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The Effect of Mannanolytic Fungi and Humic Acid Dosage to Improve the Nutrient Content and Quality of FermentedPalm Kernel Cake

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Abstract: An experiment was conducted to understand the effect of mannanolytic fungi and dosage of humic acid to improve the nutrient content and quality of fermented palm kernel cake (PKC). The experiment used completely randomized design (CRD) with 2 x 3 factorial and 3 replications. The first factor wastwo kinds of mannanolytic fungi: (1) *Sclerotium rolfsii (2) Eupennicillium javanicum.* The second factor was dosage of humic acid: (1) 100 ppm (2) 200 ppm (3) 300 ppm. The parameters were Crude Protein, Nitrogen Retention, Crude Fiber and Digestibility of Crude Fiber of fermented palm kernel cake. The result of study showed hat there was highly significant interaction between manannolytic fungi and dosage of humic acid (P<0.01), also types of fungi and dosage of humic acid had significant (P<0.01) effect to crude protein, nitrogen retention, crude fiber and digestibility of crude fiber of fermented palm kernel cake. The conclusion was fermented palm kernel cake by *Sclerotium rolfsii* and dosage of humic acid 200 ppm had better nutrient content and quality than other treatments. This condition can be seen in crude protein (27,43%), nitrogen retention(59.17%), crude fiber (11,53%) and digestibility of crude fiber (55,40%) of fermented palm kernel cake.
Key words : Mannanolytic Fungi, Humic acid, Nutrient, Fermented, Palm kernel cake.

Introduction

Palm Kernel Cake (PKC) is one of by-products of palm oil processing. Currently, Indonesia is the largest palm oil producer in the world and 70% of palm oil production came from the island of Sumatera. Furthermore, West Sumatera province is the fourth largest oil producer with CPO production up to 30.948.931 tons/year. The continued development of palm oil processing is certainly going to generate a high volume of waste product in the form of palm kernel cake (PKC), which is found about 45-46% of PKC as by-product of palm oil processing.

Nutrient contents of the PKC were as follows: 16.07% crude protein, 21.30% crude fiber, 8.23% crude fat, 0.27% Ca, 0.94% P and 48.4 ppm Cu, it can be concluded that PKC can be used as animal feed[1]. Although PKC contains a relatively high crude protein, its usage in poultry ration is still limited[2]and the research conducted by Rizal (2000) discovered that in broiler rations, the amount of PKC can be up to 10% or PKC may become a substitute for 40% of soybean meal.

The limited usage of PKC in poultry rations was due to a low nutritional quality which is caused by a high crude fiber content, low of amino acid content[4], high Cu content[1]and a high β-manan / mannose polymer content as much as 56.4% of crude fiber. Crude fiber ofPKC contains β-manan[5-7]. Furthermore, poultry has a limited ability in digesting crude fiber.

In order to increase the utilization of PKC in poultry rations, it is necessary to improve the quality of PKC through biotechnological fermentation using cellulolytic and mananolytic fungiwhich can reduce the crude fiber and β-manan content[7]. The reduced crude fiber and β-manan content will increase the quality of PKC and may become a substitute for soybean meal in poultry rations.Mananolytic and Cellulolytic fungus that can be used for the fermentation of PKC are *Aspergillus niger, Eupenicillium javanicum* and *Sclerotium rolfsii*. Research conducted by Mirnawati *et al.,* (2014) stated that the enzyme activity of the *Sclerotium rolfsii* provides activity for cellulose enzyme (21.89 U/ml), and mannanase enzyme (24.58U/ml) higher than *Eupenicillium javanicum* and *Aspergillus niger*[8].According to Purwadaria and Sari(2004),*Eupenicillium javanicum*can produce β-mannanase in 1% of locust bean gum with the highest activity at 49 U/ml and β-mannanase with a much higher activity, if the fungi are grown in coconut cake[9]. In reference to the research conducted by Razak (2006),mannanase enzyme activities in *Sclerotium rolfsii* are much higher than in *Aspergillus niger*[10].

On the other hand, palm kernel cake (PKC) also has a highheavy metal content such as Cu and Zn, where heavy metal is an obstacle in the utilization of PKC in poultry rations. This is due to the high heavy metals such as Cu after fermentation or showed significant reduction as compared prior to fermentation. This may be due to the high existence of limiting factors such as Cu and Zn on the PKC. Added by Vidal *et al.,* (2001)that Cu becomes the limiting factor in the fermentation process[11].

This study introduced the humic acid role in the processing of the PKC in order to obtain the optimal conditions for improving the quality of PKC. It is necessary to find substances or compounds which is able to reduce Cu on PKC. Humic acid is effective in binding nutrient - micronutrients, such as Cu, Zn, and Mn[12].Humic acid fraction can interact with the metal through the formation of chelate compounds[13,14]. Humic acid can also provide nutrients like N, Pand S in the soil and energy for the activities of microorganisms[15]. Enviromate TM (2002)stated that humic acid also used as a source of mineral and organic substances that play an important role in the life of the microorganisms[16,17],while the fermentation process of microorganisms also need nutrients such as N, S, and P for its growth.

