THE EFFECT OF Trichoderma viridae USAGE OF NUTRITIONAL VALUE ON Goroho BANANA STEM (Musa acuminafe, sp)

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THE EFFECT OF *Trichoderma viridae* USAGE OF NUTRITIONAL VALUE ON *Goroho* BANANA STEM (*Musa acuminafe, sp*)

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Abstract

Research on the nutritional value of goroho banana stem (Musa acuminafe, sp) fermented by Trichoderma viridae has been carried out in the Laboratory of Technology and Animal Feed Industry of the Faculty of Animal Husbandry at Universitas Sam Ratulangi, Manado. The purpose of this study was to evaluate the effect of Trichoderma viridae inoculum dose and fermentation time to changes in nutritional value (Ash, Crude Protein, Crude Fibers and Crude Lipid) of the fermentation goroho banana stem flour products. This study was designed using completely randomized design with nested pattern. Factor A is the inoculum dose of the Trichoderma viridae fungi of 0.3, 0.6 and 0.9 percent, and factor B is the fermentation time of 4, 8 and 12 days; each treatment was repeated 3 times. Factor B is nested in factor A. Statistical tests with analysis of variance and differences between treatments using Duncan's multiple range test. The results obtained are fermented goroho banana stem flour (Musa acuminafe, sp) with Trichoderma viridae dose of 0.6 percent for 8 days produced the best nutritional content changes (Ash 20.17%, Crude Protein 21.69%, Crude Fiber 14.94%, and Crude Fat 20.17%).

Key words: *Goroho banana flour, Trichoderma viridae, ash, crude protein, crude fiber, crude fat*

INTRODUCTION

Efforts to locate and utilize other materials, including waste materials or whose primary purpose is not to be used as food, is a right step, enabling to increase the diversity of feed ingredients making up the ration, and rising the materials economic value and, even further, reduce the environmental pollution.

Goroho banana *(Musa acuminafe, sp)* is a unique type of banana that only grow in this area; is highly favored by consumers, particularly in the city of Manado and Minahasa region. The favor is marked by so many fried snack outlets that offer fried *goroho* banana, because it has a distinctive taste and is could be consumed by diabetics. *Goroho* banana has special characteristic, where its skin color remains green despite being overharvested [21]. The largest component of the banana plant is the stem (60%), compared to the leaves (10%) and fruit (10%) [11]. When the fruit is being harvested, the stem of bananas will be discarded and left to rot unused. The nutrient content of banana stem are as follows: crude protein (4.81%), crude fibers (27.73%), crude fats (11.23), lignin (9.92) and ash (23.12%) [9]. Another research also reported that the banana stems nutrient compositions are, 2.4 to 8.30% crude protein, crude fiber 13.40 to 31.70%, crude fat from 3.20 to 8.10% and ash from 18.24 to 24.76% [23]. Banana stems contain lignin and tannin which act as inhibitors in reducing dry and organic materials [15][25]. The tannin, as a phenol compound, may degrade organic material digestibility, mostly protein, to form a complex bond of tannin-protein [22]. The tannins may cause decrease the protein digestibility and nitrogen retention on poultry [17].

The weakness of banana stem as feed ingredients for livestock directly in its natural form is the high palatability value and high crude fiber content. Besides, the existence of tannin as a phenol compound that will affect the organic material digestibility, particularly protein, with the formation of protein-tannin complex bond that is hard to be digested in the digestive system, and high fiber content. One technology that can be used to overcome the constraints of banana stem utilization as a ration component is by using microorganisms through a fermentation process. This is due to the enzyme activity produced by the microorganism which may cause changes in the nutrient composition of the media, covering changes of complex molecules such as protein, carbohydrate and fat, into simple molecules.

