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Improving the Nutrient Quality of Durian (*Durio zibethinus*) Fruit Waste Through Fermentation by Using *Phanerochaete chrysosporium* and *Neurospora crassa* for Poultry Diet

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Abstract: Durian fruit waste (peel and seed) can be used as an alternative feed based on the potential availability and nutrient content. Three experiments were conducted to improve the nutrient quality of durian fruit waste (DW) and tofu waste (TW) mixture through fermentation by using *Phanerochaete chrysosporium* and *Neurospora crassa*. The substrates consists of 70% DW (peel and seed in the same proportion) and 30% TW. Experiment 1 was performed in a 3 x 3 factorial experiment of completely randomized design with 3 replicates. First factor was inoculum composition of *P. chrysosporium* and *N. crassa* (1: 1, 2: 1 and 1: 2) and second factor was inoculums doses (6, 8 and 10%). Measured variables were Crude Fiber (CF) and Crude Protein (CP). Experiment 2 was to study incubation period (7, 9, 11 and 13 days). Measured variables were DTW's CF, CP, cellulose, hemi-cellulose and lignin. Experiment 3 was to compare nutrient content between fermented (the best treatment at experiment 2) vs. unfermented one. Measured variables were DTW's nitrogen retention, amino acid profile, B carotene, tannin and Metabolizable Energy (ME). Result of the experiment 1, there was a very significant ($p < 0.01$) interaction between inoculum compositions and inoculum doses in which *P. chrysosporium* and *N. crassa* inoculum composition (1:1) and 8% inoculum dose reduced CF and increased CP content. Experiment 2, incubation period of 9 days reduced CF, NDF, ADF, lignin and cellulose from 22.33, 32.49, 27.50, 15.81 and 17.62 to 8.30, 30.58, 20.26, 6.20 and 11.58%, respectively. Meanwhile increased CP and hemicellulose from 11.73 and 4.99 to 19.37 and 9.52%, respectively. Experiment 3, Nitrogen retention, B-carotene, tannin and ME content of fermented was better than unfermented DTW (42.50 vs 68.54%, 0 vs. 119.07 mg/kg, 3,76 vs 0.43% and 2586.84. vs. 2728.27 kkal/kg, respectively). *Phanerochaete chrysosporium* and *Neurospora crassa* treatment improved DTW's amino acids profile. In conclusion, fermentation by using *Phanerochaete chrysosporium* and *Neurospora crassa* (1:1), 8% inoculum dose and 9 day incubation period was the best treatment for improving DTW's nutrient quality.

Key words: Durian tofu wastes mixture, fermentation, *Phanerochaete chrysosporium*, *Neurospora crassa*, nutrient quality

INTRODUCTION

Different kinds of agricultural by-products or fruit wastes such as cocoa, coffee and durian fruit waste are the alternative solutions to overcome the shortage of feed, especially corn in poultry diets. Waste of durian (*Durio zibethinus*) fruit could be used as unconventional animal feed in terms of availability and nutritional content. Durian fruit production in West Sumatra in 2013 about 82, 69 tones (Central Bureau of Statistics of West Sumatra, 2011). If the composition of durian consists of 60% peel, 20% meat and 20% seed, it can be estimated the peel and seeds of durian fruit as much as 66.15 tones in 2014.

Nutrient content of durian fruit waste according to Nuraini *et al.* (2012) reported that durian fruit waste (50% peel and 50% seed) have 10.31% crude protein, 3.24% fat, 50.51% Nitrogen Free Extract and 22.33% crude fiber (10.32% lignin and 9.50% cellulose).

The experiment about the utilization of waste from durian fruit have been reported. Nuraini and Mahata (2008) reported that up to 15% inclusion of durian seed could be to replace up to 40% of the maize in the diet of broilers without adverse effects on growth and carcass measurements. The utilization of the durian peel in poultry diet not yet been reported, possibly because of high crude fiber content.