Kucukersan *et al.,* (2005) stated that the use of humic acid in animal feed provides a number of advantages for the health and growth of livestock, for it has the ability to metabolize carbohydrates and proteins through catalytic process[18]. Some researchers have research the use of humic acid in broiler rations to stimulate growth[19,20]. The addition of humic acid in drinking water improves body weight gain, carcass persentage and feed efficiency in broiler chickens[21]. This is due to the fact that humic acids stimulate growth of microbes in the gut[18,19,22].Mirnawati *et al.,* (2010) stated that fermented PKC by *A. niger* combine with 100 ppm of humic acid for 7 days provides increased protein 23.20 % and reduction of crude fiber 10.59 %[1].

Based on the above explanation, we should do an experiment to determine the type of mananolytic fungi and optimum dosage of humic acid that can improve the nutrient content and quality of palm kernel cake so it can be used as a substitute of imported feed ingredients (soybean meal and corn) in poultry rations. It is necessary to study the combination of various type of fungi and optimum dosage of humic acid (300ppm)so that it canincrease thenutrientcontent and quality offermented palm kernelcake. The variables measured were the crude protein, nitrogen retention, crude fiber and digestibility of crude fiber of fermented palm kernel cake. The data is analyzed by using variation of investigation. To determine difference on the effect of the treatment, Duncan's Multiple range test (DMRT)was used[23].

Result and Discussion

The effects of treatments on the the crude protein, nitrogen retention, crude fiber and digestibility of crude fiber of fermented palm kernel cake were illustration in Table 1.

Table 1. The average value of the crude protein, nitrogen retention, crude fiber and digestibility of crude fiber of fermented palm kernel cake.

Parameters	Factor A (Manannolytic Fungi Types)	Factor B (Doses of Humic acid)			Average
		B1 (100 ppm)	B2 (200 ppm)	B3 (300 ppm)	
Crude Protein (CP) %	A1 (*S.rolfsii*)	26.38^{bA}	27.43^{aA}	24.05^{cA}	25.95
	A2 (*E. javanicum*)	21.86^{aB}	20.09^{bB}	19.01^{cB}	20.32
	Average	24.12	23.76	21.53	
Nitrogen Retention	A1 (*S.rolfsii*)	57.12^{bA}	59.17^{aA}	54.57^{cA}	56.96
	A2 (*E. javanicum*)	52.23^{aB}	50.61^{bB}	49.93^{bB}	50.92
	Average	54.68	54.89	52.25	
Crude Fiber (CF) (%)	A1 (*S.rolfsii*)	12.45^{bB}	11.53^{cB}	14.05^{aA}	12.68
	A2 (*E. javanicum*)	15.04^{aA}	13.68^{cA}	12.57^{bB}	13.76
	Average	13.74	12.61	13.31	
Digestibility of Crude Fiber (DCF)	A1 (*S.rolfsii*)	52.13^{bA}	55.40^{aA}	50.00^{cA}	52.51
	A2 (*E. javanicum*)	48.61^{bB}	52.78^{AB}	46.11^{cB}	51.77
	Average	50.37	54.09	51.96	

Note : Different superscripted capital letter on the same column and different superscripted smallletter on the same row indicated highly significant(P<0,01).

Crude Protein Content of Fermented Palm Kernel Cake

The result of analysis of variance showed that there was an interaction (P<0,01) between types of mannanolytic fungi and doses of humic acid. However each of factor were significantly (P<0.05) effected to crude protein content of fermented PKC.

The DMRT Test for interaction between A and B shows that treatment combination A1B2 was highly significant (P<0.01) compared to others. This indicates that *S. rolfsii* with doses of humic acid 200 ppm has the highest crude protein content than the other treatment. The high crude protein content of A1B2 treatment (*S. rolfsii* and doses of humic acid 200 ppm) compared with others are caused by the growing of *S. rolfsii*. The more the growth of microbe, the more the contribution of microbe body protein because microbe body also contain a single cell protein. This is appropriate with the opinion of Sukaryana *et al.,* (2010) that the high population of fungus could increase crude protein content of the substrate as the fungi is a source of single cell protein[24]. The high protein content of palm kernel cake after fermentation with the *S. rolfsii* fungi caused by higher enzyme activity of *S. rolfsii* than other fungi[25].

The high crude protein of A1B2 treatment was caused by 200 ppm of humic acid dosage which was able to reach the right condition for the growth of fungi, while humic acid with available constituent and energy is really needed in fungi growth. According to Enviromate TM (2002) that humic acid supplied constituent and energy for the growth of microorganism in soil[16]. Sukaryana *et al.,* (2010)have a notion that the better growth and development of fungi will change more media component composer into a mass cell, so the protein of fungi formed and will increase crude protein[24].