One of microorganisms that can be used is *Trichoderma viridae.* Fungus is well known as cellulolytic organisms and produce cellulolytic enzymes, including cellobiohydrolase, endoglucanase and ß-glucosidase [5]. In the process of fermentation factors that must be considered, namely, inoculum dose, temperature, substrate, pH and nutrients, as well as the time required by the microbes to grow and multiply. Through the process of fermentation flour made from the *goroho* banana stem is expected to have increased nutritional value, so it can be used as feed material of better quality than the original material. There are any changes made in the substrate through the fermentation process [24]. Coconut cake fermentation with *Trichoderma viridae* on inoculum dose of 0.6% and 6-days fermentation time may increase the protein content and lower crude fiber.

Nutrition supplied by the *goroho* banana stems is not yet sufficient, but when it is being related to the nature of carbohydrates contained in plants and the use of fermentation technology, it is expected that the quality can be increased and later allowed this waste to be used as the ration. The use of *goroho* banana stem in the ration of broiler without fermented resulted in limited role, replacing only 5 percent of the corn in the ration measured by feed intake, weight gain, feed conversion, carcass percentage and abdominal fat [13].

MATERIALS AND METHODS

This research was conducted at the Laboratory of Technology and Animal Feed Industry of the Faculty of Animal Husbandry at Universitas Sam Ratulangi, Manado. The method used was a laboratory experiment using a completely randomized design with nested pattern. Treatment at this stage of research: factor A is the dose of *Trichoderma viridae* fungi consisted of: D1 = 0.3%; D2 = 0.6%; D3 = 0.9%. Factor B is the fermentation duration, comprised of: W1 = 4 hours; W2 = 8 days; and W3 = 12 days. Each treatment combination was repeated three times, thus obtained 27 experimental units. During the fermentation process of *goroho* banana stem flour, *pocari sweat* was added as much as 50 percent in the *autoclave* for 15 minutes. The substrate is cooled, then the inoculation and incubation process is carried out in accordance with the treatment on each factor. After that, the substrate is dried and the nutrients content is analyzed using Proximate Analysis Method according to *AOAC (1995)* [1].

Data were statistically analyzed according to completely randomized design with nested pattern [10]. The real difference between treatments were being tested further by *Duncan's Multi Range Test* [19].

RESULTS AND DISCUSSIONS

Effect of inoculum dose and fermentation time to change Ash Content

Results showed that changes in the content of Ash depend on inoculum dose and the duration of fermentation. The average of change in Ash content can be seen in Table 1.

Table 1. Average of Ash Content Reduction in Fermentation Products on each Treatment

Treatment		Repetition			Amount	Average
Dose	Time	1	2	3		
		----------------------	%	----------------------		
D1	W1	3.24	3.23	4.01	10.48	3.49
	W2	5.06	4.95	5.02	15.03	5.01
	W3	5.10	6.12	6.33	17.55	5.85
Average						4..78[a]
D2	W1	8.54	7.69	8.40	24.63	8.21
	W2	11.57	12.33	11.03	34.93	11.64
	W3	12.10	11.56	12.90	36.56	12.21
Average						10.68[b]
D3	W1	12.00	11.10	10.70	33.80	11.27
	W2	13.25	12.90	11.23	37.38	12.46
	W3	12.50	11.10	13.00	36.60	12.20
Average						11.98[b]

Notes: D1= 0.3 %; D2 = 0.6%; D3= 0.9%; W1 = 4 days; W2= 8 days; W3 = 12 days

The data on Table 1 show the reduction in Ash content; with the most reduction happened on treatment D2W3 *(T.viridae* 0.6 per cent, 12 days) and the least reduction on treatment on D1W1 *(T.viridae* 0.3 percent, 4 days). Results of variance analysis showed that the treatment of inoculum dose and the fermentation duration have significant influence (P> 0.05) to the decrease of ash content. Decrease in ash content identified an increase in the number of organic material substrates. Organic materials contained important nutrients, such as proteins, fats and carbohydrates as well as vitamins. Therefore, loss of organic material means it will also lose nutrient substances which are quite important. In terms of nutrients, the amount of ash is not so important, but in the proximate analysis the ash is necessary to calculate or measure the NPF value (extract ingredients without N) [4]. This study is in line with research on rice straw fermentation using *Trichoderma viridae*, in which the ash content also happened to decrease, which showed an increase of organic material during the fermentation process [20]. Increase of organic material content is allegedly because after fermentation, the substrates experienced nutrient contents recast by microorganic enzymes so that the percentage of nutrients that can be utilized was increased, which was reflected by the increased protein value and reduced level of ash [14].

