The effect of palm kernel meal (PKM) fermentation by different level and time using *Aspergillus Niger* to nutrition composition and digestibility on the sensi Agrinak-1 Chicken

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Abstract. The study consisted of two stages; the first stage was to evaluate the chemical nutritional content of palm kernel meal fermented *Aspergillus niger* then followed by a biological test of the palm kernel meal treatment. The research was conducted using a factorial completely randomized design. The first factor was the dose of *Aspergillus niger* inoculum: D1 = 4 g/kg DM PKM, D2 = 8 g/kg and D3 = 12 g/kg. The second factor is fermentation time: T1 = fermentation for 3 days, T2 = 6 days and T3 = 9 days. The fermented PKM results were analysed for chemical composition followed by a biological test. The biological test was carried out by placing 30 Sensi Agrinak-1 chickens in individual cages. Chickens were fed palm kernel meal in the first treatment by force feeding as much as 40 g/head. Observation data were tabulated and statistically analysed. The results showed that fermentation of oil palm kernel meal using a dose of *Aspergillus niger* and different fermentation times could increase the nutrients (chemicals) of palm kernel meal, especially the content of organic matter, besides that it can also increase the digestibility value of dry matter and organic matter in native chickens.

1. Introduction
Palm kernel meal is a by-product of the oil palm processing industry and its availability in Indonesia is very high. The use of PKM as a component of animal feed has been widely used [1], chicken [2]. The limiting factor for the use of PKM as feed especially for poultry is its high fibre content (mannose; 56.4% of the total cell wall of PKM in the form of β-mannan bonds [3]. Because of its high crude fibre content, it is necessary to ferment it to hydrolyse the mannan substrate into mannoooligosaccharides, a little mannose, glucose and galactose which are simple forms of material that are more easily absorbed by livestock [4].

Native chickens or free-range chickens are known to be more tolerant of low-quality feed ingredients [5]. Providing rations with 10.0-15.0% crude fibre content to native chickens showed that egg production and efficiency of use of rations were better compared to low crude fibre (5.0-9.6%) [6], but the crude fibre content was higher than 15%, it can lead to decrease feed productivity and efficiency [7].

*Aspergillus niger* is a type of fungus that is most commonly used to ferment animal feed ingredients. *Aspergillus niger* can produce enzymes, especially manhanase, galactomannans and cellulase enzymes so that fermentation of high starch solid substrates using Aspergillus niger can reduce crude fibre levels, increase protein levels and digestibility in vitro [8]. Fermentation of PKM using *Aspergillus niger* is better able to increase crude protein and reduce neutral detergent fibre (NDF) and acid detergent fibre.
than using *Trichoderma harzianum* [9]. The quality of PKM fermentation can be determined by looking at its metabolic energy and digestibility values [10]. Based on the description above, it is necessary to conduct research to determine the nutritional content of palm kernel meal with *Aspergillus niger* fermentation, both chemically and biologically

2. Materials and methods

The study consisted of two stages; the first stage was to evaluate the chemical nutritional content of palm kernel meal fermented with *Aspergillus niger* then followed by a biological test of the palm kernel meal treatment of 5 months old Sensi Agrinak-1 chickens.

Phase evaluation of the nutritional content of fermented palm kernel meal conducted using a factorial completely randomized design. The first factor was the dose of *Aspergillus niger* inoculum: D1 = 4 g/kg dry matter PKM, D2 = 8 g/kg PKM dry matter and D3 = 12 g/kg dry matter. The second factor is fermentation time: T1 = fermentation for 3 days, T2 = fermentation for 6 and T3 = fermentation time for 9 days. The parameters observed were the chemical composition (nutrition) of PKM in the form of: moisture content, ash, crude protein, crude fat, crude fibre, gross energy, NDF and ADF.

Phase biological test of palm kernel meal treat digestibility on the Sensi Agrinak-1 Chicken. The analysis results of ash, nitrogen, crude protein and metabolic energy were used to calculate the digestibility of organic matter, protein, nitrogen retention and metabolic energy.