To improve the quality of durian fruit waste as feedstuff, must reduce their crude fiber (lignin and cellulose) as limiting factor through fermentation by using *Phanerochaete chrysosporium*. Fungus *P. chrysosporium* can produce cellulase and ligninase enzymes (Howard *et al.*, 2003). *P. chrysosporium* is a white rot fungus known the ability to degrade lignin. Some species of white rot fungi of the class *Basidiomycetes* capable breaking down all the components of lignocellulose (Zeng *et al.*, 2010). *Phanerochaete*

chrysosporium is a microorganism capable of degrading the lignocellulose selectively by firstly degrading the lignin component followed by cellulose component.

The crude protein of cacao shell inoculated with *P. chrysosporium* and incubated for 15 days increased from 8.57 to 11.52%, with decreased crude fiber from 44.21 to 29.94% and increase ash content from 6.79 to 7.12% (Nelson and Suparjo, 2011). The increased protein content was due to bioconversion of organic materials that had been broken down into one of the fungi body components. Secondary metabolite secretions such as extracellular enzymes by the fungi also contributed to the increased protein content of treated corn stover.

According to Fadillah *et al.* (2008) found that the lignin content of corn stalks reduced 81.40% and cellulose content decreased by 43.03% through fermentation by using *Phanerochaete chrysosporium* with 7% inoculum dose and fermentation time of 14 days. According to Nuraini *et al.* (2013) substrate composition 80% cacao pulp and 20% tofu waste fermented by *Phanerochaete chrysosporium* increased crude protein 33.79% and decreased crude fiber 33.02% with 10% inoculum dose and 8 days incubation. Nuraini *et al.* (2013) reported coffee fruit peel 80 and 20% tofu waste mixture fermented by *Phanerochaete chrysosporium* with 7% inoculum dose and fermentation time of 10 days decreased crude fiber 43.89% and increased crude protein. Fermentation was also carried out with *Neurospora crassa* to produce beta-carotene high feed. *Neurospora crassa* was carotenogenic fungi that produced the highest of beta-carotene (270.60 U/g) isolated from corn cobs. Besides, *Neurospora crassa* produced amylase, cellulose and protease enzyme and the enzymes activity were 17.21, 0.33 and 15.06 U/g, respectively (Nuraini, 2006). According to Nuraini *et al.* (2009) reported that the mixture of 80% cassava pulp and 20% tofu waste fermented by using *Neurospora crassa* with inoculum dose of 9%, for 7-day fermentation with thickness 2 cm increased crude protein 83.84%, decreased crude fiber 13.88% and the content of other nutrients are 2.25% fat, 0.22% calcium, 0.02% phosphor, 52.25% NFE and beta-carotene 295.16 mg/g. Fermentation using two fungi *Phanerochaete chrysosporium* and *Neurospora crassa* to reduce the content of crude fiber and to obtain beta-carotene has been done Nuraini *et al.* (2013) reported that 80% banana peel and 20% tofu waste fermentation by using *Phanerochaete chrysosporium* and *Neurospora crassa* 2:1, with 7% inoculum and fermentation time of 10 days could reduced 48.11% crude fiber (reduced cellulose and lignin as much as 45.01 and 53.82%, respectively) and increase crude protein and hemicellulose as much as 33.73 and 57.58%, respectively and increase the digestibility of crude fiber as much as 32.73% and gained 66.83% nitrogen retention.

The success of a solid media fermentation depends on optimum conditions such as substrate composition, inoculum dose and incubation period that affect the nutrients content of fermentation product (Nuraini *et al.*, 2009). The most important thing that should be present in the fermentation medium is a source of carbon, nitrogen and other essential elements in the number and the appropriate balance (Carlile and Watkinson, 1995).

Comparison of the composition of the inoculum, the inoculum dose and fermentation period using two kinds of fungus *Phanerochaete chrysosporium* and *Neurospora crassa* to improve the nutrition quality of the durian waste fermentation product are remain unknown.

MATERIALS AND METHODS

The main objective of this experiment was to study the improvement of the nutrient quality of durian and tofu wastes mixture through fermentation by using *Phanerochaete chrysosporium* and *Neurospora crassa*. The specific objectives were: to determine the effects of *Phanerochaete chrysosporium* and *Neurospora crassa* composition, inoculums doses and incubation period on nutrient quality of durian and tofu wastes mixture.

