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Research Article

Effect of Inoculum Dose and Fermentation Time on Enzyme Activity and Nutrients of Palm Kernel Cake Fermented with *Sclerotium rolfsii* Powder Inoculum

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ABSTRACT

The study aimed to determine the inoculum dose and fermentation time of palm kernel cake with *Sclerotium rolfsii* powder inoculum on the enzyme activity of cellulase, mannanase, crude protein, and crude fiber. This study used an experimental method with a completely randomized design of a factorial 3x3 with two replications. Factor A is the inoculum dose consisting of A1: 6%, A2: 8%, and A3: 10%. Factor B is the fermentation time, which consists of B1: 5 days, B2: 7 days, and B3: 9 days. The measured parameters were cellulase and mannanase enzyme activity, crude protein, and fiber content. The data obtained were analyzed using ANOVA and continued with Duncan's multiple range test. The results show that the inoculum dose and fermentation time significantly affect ($P<0.01$) cellulase and mannanase enzyme activity, crude protein, and crude fiber content. The use of an inoculum dose of 8% and a fermentation period of 7 days gave the best results with an average cellulase enzyme activity of 26.77 U/ml, mannanase enzyme activity of 27.35 U/ml, crude protein of 24.07 U/ml and crude fiber of 19.26%.

Keywords: enzyme activity, fermentation time, palm kernel cake, powder inoculum

INTRODUCTION

Palm kernel cake (PKC) is a by-product of palm oil processing. Currently, palm kernel cake has been commercialized as a poultry feed ingredient, but its utilization is very limited, which can only be given as much as 10% (Rizal, 2000). The low utilization of BIS in poultry rations is due to the high crude fiber content, most of which or around 56.4% is in the form of β-mannan (Daud and Jarvis, 1992). The low utilization of PKC in poultry rations is also caused by the low crude protein content or around 16.07% (Mirnawati *et al*., 2010). Efforts to increase the utilization of PKC in poultry rations have been carried out by several studies by means of fermentation.

Fermentation is a substrate processing technique using the help of microorganisms. Mirnawati *et al*. (2011) stated that BIS fermentation with 100% *Aspergillus niger* can replace soybean meal protein or can be utilized as much as 17% in broiler rations. Mirnawati *et al*. (2014) have carried out BIS fermentation using molds that can produce mannanase enzymes such as *Sclerotium rolfsii*, *Eupenicillium javanicum* and *Aspergillus niger*. Mirnawati *et al*. (2016)

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that the use of BISF with *Sclerotium rolfsii* in broiler rations can only be given as much as 25%, if the provision is increased by 30% then there will be a decrease in broiler performance.

The decrease in broiler performance is thought to be because the substrate used for the growth of the fermentation inoculum is only bran. Bran does not contain β-mannan so that the enzyme activity and growth of *Sclerotium rolfsii* mold as an inoculum are not optimal. Substrate is one of the factors that affects the success of fermentation. Fajrona *et al*. (2024) have added PKC as a substrate to maximize the production of *Sclerotium rolfsii* enzymes, with a substrate composition of 80% bran and 20% BIS producing optimal enzyme activity compared to other substrate compositions.

Powdered inoculum is an inoculum that has been ground into flour. Information on the use of powdered inoculum as an inoculum in fermentation has not been found. Another factor that affects the results of fermentation is the fermentation time. A short fermentation time will limit microorganisms in decomposing the substrate, but the longer the fermentation will also reduce the quality of the substrate. Based on the description above, a study was conducted to see the potential of *Sclerotium rolfsii* inoculum with a substrate composition of 80% bran and 20% PKC as a powdered inoculum.

MATERIAL AND METHODS

Materials

The materials used are *Sclerotium rolfsii* powder inoculum, palm kernel cake, bran, alcohol and distilled water. Laboratory equipment for making fermentation products, enzyme activity analysis and proximate analysis.

Methods

This study used an experimental method with a completely randomized design of a factorial 3x3 with two replications. Factor A is the inoculum dose consisting of A1: 6%, A2: 8% and A3: 10%. Factor B is the fermentation time consisting of B1: 5 days, B2: 7 days and B3: 9 days.

Work Procedure

The research work procedure consists of making *Sclerotium rolfsii* powder inoculum, fermenting palm kernel cake with *Sclerotium rolfsii* powder inoculum, enzyme activity analysis and proximate analysis.

Measured Parameters

The parameters measured were cellulase and mannanase enzyme activity, crude protein and crude fiber content.

Data Analysis

Data analysis was obtained using analysis of variance (ANOVA) in accordance with the Completely Randomized Design (CRD) factorial pattern. Differences between treatments were tested using the Duncans Multiple Range Test (DMRT) according to Steel and Torrie (1991).

RESULTS AND DISCUSSION

Effect of Treatment on Cellulase Enzyme Activity

The average cellulase enzyme activity of fermented palm kernel cake with *Sclerotium rolfsii* powder inoculum can be seen in Table 1.

Table 1. Average cellulase enzyme activity of fermented palm kernel meal (BISF) with *Sclerotium rolfsii* powder inoculum

Factor A (inoculum dose)	Factor B (fermentation time)			Mean
	B1 (5 days)	B2 (7 days)	B3 (9 days)	
A1 (6%)	20.05^{cC}	24.44^{aC}	22.64^{bC}	22.38
A2 (8%)	24.73^{cA}	26.77^{aA}	25.57^{bA}	25.69
A3 (10%)	22.74^{cB}	$25,12^{aB}$	23.53^{bB}	23.80
Mean	22.51	25.45	23.91	

Note: superscripts with lower case letters in the same row and upper-case letters in the same column indicate a very significant effect (P<0.01).