2.1. Observed variables

Protein Digestibility: Consumed CP was level CP feed x total consumption and Excreta CP was total of excreta x CP excreta. Urine CP was 30% x excreta C, Excreta CP corrected was Excreta CP – Urine CP [11]

\[
\text{Protein Digestibility} = \frac{\text{consumed CP} - \text{crude protein of excreta corrected}}{\text{consumption of crude protein}}
\] (1)

Nitrogen Retention (%): This value can be obtained from the difference between the value of crude protein consumption (CPC) and the value of protein excreted (EP) after being corrected by the value of endogenous protein excretion (EPE). In other words, nitrogen retention (RN) is the difference between the value of crude protein consumption and the value of crude protein excreted after being corrected by the value of endogenous protein excretion

\[
\text{RN}\% = \frac{(\text{Consumed Nitrogen (g/head)}) - (\text{Excretion Nitrogen (g/head)} - \text{Endogenous Nitrogen (g/head)})}{\text{Consumed Nitrogen}} \times 100\% \tag{2}
\]

Dry Matter Digestibility (DMD): Measurement of dry matter digestibility (DMD) based on formula [12] is carried out with the formula:

\[
\text{DMD(\%)} = \frac{\sum \text{DM Consumed (g)} - \sum \text{DM in feces (g)}}{\sum \text{DM Consumed (g)}} \times 100\% \tag{3}
\]

Organic matter digestibility (OMD): The measurement of digestibility of organic matter (OMD) based on formula [13] was carried out with the formula:

\[
\text{OMD(\%)} = \frac{\sum \text{organic material consumed (g)} - \sum \text{organic material in feces (g)}}{\sum \text{organic material consumed (g)}} \times 100\% \tag{4}
\]

Metabolic energy (kcal/kg): Metabolic energy is the difference between the gross energy content of the ration and the gross energy lost through excreta. Metabolic energy is expressed by 3 variables [14], namely:
Pseudo Energy Metabolism (PEM) (kkal/kg):

\[ PEM = \frac{(FGE \times X) - (EGE \times Y)}{X} \]  \hspace{1cm} (5)

Pure Metabolic Energy (PME) (kkal/kg):

\[ PME = \frac{(FGE \times X) - (EGE \times Y) - (EGE \times Z)}{X} \]  \hspace{1cm} (6)

Pseudo Metabolic Energy Corrected Nitrogen (PMECN) (kkal/kg):

\[ PMECN = \frac{(FGE \times X) - (EGE \times Y) + (8.22 \times RN)}{X} \]  \hspace{1cm} (7)

Information
FGE : Feed gross energy (kkal/kg)
EGE : Excreta gross energy (kkal/kg)
X : Feed consumption (gram)
Y : Excreta weight of fed chickens (gram)
Z : Fasted excreta weight of chicken (gram) [15]
NR : Nitrogen Retention

PMECN / FGE conversion: Digestibility is not determined by the value of metabolic energy either pseudo (PEM), Pure Metabolic Energy (PME) Pseudo Metabolic Energy Corrected Nitrogen (PMECN) or pure nitrogen corrected (PNC), but is determined by the conversion of PMECN to gross energy or the FGE / PME ratio of the ration.

\[ \frac{PMECN}{FGE} = \frac{PMECN_{FGE}}{FGE} \]  \hspace{1cm} (8)

Information
PMECN : Metabolic energy is pseudo-nitrogen corrected (kkal/kg)
RGE : Ration gross energy (kkal/kg)

The calculated data obtained were analysed using variance to determine the difference between each treatment. If there is a real effect, it is followed by Duncan's Multiple Range Test.

2.2. Implementation of research
Evaluation of the nutritional content of fermented palm kernel meal. PKM fermentation is carried out by: 1 kg of fine PKM added 600 ml of water and mineral solution containing 0.5% sugar (to stimulate microbial growth) then sterilized by steaming for 30 minutes. Then left at room temperature so that the PKM mixture cools down. After cold then mixed with mold starter (Aspergillus niger according to treatment). Then the mixture is placed in a plastic pan and stored at room temperature (30°C) for aerobic fermentation according to the treatment. After the PKM fermentation, the treatment was complete, the PKM was heated at 60°C for 2 days and then analysed the data on the observed parameters [16]. The parameters observed were the chemical composition (nutrition) of PKM in the form of: moisture content, ash, crude protein, crude fat, crude fibre, gross energy, NDF and ADF. Observation data were tabulated and statistically analysed using the t test to determine the effect between treatments [17].

