuel OK, James HO. 2009. Enzyme cocktail for enhancing poultry utilization of cocoa pod husk. Francis Sci Res Essay**.** 4:555-559.

Chalall DS. 1985. Solid-substrate fermentation with *Trichoderma reseei* for cellulase production. Appl Env Microbiol. 49:205-210.

Elmakeel EA, Titgemeyer EC, Johnson BJ, Armendariz CK, Shirley JE. Fibrolytic enzymes to increase the nutritive value of dairy feedstuffs. J Dairy Sci. 90:5226-5236.

[FAO] Food and Agricultural Organization. 2002. Animal feed resources information system. USA: Food and Agricultural Organization. p. 76-82.

Haryati T, Sutikno I. 1994. Peningkatan nilai nutrisi kulit biji coklat melalui bioproses menggunakan beberapa jenis kapang. Ilmu Peternakan. 8:34-37.

Iyayi EA, Aderolu ZA. 2004. Enhancement of the feeding value of some agro-industrial by-products for laying hens after their solid state fermentation with *Trichoderma viride*. Afr J Biotechnol. 3:182-185.

Iyayi EA. 2004. Changes in the cellulose, sugar and crude protein contents of agro-industrial by-products fermented with *Aspergillus niger, Aspergillus flavus* and *Penicillium* sp. Afr J Biotechnol. 3:186-188.

Kompiang IP. 1994. Cassapro, a promising protein enriched cassava as animal and fish feed. Indones Agric Res Develop J. 16:57-63.

Lateef A, Oloke JK, Gueguim Kana EB, Oyeniyi SO, Onifade OR, Oyeleye AO, Oladosu OC, Oyelami AO. 2008. Improving the quality of agro-wastes by solid-state fermentation: Enhanced antioxidant activities and nutritional qualities. World J Microbiol Biotechnol. 24:2369–2374.

Leifa F, Padey A, Soccol CR. 2001. Production of *Flammulina velutipes* on coffee husk and coffee spend-ground. Braz Arch Biol Tech. 44:205-212.

Lowry OH, Rosebrough NJ, Farrand AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. J Biol Chem. 193:265-275.

Marais JP, Evenwell TK. 1983. The use of trichloroacetic acid as precipitant for the determination of 'true protein' in animal feeds. S Afr J Anim Sci. 13:138-139.

Mekala NK, Singhania RR, Sukumaran RK, Pandey A. 2008. Cellulase production under solid state fermentation by Trichoderma reesei RUT C30: Statistical optimization of process parameters. Appl Biochem Biotechnol. 151:122-131.

Miller GL. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chemist. 31:426–428.

Purwadaria T, Haryati T, Darma J. 1994. The development of inoculum production of *Aspergillus niger* for cassapro fermentation. Proceeding Seminar on National Science and Technology of Animal Husbandry. Bogor (Indones): Indonesian Research Institute for Animal Production.

Purwadaria T, Nirwana N, Ketaren PK, Iswantini D. 2003. Synergistic activity of enzymes produced by *Eupenicillium javanicum* and *Aspergillus niger* NRRL 337 on palm oil factory wastes, Biotropis. 20:1-10.

Singhania RR, Sukumaran RK, Pillai A, Prema P, Szakacs G, Pandey A. 2006. Solid-state fermentation of lignocellulosic substrates for cellulase production by Trichoderma reesei NRRL 11460. Indian J Biotechnol. 5:332-336.

Smith OB, Adegbola AA. 1985. Studies on the feeding value of agro-industrial by-products. III. Digestibility of cocoa-pod and cocoa-pod-based diets by ruminants. Anim Feed Sci Technol. 13:249-254.

Sobamiwa O, Longe OG, 1993. Utilization of cocoa pod pericarp fractions in broiler chick diets. Anim Feed Sci Technol. 44:237-244.

Sobamiwa O. 1998. Performance and egg quality of laying hens fed cocoa husk based diets. Nigeria J Anim Prod. 25:22-24.

Swe KH, Alimon AR, Ramin M. 2009. Effect delaying sporulation by addition of ammonium sulphate on the fermentation of palm kernel cake based subtrate by *Aspergillus niger*. Am J Agric Biol Sci. 4:262-265.

World Cocoa Foundation. 2010. In: FAOstat, cocoa market update. Published repports and ressources ed. Washington DC (USA): Food and Agricultural Organization.