The highest cellulase enzyme activity was found in the combination of 8% inoculum dose treatment with 7 days fermentation time (A2B2) which was 26.77 U/ml. The high cellulase enzyme activity in this treatment was caused by the combination of the right inoculum dose and fermentation time so that the mold grew better and could break down the substrate nutrients during fermentation. The substrate nutrients that were broken down were cellulose, the more cellulose that was broken down into glucose as an energy source, the mold could produce cellulase enzymes with maximum activity. In accordance with the opinion of Pujiati *et al*. (2014) stated that the fermentation process with the appropriate inoculum dose and fermentation time can produce cellulase enzymes with maximum activity.

Effect of Treatment on Mannanase Enzyme Activity

The average mannanase enzyme activity of fermented palm kernel cake with *Sclerotium rolfsii* powder inoculum can be seen in Table 2.

Table 2. Average mannanase enzyme activity of fermented palm kernel meal (BISF) with *Sclerotium rolfsii* powder inoculum

Factor A (inoculum dose)	Factor B (fermentation time)			Mean
	B1 (5 days)	B2 (7 days)	B3 (9 days)	
A1 (6%)	19.67^{cC}	23.51^{aC}	21.93^{bC}	21.70
A2 (8%)	22.30^{cA}	27.35^{aA}	24.88^{bA}	24.84
A3 (10%)	21.08^{cB}	25.88^{aB}	23.33^{bB}	23.43
Mean	21.02	25.58	23.38	

Note: superscripts with lower case letters in the same row and upper-case letters in the same column indicate a very significant effect (P<0.01).

The highest mannanase enzyme activity was found in the combination of 8% inoculum dose and 7 days fermentation time (A2B2) which was 26.24 U/ml. The high mannanase enzyme activity in this treatment was due to better mold growth. The growing *Sclerotium rolfsii* mold will hydrolyze the mannan in the fermentation substrate so that the resulting mannanase enzyme activity is higher. In accordance with the opinion of Sachslehner *et al*. (2000) stated that *Sclerotium rolfsii* mold can produce mannanase enzyme with high enzyme activity due to the mold activity in hydrolyzing the mannan in the substrate.

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Effect of Treatment on Crude Protein Content

The average crude protein of fermented palm kernel cake with *Sclerotium rolfsii* powder inoculum can be seen in Table 3.

Table 3. Average crude protein of fermented palm kernel meal (BISF) with *Sclerotium rolfsii* powder inoculum

Factor A (inoculum dose)	Factor B (fermentation time)			Mean
	B1 (5 days)	B2 (7 days)	B3 (9 days)	
A1 (6%)	16.48^{cC}	21.57^{aC}	19.72^{bC}	19.26
A2 (8%)	19.17^{cA}	24.07^{aA}	21.37^{bA}	21.53
A3 (10%)	17.52^{cB}	22.52^{aB}	20.77^{bB}	20.27
Mean	17.72	22.72	20.62	

Note: superscripts with lower case letters in the same row and upper-case letters in the same column indicate a significant effect ($P<0.05$)

The highest crude protein was found in the A2B2 treatment (inoculum dose of 8% and fermentation time of 7 days) which was 24.07%. This was due to the combination of the right inoculum dose and fermentation time so that the mold grew more in utilizing the substrate. In its growth, mold uses carbon and nitrogen to form body cell components. In accordance with the opinion of Musnandar (2004) that mold will grow more and faster by using carbon and nitrogen to form body cell components. In addition, the high crude protein content in the A2B2 treatment (inoculum dose of 8% and fermentation time of 7 days) was also due to the contribution of the mold body and enzymes produced during fermentation (Iyayi, 2004).

Effect of Treatment on Crude Fiber Content

The average crude fiber of fermented palm kernel cake with *Sclerotium rolfsii* powder inoculum can be seen in Table 4.

Table 4. Average crude fiber of fermented palm kernel meal (BISF) with *Sclerotium rolfsii* powder inoculum

Factor A (inoculum dose)	Factor B (fermentation time)			Mean
	B1 (5 days)	B2 (7 days)	B3 (9 days)	
A1 (6%)	22.91^{aA}	20.41^{bB}	23.48^{aB}	22.38
A2 (8%)	21.47^{aB}	19.26^{bC}	22.00^{aC}	20.91
A3 (10%)	23.55^{bA}	21.55^{cA}	27.14^{aA}	24.08
Mean	22.64	20.40	24.32	

Note: superscripts with lower case letters in the same row and upper-case letters in the same column indicate a very significant effect ($P<0.01$)

The results of the analysis showed that there was a very significant interaction ($P<0.01$) between Factor A (inoculum dose) and Factor B (fermentation duration) on crude fiber content. The lowest crude fiber was found in the A2B2 treatment (inoculum dose of 8% and fermentation time of 7 days) which was 19.26%. This was due to the combination of the right inoculum dose and fermentation time so that the activity of cellulase and mannanase enzymes in this treatment

was high. Cellulase enzyme is an enzyme that can degrade cellulose into glucose, while mannanase enzyme is an enzyme that can degrade mannan content.

CONCLUSION

The results of the study showed that fermentation of palm kernel cake using 8% *Sclerotium rolfsii* powder inoculum and a fermentation time of 7 days gave the best results seen from the cellulase enzyme activity of 26.77 U/ml, mannanase enzyme activity of 27.35 U/ml, crude protein content of 24.07%, reducing crude fiber content by 19.26%.

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