Experiment 1: The experiment 1 was designed in a 3 x 3 factorial experiment in a completely randomized design with 3 replication per treatment. The first factor was the composition of *Phanerochaete chrysosporium* and *Neurospora crassa* (1:1, 2:1 and 1:2) and the second factor was inoculums doses of *Phanerochaete chrysosporium* and *Neurospora crassa* (6, 8 and 10%). In the experiment 1: 100 g substrate contain of 70% durian waste (peel and seed in the same proportion) and 30% tofu waste mixture per experimental unit was treated with *Phanerochaete chrysosporium* and *Neurospora crassa* (1:1, 2:1 and 1:2) and the inoculum doses (6, 8 and 10%). There were 27 samples or experimental units in this experiment. Measured variables in this experiment were crude fiber (CF) and crude protein (CP).

Experiment 2: This experiment was designed in a completely randomized design (CRD) with 4 treatment (the incubation period 7, 9, 11 and 13 days) and 5 replication. The best composition of *Phanerochaete chrysosporium* and *Neurospora crassa* and inoculum dose at experiment 1, used at experiment 2. Measured variables were crude fiber, crude protein content and fiber profile (NDF, ADF, cellulose, hemicellulose and lignin) of durian and tofu wastes mixture. There were 20 samples of experimental units in this experiment. All of the data were statistically analyzed by analysis of variance of a factorial experiment in a completely randomized design (experiment 1) and a completely randomized design (experiment 2). The difference

among treatments was determined by DMRT procedure according to Steel and Torrie (1980).

Analyses of crude fiber and crude protein were performed according to the proximate analysis procedures (AOAC, 2000), while NDF, ADF, cellulose, hemicellulose and lignin contents were determined according to Goering and Van Soest (1970).

Experiment 3: The experiment was to compare nitrogen retention, beta carotene, tannin, amino acid profile and metabolizable energy between fermented and non-fermented durian and tofu wastes mixture numerically. The fermented sample was obtained from the best of fermented durian and tofu wastes mixture in the experiment 2.

Amino acids, tannin and beta carotene contents of the durian and tofu wastes mixture were performed by using HPLC. Nitrogen retention and metabolizable energy were assayed according to Sibbald (1986). All of the data obtained from untreated versus treated durian and tofu wastes mixture were compared numerically.

RESULTS

Experiment 1

Effect of *Phanerochaete chrysosporium* and *Neurospora crassa* composition and inoculums dose on crude fiber: The effect of *Phanerochaete chrysosporium* and *Neurospora crassa* composition and inoculums dose on crude fiber content of durian and tofu wastes mixture is shown in Table 1. The crude fiber content of durian and tofu wastes mixture was a very significant interaction ($p < 0.01$) by the *Phanerochaete chrysosporium* and *Neurospora crassa* composition and inoculums dose.

Effect of *Phanerochaete chrysosporium* and *Neurospora crassa* composition and inoculums dose on crude protein: The effect of *Phanerochaete chrysosporium* and *Neurospora crassa* composition and inoculums dose on the crude protein content of durian and tofu wastes mixture are depicted in Table 2.

The *Phanerochaete chrysosporium* and *Neurospora crassa* composition very significantly ($p < 0.01$) affected the crude protein content of durian and tofu wastes mixture. The inoculums dose also influenced very significantly ($p < 0.01$) this crude protein content. There was a very significant interaction ($p < 0.01$) between the *Phanerochaete chrysosporium* and *Neurospora crassa* composition and inoculum dose on this crude protein content.

Experiment 2

Effect of incubation period of *Phanerochaete chrysosporium* and *Neurospora crassa* on crude fiber, NDF, ADF, lignin, cellulose and crude protein: The effect of incubation period of *Phanerochaete chrysosporium* and *Neurospora crassa* on crude fiber,

Table 1: Effect of *Phanerochaete chrysosporium* and *Neurospora crassa* composition and inoculum dose on crude protein