From the above data, there is an increase of crude protein in palm kernel cake as much as 67%, which is from 16.07% to 26.90% after fermentation. This result was way too higher than one obtained by Mirnawati *et al.,* (2010), Mirnawati *et al* (2011), Iyayi (2004), and Sari and Purwadaria (2004)[1,2,26,27].

Nitrogen Retentionof Fermented Palm Kernel Cake

Results of analysis of variance showed that there were highly significant interaction (P<0.05) between factor A (type of fungus) and factor B (dose of humic acid), each factor A and factor B also showed highly significant influence (P<0.01) to the nitrogen retention of fermented palm kernel cake.

From the table above, it can be seen that the retention of nitrogen is highest in A1B2 treatment (*Sclerotium rolfsii* and 200 ppm of humic acid) which is 59.17% of nitrogen retention in treatment A1B2 due to the more consumed protein than the protein excreted through feces and urine. Nitrogen retention will be positive if the nitrogen consumed more than excreted through feces and urine. The high nitrogen retention in treatment A1B2 also caused by fungi that can alter protein structure of the substrate, so that when given to poultry, it will facilitate the work of protease enzymes in the digestive tract of poultry to break down the protein component contained in the feed[28]. It happens since fermentation is an application of microbial metabolism through vitamin and essential amino acid biosynthesis as well as to increase protein and fiber by reducing crude fiber composition[29].

The high retention of nitrogen in A1B2 treatment was also due to the addition of 200 ppm humic acid. This is in accordance with the opinion of Kucukersan *et al.*, (2005) that the usage of humic acid in livestock feed gives some advantages for health and livestock growth, for example humic acid has an ability to metabolic carbohydrate and protein throughcatalytic[18].

Crude Fiber Content of Fermented Palm Kernel Cake

The result of analysis of varianceshowed that there was an interaction (P<0,01) between factor A and B. However, each factor A and B also shows significant different (P<0.05) effect of crude fiber content of fermented palm kernel cake.

The low content of crude fiber in A1B2 treatment (*Sclerotium rolfsii* and humic acid 200 ppm) is caused by the better growth of *Sclerotium rolfsii* than other fermented time. The longer the time for fungi to grow, the more the cellulose enzyme will be retained to tear down cellulose, so at the end of fermentation, the crude fiber decrease[30]. The research of Mirnawati *et al.*, showed that the cellulase enzyme activity of *Sclerotium rolfsii* is higher than *Eupennicilium javanicum* and *Aspergillus niger*[8].Appropriate with research by Madonna *et al.*, andRizal *et al.*, which stated that there is a decrease of crude fiber in fermented PKC by *A. niger*[31,32]. Also added by Mirnawati *et al.*, that there is a decrease of crude fiber content in fermented PKC by *Eupenicilium javanicum*[21].

The low content of crude fiber in A1B2 treatment is caused by the addition of humic acid 200 ppm because humic acid can activate microorganism growth. Appropriate with Kucukersan *et al.*, that the function of humic acid in ration gave an amount of profit for health and growth of livestock, for example humic acid had an ability to metabolic carbohydrate and protein through catalytic[18]. The higher the activity of microorganisms, the higher the retained enzyme produced by fungi to tear down cellulose, so at the end of fermentation, the crude fiber decrease.

Digestibility of Crude Fiber of Fermented Palm Kernel Cake

The results of analysis of variance showed that there is no interaction (P>0.05) between factor (A) the type of fungi and (B) the doses of humic acid, but the factor A and factor B gives highly significant (P<0.01) effect to the digestibility of crude fiber .

From table 1, it can be seen that the treatment A1B2 of fermented PKC by *S. rolfsii* with doses of humic acid 200 ppm showed an increase in the digestibility of crude fiber which is 55.40 % higher than the other treatments. This is in accordance with the opinion of Sukaryana *et al.*, which stated that the fermented food has a higher digestibility because the fermentation process causes breakdown of materials that can't be digested by specific enzymes such as cellulose, hemicellulose and other polymers into simple sugars that are easily digested[24]. The high digestibility of crude fiber in A1B2 treatment was due to the low content of crude fiber consumed, resulting in many ingredients stored and put to good use.In accordance with the opinion of Alshelmani *et al.*, that the decline in crude fiber content will increase the digestibility of other nutrients.

Improved digestibility of crude fiber is caused by cellulose enzymes that examine thoroughly the crude fiber substrate[33].

Conclusion

The conclusion was palm kernel cake which was fermented by *Sclerotium rolfsii* and dosage of humic acid 200 ppm showed a better content and quality of fermented of palm kernel cake. This condition can be seen in crude protein 26,43%, nitrogen retention 59.17%, crude fiber 12,72% and digestibility of crude fiber 55.40%.

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