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 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. mutan* 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 × 10⁶ 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
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. mutant 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 were lignin 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.
Multiple Range Test (DMRT)

T3:

T1: The rice straw fermentation

T0: The rice straw without fermentation

The treatments are: chopper, blender, and laboratory.

T0: Molasses, urea, lime, and water.

Amylase

15 protein

(rice straw fermentation by using

8.23 % and decreased the crude fiber from 54.24 % to 38.83 %.

While the fermentation

Silica about

8

8

- phenol

- sulfuric acid

- methanol

Lignin

Cellulose

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 Efifyanti [20] that

| 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 T3 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 Efifyanti [20] that the biological treatment of the substrates Albizia chinensis, 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.

| The replication | Cellulose content (%) | T0 | T1 | T2 | T3 |
|-----------------|-----------------------|----|----|----|----|
| 1               | 16.709                | 17.408 | 17.860 | 20705 |
| 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 )
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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Further research on fermentation can be done by using a combination of 

\[ \text{Fermented of the rice straw using} \]

\[ \text{A. niger}\]

The results of the research can be concluded that:

\[ \text{4. by DMRT} \]

\[ \text{midrib} \]

\[ \text{b} \]

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iii.

Conclusion

T. mutant

Composition

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