Phase biological test of palm kernel meal treat digestibility on the Sensi Agrinak-1 Chicken. Sensi Agrinak-1 chickens aged 5 months as many 30 head were adapted for 3 days in individual cages with a size of 35 x 35 x 45 cm. Next, the chickens were fasted for 36 hours and the excreta during fasting were collected then weighed and were heated at a temperature of 60°C for 2 days. After being fasted, the chickens are given fermented PKM feed according to the first stage of treatment by force feeding in the
form of flour which is put into the oesophagus using a tool (modified infusion tube) of 40 gr/head, and drinking water is given ad libitum. The excreta were collected after being given treated feed for 24 hours, and the excreta that came out was sprayed with 5% boric acid every 3 hours. Then the excreta were weighed and dried using an oven at 60°C for three days. The dry excreta were analysed for the content of ash, nitrogen, crude protein and metabolic energy. The analysis results of ash, nitrogen, crude protein and metabolic energy were used to calculate the digestibility of organic matter, protein, nitrogen retention and metabolic energy.

3. Results and discussion

3.1. Nutritional composition of fermented palm kernel meal

The results of fermentation of palm kernel meal with a dose of Aspergillus niger and different fermentation time to change in chemical composition (nutrition) compared to unfermentation can be seen in Table 1.

| Parameter          | Treatment | D1T1 | D1T2 | D1T3 | D2T1 | D2T2 |
|--------------------|-----------|------|------|------|------|------|
| Moisture Content (%) |           | 6.91a | 7.73a | 6.16a | 6.72a | 7.91a |
| Ash Content (%)    |           | 4.91a | 4.69ab | 4.59b | 4.67ab | 4.62ab |
| Dry Material (%)   |           | 95.09a | 95.31a | 95.41a | 95.33a | 95.38a |
| Crude protein (%)  |           | 17.59a | 18.17a | 15.82a | 15.84a | 17.71a |
| Crude Fibber (%)   |           | 16.25a | 16.92a | 16.11a | 16.47a | 18.92a |
| Crude Fat (%)      |           | 4.71a | 4.58a | 4.52a | 4.43a | 4.74a |
| ADF (%)            |           | 39.71a | 37.19a | 37.13a | 36.93a | 33.98a |
| NDF (%)            |           | 66.57a | 69.67a | 72.48a | 70.02a | 70.49a |

From Table 1, it can be seen that the fermentation treatment of palm kernel meal with a combination of Aspergillus niger inoculum dose (4, 8, 12 gr / kg DM) and fermentation time (3, 6, 9 days) causes changes in the chemical composition (nutrition) of palm oil even though statistical test did not provide a significant difference. There was an increasing in crude protein content while the water content, crude fat and NDF decreased. This occurs due to the activity of the Aspergillus niger fungi’s which carries out metabolic processes. The aerobic fermentation process will produce CO2 and H2O as well as evaporation of water, resulting in a decreasing in the water content of the media and an increasing in the ash content of the fermentation products. The real ash content (P <0.05) affected the D1W1 treatment (Aspergillus niger dose of inoculum 4 gr / kg BK and 3 days fermentation time) this indicates that the fermentation process in this treatment optimally increases the biomass of the media so that it also affects changing in other chemical composition.
The higher the inoculum used causes more mycelium to be produced so that it will increase the total nitrogen content proportionally due to the degradation of crude fibre and changes in carbohydrates into energy needed for the growth process of fungi, resulting in an increasing in the protein content of fermented PKM. This protein increasing due to the work of microbes and the contribution of protein from microbes during their growth. Also described in the process of lignin degradation by the fungus Aspergillus niger, due to the activity of lignocellulosic enzymes that can break lignin bonds with cellulose, lignin bonds with hemicellulose and lignin bonds with proteins. By breaking the lignin bonds, it will indirectly result in a decrease in the NDF content of PKM.

NDF is a feed substance that is insoluble in neutral detergents and NDF is the largest part of the plant cell wall. This material consists of cellulose, hemicellulose, lignin and silica as well as fibrous protein [18]. The NDF content of palm kernel meal decreased after fermentation but the crude fibre and ADF content did not decrease even though it was fermented, this occurred because the degradation of NDF was higher than ADF by the enzyme produced by Aspergillus niger, because NDF contains a soluble fraction, namely hemicellulose [19]. NDF content has a negative correlation with the breakdown rate [20]. Meanwhile, ADF is a food substance that is insoluble in acidic detergents consisting of cellulose, lignin and silica [16]. The easily digested ADF component is cellulose, while lignin is difficult to digest, has double bonds, so that the lignin content is more difficult to degrade [21].

