

Lignin and Cellulose Content of Fermented Rice Straw with *Aspergillus niger* (van Tieghem) and *Trichoderma mutan* AA1

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Abstract. The rice straw has potential to be used as an alternative ruminant feed. However, it has limiting factors i.e low crude protein, high crude fiber, lignin, cellulose, and silica content. To overcome the limiting factors, immersion in a solution of alkaline (lime) or fermentation by using inoculum microbial cellulolytic and lignocellulolytic (*Trichoderma mutan* AA1 and *Aspergillus niger*). The research method was experimental, with four treatments and repeated five times. Completely randomized design was used and if there are differences among treatments a further test with DMRT was carried out (level 1 % and 5 %). These treatments were T0: The rice straw without t fermentation; T1: Fermented with *A. niger*; T2: Fermented with *T. mutan* AA1; T3: Fermented with a combination *A. niger* and *T. mutan* AA1. The results showed that the rice straw fermented with *A. niger* and *T. mutants* AA1 very significantly increased the cellulose and decreased lignin content. The highest cellulose reached on T3 (20.297 %) followed by T2 (18.191 %), T1 (17.712 %) and T0 (16.747 %), respectively. While the lowest content of lignin reached on T3 (14.793 %), followed T2 (26.063 %), T1 (26.421 %) and T0 (38.164 %).

Keywords: Fermentation, *fungus*, increase feed quality, inoculum, waste to feed

1 Introduction

The rice production in Indonesia is abundant, namely in 2017, the production of dry rice around $81\,382\,451 \times 10^6$ t. Based on the calculations, each of ton dry grain will produce the rice straw with equivalent weight. Therefore, rice straw availability enormous potential in Indonesia not yet utilized optimally for ruminant feed. The area of the rice straw is quite

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wide and spread, proper management is needed. But rice straw as feed has a limiting factor i.e. low crude protein, high crude fiber, lignin, and cellulose content [1–3]. Rice straw contains crude protein about 3.80 % [4] and fiber around 39.81 %, lignin around 15 % [5] and silica about 10 % to 15 % [6, 7]. The high fiber content will delay the hydrolysis process by fermentation, i.e. microbes enzyme in rumen resulting lower absorption [8–10]. Previous study Sukaryani [11] showed that 4 d fermentations of rice straw with MA–11 started by submersion in alkaline solution improved the crude protein from 5.22 % to 8.23 % and decreased the crude fiber from 54.24 % to 38.83 %. While the fermentation of rice straw from 2 d to 6 d by MA–11 starting with submersion of rice straw in the alkaline solution, reducing NDF from 69.22 % to 62.20 % and ADF from 50.21 % become 36.93 % [12].

To overcome the limiting factors of rice straw, an alternative effort that can be done are to provide the treatment of immersion in a solution of alkaline (lime), and the process of the rice straw fermentation by using inoculum microbial cellulolytic and lignocellulolytic (*Trichoderma* sp. and *Aspergillus* sp.). *Trichoderma* sp producing the extracellular prolific protein [15] and it has the ability to produce the enzyme to degradation the cellulose [14, 15]. Similarly, *A. niger* produce some enzymes such as enzyme cellulolytic [14, 15], amylase [18], and glucoamylase [19].

This research aimed to assess the content of lignin and cellulose in fermented rice straw with *A. niger* and *T. mutants* AA1.

2 Materials and methods

The study was conducted in Laboratory Agriculture Faculty Veteran Bangun Nusantara University of Sukoharjo, Central Java, Indonesia.

The material used in the form of rice straw obtained from paddy fields in Karanganyar district, Central Java, Indonesia microbial (*A. niger* and *Trichoderma mutant* AA1), molasses, urea, lime, and water.

The tool used in this study was a bucket and place of manufacture fermentation jar, chopper, blender, and laboratory devices for content analysis lignin and cellulose.

This research uses a design completely randomized design with the pattern of four kinds of treatments and five replications. The treatments are:

T0: The rice straw without fermentation

T1: The rice straw fermented with *A. niger*

T2: The rice straw fermented with *T. mutant* AA1

T3: The rice straw fermented with a combination *T. mutans* AA1 dan *A. niger*

Before fermentation, rice straw was submersed in a lime solution for 2 d.