The treatment combination of 0.9 percent inoculum dose and fermentation duration of 12 days resulted in higher rate of ash content reduction compared to the treatment combination of 0.3 per cent inoculum dose with fermentation duration of 4 days and the treatment combination of 0.6 per cent with fermentation duration of 8 days. Regardless of the results, statistically there is no significant difference (P>0.05) between the treatment combination of 0.6 percent inoculum dose with fermentation duration of 8 days and the treatment combination of 0.9 percent inoculum dose with fermentation duration of 12 days. This means that inoculum dose of 0.6 percent combined with fermentation duration of 8 days is more effective in increasing the percentage of the ash content reduction of the *goroho* banana stems fermentation products.

This is in accordance with the opinion of Fardiaz, which states that an increase in the reduction percentage on ash content during the fermentation process is caused by the increase in fungi body cell mass and the increase in the product concentration due to various changes in organic material resulted by the bioconversion processes that produced H_2O and CO_2 [7].

The Effect of Inoculum Dose and the Fermentation Duration on Crude Protein Content Changes

The results showed that an increase in the reduction percentage on crude protein content depend on inoculum dose and the duration of fermentation. The average of the increasing crude protein content level can be seen in Table 2.

Table 2. Average of the Increasing Crude Protein Content Level of Fermentation Products of each Treatment

Treatment		Repetition			Amount	Average
Dose	Time	1	2	3		
		----------------	----------	%----------	----------	----------
D1	W1	17.59	16.08	19.85	53.52	17.84
	W2	16.83	18.09	17.21	52.13	17.38
	W3	18.40	19.33	20.88	58.61	19.54
Average						18.25[a]
D2	W1	18.79	20.50	20.55	59.84	19.95
	W2	20.34	21.86	22.88	65.08	21.69
	W3	21.12	24.38	23.04	62.14	20.72
Average						21.50[b]
D3	W1	21.60	20.44	21.34	63.38	21.13
	W2	21.87	22.61	23.13	67.61	22.54
	W3	20.35	20.85	22.99	64.19	21.40
Average						21.69[b]

Notes: D1 = 0.3%; D2 = 0.6%; D3 = 0.9%; W1 = 4 days; W2 = 8 days; W3 = 12 days

The data on Table 2 show the crude protein content increase; with the most happened on treatment D2W2 (*T.viridae* 0.6 percent, 8 days) and the least happened on treatment D1W1 (*T.viridae* 0.3 percent, 4 days). Variance analysis test showed that the treatment of inoculum dose and the duration of fermentation have significant influence (P> 0.05) to increase the protein content. Furthermore, the Duncan's multiple range test was done to determine the effect between treatments.

Treatment D2 (*T.viridae 0.6 percent*) did not differ significantly (P> 0.05) from treatment D3 (*T.viridae 0.9 percent*) against the crude protein content of the *goroho* banana stem flour obtained from fermented products. Table 2 shows that the highest crude protein content was obtained on treatment D3 (fermentation with 0.9% inoculum dose), but statistically it did not differ significant with treatment D2 (fermentation with 0.6% inoculum dose). This is due to the fungus *Trichoderma viridae* capability of utilizing organic materials contained in the substrate to be converted to crude protein. During the fermentation process the growth of crude protein level causes an increase in the number of microbial biomass [3]. The fungus, which has the ability to produce the enzyme protease, will remodel the protein. Protein changed into polypeptides, then into small peptides which ultimately further recasted into amino acids, which will be used by microbes to proliferate. The growth on the number of microbe colonies, which is a single cell protein, during fermentation indirectly increased the crude protein substrate content [2][3]. Afterwards, the Duncan's multiple range test was done to determine the fermentation duration on treatment D2 (fermentation with 0.6% inoculum dose) on the reduction in the crude protein content level of the flour banana stems fermented products.