Treatments	B1(6%)	B2(8%)	B3(10%)	Averages
A1(1:1)	18.11 ^{Ba}	19.92 ^{Aa}	19.85 ^{Aa}	19.29
A2(2:1)	18.14 ^{Ba}	19.54 ^{Aa}	19.64 ^{Aa}	19.11
A3(1:2)	16.94 ^{Bb}	17.41 ^{Ab}	17.58 ^{Bb}	17.31
Averages	17.73	18.96	19.02	

Table 2: Effect of *Phanerochaete chrysosporium* and *Neurospora crassa* composition and inoculum dose on crude fiber

Treatments	B1(6)	B2(8)	B3(10)	Averages
A1(1:1)	12.12 ^{Aa}	11.51 ^{ABab}	11.04 ^{Bab}	11.56
A2(2:1)	11.48 ^{Ab}	10.49 ^{Bb}	10.13 ^{Bb}	10.70
A3(1:2)	14.79 ^{Ab}	14.24 ^{Aa}	14.10 ^{Aa}	14.38
Averages	12.80	12.08	11.76	

NDF, ADF, lignin, cellulose and crude protein content of durian and tofu wastes mixture is shown in Table 3.

The crude fiber content of durian and tofu wastes mixture was a very significant affected ($p < 0.01$) by the *Phanerochaete chrysosporium* and *Neurospora crassa* incubation period.

Experiment 3

Comparison of nitrogen retention, B carotene, tannin, metabolizable energy and amino acid profile between unfermented vs. fermented: Nitrogen retention, beta carotene, tannin and metabolizable energy of durian and tofu wastes mixture is figured in Table 4. Amino acid profile shown in Table 5.

DISCUSSION

When inoculum doses was increased (8% and 10%) at the composition of *Phanerochaete chrysosporium* and *Neurospora crassa* 1:1 and 2:1 at experiment 1, the crude protein content increased. This result is in accordance with the result of experiment by Nuraini *et al.* (2013) in cacao pulp and tofu waste mixture, in which its crude protein content was increased from 11.71 to 21.69% by fermentation using *Phanerochaete chrysosporium* and *Monascus purpureus* at the level of 8%. Nuraini *et al.* (2009) reported that crude protein cassava and tofu waste mixture also increased from 10.70 to 20.44% when fermented by using *Neurospora crassa* at inoculums dose 9%.

At experiment 2, crude protein also increased at 7, 9, 11 and 13 days incubation time than the control. This finding is in accordance with the result obtained by Islamiyati *et al.* (2013) who reported that protein content of the corn stover treated with *P. chrysosporium* was significantly higher at 1, 2 and 3 weeks incubation time than the control.

An increase in crude protein content of fermented product was clearly found that there was the growth of fungi in each fermented. The growing fungi contribute to the increase in crude protein content of durian and tofu waste, so that there was an augmenting in crude protein of fermented DTW. Crueger and Crueger (1989) reported that fungi contained high crude protein (40-60%). According to Carlile and Watkinson (1995),

Table 3: Effect of *Phanerochaete chrysosporium* and *Neurospora crassa* incubation period on crude fiber, NDF, ADF, lignin, cellulose and crude protein

Incubation period (day)	L1(7)	L2(9)	L3(11)	L4(13)
CF	10.60±0.62 ^A	8.30±0.50 ^B	8.75±0.72 ^B	9.27±0.59 ^{AB}
CP	18.06±0.99 ^{AB}	19.37±0.87 ^A	18.17±0.82 ^{AB}	18.08±0.93 ^{AB}
NDF	32.11±0.42 ^A	30.58±0.32 ^B	31.98±0.45 ^{AB}	31.58±0.34 ^{AB}
ADF	25.20±0.83 ^A	20.26±0.70 ^B	21.26±0.73 ^B	22.26±0.65 ^{AB}
Lignin	13.33±0.62 ^A	6.20±0.52 ^B	6.26±0.55 ^B	7.26±0.62 ^B
Cellulose	14.26±0.72 ^A	11.58±0.79 ^B	11.98±0.82 ^B	12.26±0.80 ^B
Hemicellulose	6.96±0.62 ^B	10.20±0.52 ^B	10.01±0.55 ^A	8.26±0.52 ^{AB}

Values bearing different superscripts in a row differ significantly, p<0.01

Table 4: Nitrogen retention, beta carotene, tannin metabolizable energy of unfermented vs. fermented durian and tofu wastes mixture

