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Optimization of Broiler Feed Production from Corn Husk Waste by *Aspergillus niger*, *Trichoderma viride* and its Consortium

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Abstract: The aim of this study was to determine the dose of inoculum and fermentation period by *A. niger*, *T. viride* and its consortium on the waste of corn husk to get the best substrate nutrient content (crude protein, crude fat and crude fiber). The experiment was arranged in a Completely Randomized Design (CRD) with three replications. The first factor is the type of microbe (M) which consists of three levels, i.e., *A. niger* (m1). *T. viride* (m2), *A. niger*+*T. viride* (m3). The second factor is the inoculum dose (D) consists of three levels, i.e., d1 = 2 g, d2 = 3 g and d3 = 4 g. The third factor is the period of fermentation (W) is w1 = 48 h, w2 = 76 h and w3 = 96 h. The existing data collected and processed by Variance Analysis (ANOVA). If there is a significant difference to the control, then followed by Duncan's multiple range test. The results showed that the increase of crude protein present in the interaction between types of microbes and dose of inoculums, that is consortium microbe of 15.40%. The interaction between microbes and fermentation period on consortium microbe is 18.38% in 96 h, while the average decline in crude fiber influenced by microbes present in *T. viride* is 1.81%. The decline average of crude fiber influenced by fermentation period of 48 h is 0.54%. The decline average of crude fat which influenced by consortium microbe type (5.75%) while the average of crude fat reduction influenced by inoculum dose is at the dose of 0.2% (b/k).

Key words: *A. niger*, *T. viride*, consortium and corn husk

INTRODUCTION

One of the sectors that has not been widely used are agricultural waste. Corn husk is one example of an agricultural lignocellulosic waste that still has economic value when there were further processing, in line with the development of biotechnology, the use of microbes in the waste bioconversion process in order to gain added value from the waste into other products such as animal feed. The chemical composition of the corn husk waste consists of 23% cellulose, 67% hemicellulose, 0.1% lignin and others 9.9% (Hardiyanto, 2004). This composition could not be digested by the chickens, so as to utilize the waste corn husks as chicken feed ingredients, it should be given treatment. Fermentation is one alternative that can be done on waste corn husks that the nutrient content in it increases. According to Winarno (1980), fermentation occurs due to microbial activity in suitable organic substrates.

Microorganism role is needed to change the chemical composition into glucose which is easily digested by the chickens. The microorganisms which are used to alter the chemical composition are *Aspergillus niger* and *T. viride* with cellulase enzyme resulted can degrade cellulose, hemicellulose and lignin, which will break down into glucose that can be digested by cattle. The Fermented corn husk waste can be then used as animal feed ingredient, for such as the broiler. Broilers are chicken whose growths do not require a long time to be harvested soon. They are generally reared by grounded.

MATERIALS AND METHODS

The study is aimed to determine the dose of inoculum and fermentation period of *A. niger*, *T. viride* and its consortium on corn husk waste to get the best substrate nutrient content (crude protein, crude fat and crude fiber). The experiment was arranged in a Completely Randomized Design (CRD) with three replications. The first factor is the type of microbe (M) consisting of 3 levels of treatment, namely, *Aspergillus niger* (m1), *Trichoderma viride* (m2), *A. niger*+*T. viride* (m3). The second factor is the dose (D) inoculums which consists of 3 levels i.e., d1 = 2 g, 3 g and d2 = d3 = 4 g. The third factor is the time of fermentation (W) which consists of three levels, those are w1 = 48 h, w2 and w3 = 76 h = 96 h. The existing data collected and processed by analysis of Variance (ANOVA). If there is a significant difference to the control, then followed by Duncan's multiple range test.

RESULTS AND DISCUSSION

Specification: Different lowercase letters on the vertical direction indicate significantly different according to Duncan's multiple range test at 5% level.

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Different capital letters on the horizontal direction indicate significantly different according to Duncan's multiple range test at 5% level:

m1 = *A. niger* m2 = *T. viride* m3 = *A. niger*+*T. viride*
d1 = 2 g d2 = 3 g d3 = 4 g

Based on the results, Table 1 indicate that the influence of the type and dose of microbial inoculum showed significant effect (α<0.05) of the crude protein content. The effect of inoculum dose of 2 g (d1) and 3 g (d2) with the type of microbe m3 was significantly higher protein content compared with other treatments. This suggests that the dosage of 2 g and microbial inoculum consortium is the best treatment to produce the highest protein content, while the dose of 4 g (d3) is generating the lowest protein among all treatments.