Treatment with fermentation time of 8 days had no significant influence (P> 0.05) with the treatment with fermentation period of 12 days on the crude protein content level of the *goroho* banana stem flour. The longer the fermentation time, the higher is the growth of the crude protein content level of the fermented banana stem flour products. This is due to fungus *Trichoderma viridae* will always take advantage of organic material on the substrate. In this study, the best treatment duration is 8 days, because statistically there are no significant difference between 8-days treatment and the 12-days treatment.

It is in accordance with the four principal stages of the microbe growth, namely: *lag phase* (adaptation phase), which at this time the growth happened slowly and microbes tend to adapt with the new environment; *exponential / logarithmic phase* (growth phase); *stationary phase* (phase where the death level is balanced with the growth level); and *death phase*, where mortality is greater than growth [6].

The Effect of Inoculum Dose and the Fermentation Duration on Crude Fiber Content Changes

Results showed that changes in the content of crude fibers depend on inoculum dose and the duration of fermentation. The average change in the content of crude fibers can be seen in Table 3.

Table 3. Average Reduction of Crude Fiber Content of Fermentation Products of each Treatment

Treatment		Repetition			Amount	Average
Dose	Time	1	2	3		
		------------------------	------------------------	% ------------------------	------------------------	------------------------
D1	W1	8.83	9.24	9.60	27.67	9.22
	W2	10.22	10.77	11.63	32.62	10.87
	W3	11.76	11.99	10.65	34.40	11.47
Average						10.52[a]
D2	W1	11.55	12.22	13.01	36.78	12.26
	W2	14.41	14.41	15.01	43.43	14.48
	W3	15.00	14.99	15.32	45.31	15.10
Average						13.99[b]
D3	W1	13.09	14.01	13.90	41.00	13.67
	W2	15.01	15.23	14.99	45.32	15.11
	W3	16.01	15.09	16.31	47.41	15.80
Average						14.27[b]

Notes: D1 = 0.3%; D2 = 0.6%; D3 = 0.9%; W1 = 4 days; W2 = 8 days; W3 = 12 days

The data on Table 3 show the reduction in crude fiber content; with the most reduction happened on treatment D3W3 (*T.viridae* 0.9 per cent, 12 days) and the least happened on treatment D1W1 (*T. viridae* 0.3 percent, 4 days). Variance analysis tests showed that the treatment dose and the duration of fermentation inoculum have significant influence (P> 0.05) in reducing the content of crude fiber. Furthermore, the Duncan's multiple range test was done to determine the effect between treatments.

The result showed that the treatment using dose of 0.6% (D2) did not differ significantly (P> 0.05) with treatment using dose of 0.9% (D3) but significantly different (P <0.05) with treatment using dose of 0.3% (D1). It showed that the treatment D2 (with dose of 0.6%) is the most effective treatment to reduce the percentage of crude fiber of the *goroho* banana stem fermented products flour. It is proved that, as lignocellulolytic microbes, fungi *Trichoderma viridae* were able to remodel the high-fiber feed so as to reduce the percentage of crude fiber on the coconut substrate [24].