The variables observed in this study werelignin and cellulose content. The data were analyzed statistically using Analysis of Variance (ANOVA) and followed by Duncan's Multiple Range Test (DMRT).

3 Result and discussions

3.1 Lignin content (%)

The results showed that the lignin content of fermented rice straw with *A. niger*, *T. mutant* AA1, and the combination were presented in Table 1.

Table 1. Lignin content of rice straw fermented with AA1 *mutan Trichoderma* and *A. niger*

The replication	Lignin content (%)			
	T0	T1	T2	T3
1	37.422	25.629	26.915	14.401
2	37.779	27.184	25.780	15.989
3	38.638	26.086	27.601	14.425
4	37.234	26.899	24.161	13.451
5	39.745	26.308	25.860	15.701
Average	38.164 ^a	26.421 ^b	26.063 ^b	14.793 ^c

Note: Different superscripts on the same line show highly significant ($p < 0.01$).

Based on Table 1, lignin content in fermented straw with *A. niger* or *T. mutant* AA1 individually or in combination degrade lignin highly significant. After further tested by DMRT showed, the lowest lignin content was reached by T3 (14.793 %), followed by T2 (26.063 %), T1 (26.421 %) and T0 (38.164 %). Treatment T1 and T2 showed lignin content was not significantly different. Visually, the lignin in T2 tends to be lower than T1. This indicates that the *Trichoderma* has higher ability to degrade lignocellulose than the *A. niger*. The content of lignin in T3 is lower significantly than T0, T1, and T2, this phenomenon indicates that both types of fungus can grow and thrive in an atmosphere of the same media and mutual synergy to meet the needs. During the fermentation, *A. niger* and *T. mutant* AA1 produced lignolytic enzyme that degrade lignocellulose, wrapping the cell wall binding micro fibril cellulose and hemicellulose apart, then the lignin in the cell wall that prevents the cellulose down. This result in accordance to Agustini and Efiyanti [20] that the biological treatment of the substrates *Albiziachinensis*, sawdust and palm leaf midrib with fungi FB1 MD-14 isolate lower the lignin content. Likewise, some studies stated fermentation with microbes can degrade lignin to improve the quality and palatability of feed [21–24].

3.2 Cellulose content

The research data from cellulose content listed in Table 2.

Table 2. Cellulose content of rice straw fermented with *T. mutant* AA1 and *A. niger*

The replication	Cellulose content (%)			
	T0	T1	T2	T3
1	16.709	17.408	17.860	20.705
2	17.812	17.821	18.030	19.142
3	16.012	17.728	18.523	20.225
4	16.725	17.649	18.170	21.046
5	16.479	17.957	18.373	20.369
Average	16.747 ^a	17.712 ^b	18.191 ^b	20.297 ^c

Note: Different superscripts on the same line show very significantly different ($p < 0.01$)

Result showed that the treatment of fermentation using *A. niger* and *T. mutant* AA1 very significantly improved the content of cellulose. This is might due to the activity of enzyme produce by cellulolytic has degraded lignocellulose. Cellulose become available for degrading and absorption. According to Prihartini, et al. [25], cellulose content in fermented rice straw tends to improve. In addition by Agustini and Efiyanti [20] that

biological treatment that applied to the substrate *A. chinensis* wood, sawdust, and palm leaf midrib with fungi MD-14 FB1 isolate tend to improve cellulose. After being further tested by DMRT, the highest cellulose content was by T3 (20.297 %) and the lowest in T0 (16.747 %).

4 Conclusions

The results of the research can be concluded that:

- i. Fermented of the rice straw using *A. niger* and *T. mutant* AA1 with monoculture and a combination of both decreased the lignin and increased cellulose content.
- ii. The lowest lignin and highest cellulose content in fermented rice straw occurred in a combination of *A. niger* and *T. mutant* AA1 inoculum.
- iii. Fermentation rice straw with *A. niger* and *T. mutant* AA1 in monoculture produced not different lignin and cellulose content.

5 Suggestion

Further research on fermentation can be done by using a combination of fungus *A. niger* and *T. mutant* AA1 to be applied on others agricultural waste.

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