According to Fardiaz (1992), fungi can synthesize proteins by taking carbon source of carbohydrates (such as glucose, sucrose or maltose), a source of nitrogen from organic or inorganic materials and minerals from the substrate. During the growth, this fungus produces two types of enzymes which will be donated to the substrates as a protein that increases protein substrate.

Suliantari and Winiati (1990) suggested that during the process of fermentation, substrates undergo physical and chemical changes. By the presence of proteolytic activity of molds, then the substrate protein broken down into amino acids that dissolved nitrogen increased, while d3 inoculum dose (3 g), the type of microbe m3 produced the lowest protein content (6.20). This was due to the high doses produce biomass that is too much lead to a lack of nutrients necessary for the formation of the product (Sastramihardja, 1989).

The best treatment that produces the highest protein content is in the microbial consortium with inoculum dose of 2 g, that is 15.40%.

Specification: Different lowercase letters on the vertical direction indicate significantly different according to Duncan's multiple range test at 5% level.

Different capital letters on the horizontal direction indicate significantly different according to Duncan's multiple range test at 5% level:

m1 = *A. niger* m2 = *T. viride* m3 = *A. niger*+*T. viride*
w1 = 48 jam w2 = 72 jam w3 = 96 jam

Based on the results in Table 2 show that the average value of the highest crude protein content (18.38)% found in m3 and w3, followed by m1 and w3 (15.96)% then m3 and w2 (14.73)% and m3 and w1 (13.09)%.

So the longer the fermentation of corn husk waste by m3 is used, the higher the protein content for a certain time limit, as long as the substrate as a nutrient is still available enough. In accordance with the opinion of Tangenjaya (1993) that the increase in the protein during fermentation is due to the work of the mold and the addition of protein donated from fungus cell growth. This is because the more the growth of mold, the higher substrate protein resulted, due to microbial fermentation process will produce enzymes that will degrade complex compounds into simpler and microbes will also synthesize proteins that constitute the process of enrichment, that is the enrichment of protein materials. This is in line with Fardiaz's opinion (1989) that the increase in the protein substrate during fermentation indicates that the fungus is able to use a portion of the substrate for microbial growth and form microbial proteins.

Table 1: Average increase in crude protein (%) on the effect of microbial interactions and inoculum dose

	Types of microbes		
Inoculum dose	m1	m2	m3
d1	11.71^{a}	6.55^{a}	15.40^{B}
	AB	A	B
d2	10.30^{a}	6.98^{a}	13.20^{Ab}
	AB	A	B
d3	9.88^{a}	6.87^{a}	6.20^{A}
	A	A	A

Table 2: Average increase in crude protein (%) on the effect of the interaction of microbes and fermentation period

	Types of microbes		
Fermentation period	m1	m2	m3
w1	6.36^{a}	9.11^{a}	13.09^{a}
	A	A	B
w2	12.82^{b}	3.83^{b}	14.73^{a}
	A	B	A
w3	15.96^{b}	6.73^{ab}	18.38^{b}
	A	B	A

Table 3: Average reduction of crude fiber (%) influenced by the type of microbe

Microbe	Mean (%)	Significance
m3	2.73	a
m1	2.20	b
m2	1.81	c

Table 4: Average reduction of crude fiber (%) effected by fermentation period

Period	Mean	Significance
w3	5.13	a
w2	2.14	b
w1	0.54	c

Table 5: Average decrease in crude fat (%) influenced by the type of microbe

Type of microbes	Mean	Significance
m2	9.66	a
m1	9.25	a
m3	5.75	b

Table 6: Average decrease in crude fat (%) affected by inoculum dose

Dose	Number	Significance
d2	10.12	a
d3	8.60	ab
d1	5.94	b

Based on Yusuf *et al.* (2004) who said that *T. viride* has the ability to increase cellulose feed ingredients protein and stimulate the release of cellulase enzymes. The best treatment that produces the highest protein content in the microbial consortium (m3) with fermentation period (w3) 96 h of 18.38%.

Specification: Different lowercase letters on the vertical direction indicate significantly different according to Duncan's multiple range test at 5% level:

m1 (*A. niger*), m2 (*T. Viride*), m3 (*A. niger+T. viride*)

Based on the results in Table 3, it indicates that the lowest crude fiber content are in m2 is equal to 1.81%, followed by m1 is equal to 2.20% and the latter by microbes m3 is equal to 2.73%. The three types of microbes showed significant differences at (α<0.05).
T. viride is producing highly efficient cellulolytic enzymes, especially enzymes which capable of hydrolyzing crystalline cellulose. The cellulase activity on cellulose is an important base in the biodegradation of cellulose. Endo-β-1, 4 glukanase is cellulase which randomly breaking the bonds of cellulose from cellulose fibers. This is in line with the opinion of Yuliasni (2009) that the presence of a highly active cellulose enzyme has a very strong bond that will only be solved by cellulase enzymes but the result enzyme has not reached the active configuration yet. It is because the energy activity has not been reached then the bonds in the substrate are not fully degraded so that it has low fiber content. Winarno (1980) said that fermentation will increase the nutritional value of feed and tend to lower crude fiber due to the activity of the enzyme so it is easy to digest. The best types of microbes to degrade crude fiber contained in the type m2 (1.81%).