The reduction of crude fiber percentage is related with the cellulase enzymes produced by *Trichoderma viridae*. Cellulase is a group of fibrolitic enzymes capable of hydrolyzing fibers on plant cell walls into glucose [12]. Furthermore, the fermentation time on the treatment dose inoculum 0.6 percent (D2) to the decrease of the content of crude fiber decline in crude fiber in the treatment of D1, D2, and D3, W3 treatment (12 days) had no significant (P.0.05) with treatment W2 (8 days) but clearly different to the treatment D1

(4 days). That is a long fermentation period of 8 days to more effectively reduce the percentage of coarse flour banana stem fiber product goroho fermentation.The decreased percentages of crude fiber, to do the role of microbes in this fungus *Trichoderma viridae,* these fungi produce cellulase enzymes. Cellulase enzyme would degrade cellulose into simpler carbohydrate compounds that can be utilized by fungi as a source of energy, it will reduce the proportion of coarse fiber content of the *goroho* banana stem flour [16].

The Effect of Inoculum Dose and the Fermentation Duration on Crude Fat Content Changes

Results showed that changes in crude fat content depend on inoculum dose and the duration of fermentation. Average changes in crude fat content can be seen in Table 4.

Table 4. Average Reduction of Crude Fat Content of Fermentation Products of Each Treatment

Treatment		Repetition			Amount	Average
Dose	Time	1	2	3		
		-----------------------	------- % -------	-----------------------		
D1	W1	14.88	14.45	15.70	45.03	15.01
	W2	17.62	19.01	18.18	54.81	18.27
	W3	18.18	18.79	19.01	55.98	18.66
Average						17.31[a]
D2	W1	18.78	17.27	18.04	54.09	18.03
	W2	20.49	21.01	19.01	60.51	20.17
	W3	20.33	20.55	19.90	60.78	20.26
Average						19.49[b]
D3	W1	19.18	20.49	20.62	60.29	20.97
	W2	21.18	22.88	21.09	65.09	21.70
	W3	20.33	25.56	23.06	65.95	21.98
Average						21.26[b]

Notes: D1 = 0.3%; D2 = 0.6%; D3 = 0.9%; W1 = 4 days; W2 = 8 days; W3 = 12 days

The data on Table 4 show the reduction in crude fat content; with the most reduction happened on treatment D3W3 *(T.viridae* 0.9 per cent, 12 days) and the least reduction happened on treatment D1W1 *(T.viridae* 0.3 percent, 4 days). Variance analysis tests showed that the treatment of inoculum dose and the duration of fermentation have significant influence ($P > 0.05$) in lowering the crude fat content. Furthermore, the Duncan's multiple range test was done to determine the effect between treatments.

The result showed that the treatment using dose of 0.6% (D2) did not differ significantly ($P > 0.05$) with treatment using dose of 0.9% (D3) but significantly different ($P < 0.05$) with treatment using dose of 0.3% (D1). It showed that the treatment D2 (with dose of 0.6%) is the most effective treatment to reduce the percentage of crude fat content of the *goroho* banana stem fermented products flour. In the fermentation process, *Trichoderma viridae* used fat as the energy source for breeding [8]. Time fermentation inoculum dose treatment D1, D2, and D3, W3 treatment (12 days) had no significant ($P > 0.05$) with treatment W2 (8 days) but clearly different to the treatment D1 (4 days). That is a long fermentation period of 8 days to more effectively reduce fat percentage of coarse flour banana stem goroho fermentation products. The changes that occur during the process of fermentation can occur in fat in the substrate, a neutral fat will be hydrolyzed into free fatty acids, which are used for the growth of fungi [18]. This occurs in flour banana stem inoculation with the fungi *Trichoderma sp* goroho looks rough can decrease the fat content of the substrate.

CONCLUSIONS

Based on the results of this study, it can be concluded that the best value of the inoculum dose for *Trichoderma viridae* is 0.6% with a fermentation time of 8 days resulted in changes to the content of ash, crude protein, crude fiber, and crude fat content of the *goroho* banana stem flour *(Musa acuminafe, sp),* respectively Ash 20.17%, Crude Protein 21.69%,Crude Fiber 14.94% and Crude Fat 20.17%.

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