3.2. Biological test of fermented palm oil
The fermented palm kernel meal with a dose of Aspergillus niger and different fermentation time was subjected to a biological test in order to obtain the digestibility value of each parameter. The digestibility value of fermented and unfermented palm kernel meal can be seen in Table 2.

| Parameter                     | Treatment       |
|-------------------------------|----------------|
|                               | D1T1  | D1T2  | D1T3  | D2T1  | D2T2  |
| Protein Digestibility (%)     | 71,25a | 64,34a | 60,94a | 61,70a | 60,57a |
| Nitrogen Retention (%)        | 45,56a | 46,67a | 48,35a | 45,02a | 55,22a |
| Dry Matter Digestibility (%)  | 21,79ab| 9,02ab | 12,81ab| 29,67a | 19,51ab|
| Organic matter digestibility (%) | 25,26ab | 12,05ab | 15,53ab | 31,80a | 22,18ab |
| Pseudo Energy Metabolism (kкал/kg) | 2.420,75a | 2.946,20a | 2.593,55a | 2.327,27a | 2.451,41a |
| Pure Metabolic Energy (kкал/kg) | 2.244,32a | 2.786,76a | 2.382,90a | 2.161,11a | 2.219,28a |
| Energy Pseudo Metabolic Energy Corrected Nitrogen (kкал/kg) | 2.428,47a | 2.955,79a | 2.603,49a | 2.334,47a | 2.462,76a |
| PMECN/ FGE conversion        | 0,57a  | 0,69a  | 0,61a  | 0,55a  | 0,58a  |
|                               | D2T3  | D3T1  | D3T2  | D3T3  | D3T3  |
| Protein Digestibility (%)     | 55,12a | 54,25a | 54,00a | 57,54a | 51,54a |
| Nitrogen Retention (%)        | 43,04a | 47,57a | 42,50a | 41,66a | 44,24a |
| Dry Matter Digestibility (%)  | 15,04ab| 12,05ab | 5,87b  | 12,17ab| 17,01  |
| Organic matter digestibility (%) | 19,45ab | 14,93ab | 8,08b  | 14,69ab| 20,64  |
| Pseudo Energy Metabolism (kкал/kg) | 2.525,97a | 2.679,49a | 2.505,15a | 2.283,80a | 2.156,63 |
| Pure Metabolic Energy (kкал/kg) | 2.340,28a | 2.489,43a | 2.299,08a | 2.084,15a | 1.947,64 |
| Energy Pseudo Metabolic Energy Corrected Nitrogen (kкал/kg) | 2.531,53a | 2.689,27a | 2.513,88a | 2.292,36a | 2.165,72 |
| PMECN/ FGE conversion        | 0,60a  | 0,64a  | 0,60a  | 0,54a  | 0,52   |
From Table 2, it can be seen that the fermentation treatment of palm kernel meal with a combination of *Aspergillus niger* inoculum dose (4, 8, 12 gr / kg DM) and fermentation time (3, 6, 9 days) was carried out by biological testing on native chickens. Significant (P <0.05) on protein digestibility, nitrogen retention, metabolic energy (both pseudo metabolic energy, pure metabolic energy, Nitrogen-corrected Pseudo Metabolic Energy and PMECN/ FGE conversion. If it is compared to unfermented palm kernel meal, it did not increase the digestibility value of palm kernel meal.

The protein digestibility of fermented palm kernel meal varied widely, the highest was in the D1T1 treatment (71.25%). Although protein digestibility between treatments did not have a significant effect, compared to unfermented palm kernel meal, protein digestibility with fermentation was higher. This is in accordance with the results of research [22], that fermentation can increase the digestibility value of the original protein. The increasing in protein digestibility due to fermentation is a reflection of the digestibility of the crude protein components. Protein digestibility is influenced by the protein content of feed ingredients, it can be seen that the protein content in fermented palm kernel meal is low (the highest is D1T2 and D3T1: 17.18%). This is different from the results of research [9] which reported that the CP content of palm kernel meal before and after fermentation with *Aspergillus niger* increased (13.98% to 25.78%). This is because the peptidoglycan content in the microbial cell walls contributes to the increasing protein [23].