Specification: Different lowercase letters on the vertical direction indicate significantly different according to Duncan's multiple range test at 5% level:

w1 (48 h), w2 (72 h), w3 (96 h)

Based on Table 4. All three treatments showed significant differences at (α<0.05), fermentation period 48 h (w1) is significantly lower than the 72-h treatment (w2) and 96 h (w3) for reduction of crude fiber content.
This is related to the growth at period of 48 h microbes were in the adaptation phase; the new cells adapt to the environment and the substrate, the enzyme has not been synthesized, the cell growth was not stable yet and dead cells still there since they have not been able to split against cellulose massively so that the microbes have low fiber content. These results are in line with Fardiaz (1992) which says that in the adaptation phase, the cells are still adjusting to the environment and the medium, there are dead cells, the enzyme has not been synthesized. The high crude fiber content of the substrate fermented due to the coarse fibers and mycelia mold. The best treatment for decreasing crude fiber is on the fermentation period w1 (0.54%).

Specification: Different lowercase letters on the vertical direction indicate significantly different according to Duncan's multiple range test at 5% level:

m1 = *A. niger*, m2 = *T. viride*, m3 = *A. niger+T. viride*

Table 5 showed that the lowest fat loss was in the microbial consortium (m3) is 5.75%, followed by (m1) *A. niger* 9.25% was not significantly different with *T. viride* (m2) 9.66%.
The low of fat content is caused by the two types of mold which grow well on the medium resulting a reshuffle on the substrate to obtain energy and fat that has become simpler compounds which are used for their growth and development. *A. niger* produces lipase enzyme which decompose fats into fatty acids and glycerol. Wang *et al.* (1988) says that the fat that is mainly in the form of fatty acid is used as an energy source for the growth of mold. It also said by Rusdi (1992) that fat reshuffle by molds is needed to get energy and fat that has been transformed into simple compounds that used by the mold for the purpose of his life. The best treatment in order to increase crude fat m3 (consortium of *A. niger* and *T. viride*) is equal to 5.75%.

Specification: Different lowercase letters on the vertical direction indicate significantly different according to Duncan's multiple range test at 5% level:

d1 = 2 g, d2 = 3 g, d3 = 4 g

Based on Table 6 showed that the lowest dose of inoculum is d1 of 5.94% which was significantly different from d2 but not significantly different from d3, while the inoculum dose of d3 was not significantly different on d1 and d2.
The low of crude fat content is caused by the increasing population of mold. The revamped fat by molds is needed to get energy and fat that has become simple compound for the purposes of his life. The treatment of inoculum dose of 2 g (d1) is the optimal dose of inoculum for the growth of mold that can remodel fat by producing lipase. The fat which has been revamped to be more easily digested by the mold and it can be seen from the crude fat content. This is in line with the opinions of Shurtleff and Aoyagi (1979) that there was a revamped of fat in the fermentation process which is approximately 35% of hydrolyzed neutral fat and free fatty acids that is equal to 40% and used by molds for his life. The best treatment to reduce crude fat contained in the inoculum dose d1 (2 g) is equal to 5.94%.

Conclusion: The best results from the fermentation of corn husk waste on increasing crude protein obtained from:

a: The interaction treatment of consortium microbes and inoculum dose d1 (*A. niger* and *T. viride* and inoculum dose of 0.2% (w/k) which is equal to 15.40%

b: The interaction effect of consortium microbes and fermentation period w3 (*A. niger*+*T. viride* by the fermentation period of 96 h) which is equal to 18.38

The best results of fermented corn husk waste to the decline of crude fiber obtained from:

a: The microbial treatment m2 (*T. viride*) which is equal to 1.81%

b: The treatment of fermentation period w1 (48) h which is equal to 0.54%

The best results of fermented corn husk waste to the decline in crude fat obtained from:

a: The treatment of microbial consortium (*A. niger* + *T. viride*) which is equal to 5.75%

b: The treatment of inoculum dose d1 (0.2% b/k)

Recommendation: The corn husk waste is suggested to be used as an alternative feed for broiler chicken in the preparation of rations and after first fermented by *A. niger*, *T. viride* and its consortium.

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