Compound and energy	Unfermented	Fermented
Nitrogen retention (%)	42.40	68.54
Beta carotene (mg/kg)	0.00	119.07
Tannin (%)	3.76	0.43
Metabolizable energy (kcal/kg)	2586.84	2728.27

Table 5: Amino acid profile of durian and tofu waste fermented and unfermented

Amino acids	Unfermented	Fermented
	----- (%DM) -----	
Aspartate	0.18	0.28
Glutamate	0.22	0.34
Serine	0.17	0.26
Histidine	0.11	0.14
Glycine	0.06	0.25
Threonine	0.11	0.25
Arginine	0.10	0.21
Alanine	0.20	0.32
Tyrosine	0.10	0.14
Tryptophan	0.13	0.14
Methionine	0.11	0.24
Valine	0.24	0.32
Phenyl alanine	0.21	0.25
Iso-leucine	0.26	0.35
Leucine	0.31	0.34
Lysine	0.24	0.25
Cystine	0.01	0.03
Cysteine	0.02	0.04
Proline	0.31	0.35

the increase in crude protein content of substrate after fermentation was associated with the process of protein enrichment which was similar to the formation of a single cell protein and in this process it was not separated between protein from fungi and substrate. The height in crude protein content of fermented DTWs was also related to the enzymes (cellulase, protease, etc.) produced by these fungi as the protein which could contribute to the height in crude protein content of the fermented DTW as compared with control. *Phanerochaete chrysosporium* produce ligninase and cellulose enzymes (Howard *et al.*, 2003) and *Neurospora crassa* produce amylase, cellulase and protease enzymes (Nuraini, 2006).

The lowest crude fiber content of fermented DTW was obtained from the *Phanerochaete chrysosporium* and *Neurospora crassa* composition 2:1 and concentration of 8 and 10% (experiment 1). The result of this experiment is similar to the result of experiment by Nuraini *et al.* (2013), where the crude fiber content of

cacao pod reduced at 8% level of *Phanerochaete chrysosporium*. At experiment 2, there are a decline in crude fiber, NDF, ADF, lignin and cellulose content of fermented durian and tofu waste mixture at the incubation time 9, 11 and 13 days. The reduce in crude fiber content of fermented DTW was due to the degradation of this crude fiber by ligninase and cellulose produced by *Phanerochaete chrysosporium*. Hossain *et al.* (2001) found *Phanerochaete chrysosporium* optimum time is 14 days for lignin biodegradation of rice straw.

When it is compared numerically, there is a decline in tannin contents of *Phanerochaete chrysosporium* and *Neurospora crassa* treated durian and tofu wastes mixture versus untreated one. On the other hand, the nitrogen retention, hemicelluloses, beta carotene and metabolizable energy are enhanced by fermentation using *Phanerochaete chrysosporium* and *Neurospora crassa*. Nuraini *et al.* (2013) also reported that the fermentation of cocoa pod with *Phanerochaete chrysosporium* and *Monascus purpureus* reduced their crude fiber, NDF, ADF, cellulose contents. According to Dhawale and Kathrina (1993) and Howard *et al.* (2003) fungi *Phanerochaete chrysosporium* can degrade lignin and its compounds effectively by producing extracellular peroxidase enzymes such as lignin peroxidase and manganese peroxidase. While *Neurospora crassa* also produce cellulase enzyme (Nuraini, 2006).

The amino acid profile of durian and tofu waste mixture was influenced by *Phanerochaete chrysosporium* and *Neurospora crassa*. Some amino acids of the treated one increased, while the others were almost the same. The first limiting amino acid in the corn-soy poultry diet, i.e., methionine and lysine slightly increased from 0.11 and 0.24 to 0.24 and 0.25%.

Conclusion: The fermentation by using 8% *Phanerochaete chrysosporium* and *Neurospora crassa* (1:1) for 9 days incubation time was the best treatment for reducing crude fiber and improving the nutrient quality of durian tofu wastes mixture. It is better than the untreated durian tofu wastes mixture nutrient content and nutrient quality.

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