Digestibility of dry matter and organic matter of fermented palm kernel meal in D2T1 treatment (dry matter digestibility: 17.01%, organic matter digestibility: 20.64%) showed a significant effect (P> 0.05). This occurs due to the degradation process of crude fibre (lignin) by *Aspergillus niger*, where the activity of lignocellulosic enzymes can break lignin bonds with cellulose, lignin bonds with hemicellulose and lignin bonds with proteins. With the breakdown of the lignin bonds, it indirectly resulted in a decreasing in the crude fibre content of PKM and a significant increasing in the digestibility of dry matter and organic matter.

The nitrogen retention value of palm kernel meal fermented using *Aspergillus niger* with various doses given to native chickens, the highest value was seen in the D2T2 treatment (55.22%), while the lowest was seen in the D2T3 treatment (43.04% 0, D3T2 (42.50%), D3T3 (41.66%) was even lower than the unfermented palm kernel meal (44.24%). The results of the analysis of variance also showed that the treatment of fermented palm kernel meal had no significant effect (P<0.05) against nitrogen retention. [24] stated that the high percentage of nitrogen causes higher retention compared to rations with lower nutrient content so that the retained nitrogen value is also lower. According to [25] who stated that ration consumption could be affected by deficiency of food substances, the better the quality of the ration, the better the level of ration consumption. The increasing in nitrogen consumption will cause an increasing in nitrogen retention, so that it has an effect on the value of metabolic energy. The higher the level of ration consumption, the higher the percentage of nitrogen that is retained. It can be seen from the excreta which contains less urine nitrogen and metabolic energy compared to cattle that do not retain nitrogen.

The results of the pseudo metabolic energy analysis in native chickens given the highest fermentation of palm kernel meal were shown by D1T2 treatment (946.20 kcal / kg), while the lowest pseudo metabolic energy was treated with D3T3 (2,283.80 kcal / kg). Pseudo metabolic energy correlates directly with pure metabolic energy, nitrogen-corrected pseudo metabolic energy and PMECN/ FGE conversion where the highest value is found in the D1T2 treatment (pure metabolic energy; 2,786.76 kcal / kg, nitrogen corrected pseudo metabolic energy: 2.955.79 kcal / kg and PMECN/ FGE conversion; 0.69 kcal / kg) and the lowest was in the D3T3 treatment (pure metabolic energy: 2,084.15 kcal / kg, nitrogen corrected pseudo metabolic energy: 2,292.36 kcal / kg and PMECN/ FGE conversion: 0.54 kcal / kg).

The results of the analysis of variance also showed that the provision of fermented palm kernel meal between treatments had no significant effect (P <0.05), but compared to unfermented palm kernel meal, there was an increasing in pure metabolic energy, nitrogen-corrected pseudo metabolic energy and PMECN/ FGE conversion (unfermented of pure metabolic energy: 1,947.64 kcal / kg, nitrogen-corrected pseudo metabolic energy: 2,165.73 kcal/kg and PMECN/FGE conversion: 0.52 kcal / kg).
This is because manan, galactomannan, glucomannan (group of NSPs) are the most carbohydrate components in palm kernel meal with the mananase-degrading enzyme (mananase) produced by Aspergillus niger which is a source of energy that can be absorbed in the digestive tract.

The role of the cellulase enzyme produced by Aspergillus niger which can depredate cellulose is also a source of glucose in livestock [24] Cellulase enzymes are enzymes that can degrade cellulose with its main products namely glucose, cellobiose and cello oligosaccharides. Cellulase has an enzyme system consisting of endo-1,4-β-glucanase, exo-1,4-β-glucanase and β-D-glucohydrodolase [25,26]. This enzyme works synergistically to degrade cellulose and release reducing sugars as the final product. Endo-1,4-β-glucanase cuts the chain bonds in cellulose to produce shorter cellulose molecules, exo-1,4-β-glucanase cuts the ends of the cellulose chains to produce cellobiose molecules, while β-D-glucosidase cuts the cellobiose molecules into two molecules glucose [25].

4. Conclusions
Fermentation of palm kernel meal using a dosage of Aspergillus niger and different fermentation times can increase the nutrients (chemicals) of palm kernel meal, especially the content of organic matter, besides that it can also increase the digestibility value of dry matter and organic matter in native chickens.

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