

THE QUALITY OF FERMENTED RICE STRAW WITH *Trichoderma viride* INOCULUM

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Supporting Information

ABSTRACT: Rice straw has several nutritional weaknesses, namely its high silica and lignin content, and its low level of protein, minerals and vitamins, so the impact on digestibility is also low. Aim of present study was to evaluating nutritional efficacy of rice straw after fermentation with *Trichoderma viride*. The study was conducted by using complete random design. There were three different treatments with four replicates for each treatment. Fermented rice straws were treated with varying concentrations of *Trichoderma viride* inoculum as follows; 0.5% (T1), 1% (T2), and 1.5% (T3). Fermented rice straw's nutrients, including dry ingredients, organic material, crude fiber, crude protein, dry matter digestibility coefficients, and organic matter digestibility coefficients were measured in this study. T3 (1.5% of *T. viride*) treatment performed a proper nutrient, with 80.02% dry ingredients, 80.03% organic materials, 31.68% crude fiber, 5.72% protein, 38.46% dry matter digestibility coefficient, and 61.05% organic matter digestibility coefficient. In conclusion, using 1.5% *Trichoderma viride* to improve the quality of rice straw, as stimulator of fermentation process can be efficient in ruminant or non-ruminant nutrition.

Keywords: Agricultural by-product; Crude fiber; Digestibility; Ruminants; *Trichoderma viride*.

INTRODUCTION

Mainly, forage is major feedstuff for ruminants (Minson, 2012). However, the limited stock of forage is the major obstacle for farms. So, to increase the nutritional efficacy of ruminants, high quality and quantity of forage are required (Guyader et al., 2016; Mahanta et al., 2020). The high sustainability of forage is directly proportional to the high productivity of ruminants (Guyader et al., 2016). Supplying ruminants with high energy food sources; such as grass, leguminosae, and agricultural by-products will increase the quality of the animal production (Roy et al., 2019; Yusriani et al., 2021).

An agricultural by-product that is commonly processed for animal feed is rice straw (Aquino et al., 2020). It is supposed to be an energy source for ruminants (Aquino et al., 2020). Even so, rice straw contains high concentrations of silica and lignin and also fewer vitamins and minerals (Malik et al., 2015; Aquino et al., 2020). It has a low digestibility level, which is not applicable enough to be a food stock (Sarnklong et al., 2010). The low digestibility level is caused by the old supporting tissue that undergoes lignification (Huang and Lo, 2019). The lignocellulose and hemicellulose of rice straws are the main causes of low digestibility levels (Tama et al., 2020).

Improving crude protein (CP) is an essential nutrient for improving rice straw quality (Sufyan et al., 2022); Yanuartono et al. (2017) reported that rice straw contained 3-5% crude protein, lower than leguminosae and grass. Setiarto (2013) explained that the fermentation and ammoniation methods can elevate rice straw nutrition. Fermentation is one of the food/feed processing technologies involving microorganisms to degrade coarse fiber and reduce lignin concentration to increase the quality of food/feed (Xia et al., 2018; Shen et al., 2018). One of fermenter agent was *Trichoderma viride* it has exhibited with high protein, and fat, also with potential of crude fiber reduction (Kasmira et al., 2023). Stand out among the cellulose-producing bacteria. The most studied species of filamentous fungi are *Trichoderma viride* (Gautam et al., 2011). However the optimum concentration of *Trichoderma viride* for improving rice straw nutritional quality is remaining unclear. Therefore, we investigated the optimum concentration of *Trichoderma viride* to improve rice straw nutrients.

MATERIALS AND METHODS

This study was conducted in July-September 2022 at Teaching Farm and Feed Chemical Analysis Laboratory of Islamic University Malang.

Production of fermented rice straws

The experimental design of this study was complete randomized design (CRD) with three treatments and four repetitions. Three various concentrations of *Trichoderma viride*, included T1: rice straw + 0.5% *T. viride* inoculum; T2: rice

RESEARCH ARTICLE
 PII: S222877012300022-13
 Received: February 16, 2022
 Revised: March 26, 2023
 Accepted: March 27, 2023

straw + 1% *T. viride* inoculum; and T3: rice straw + 1.5% *T. viride* inoculum, were used for rice straw fermentation. The fermented rice straws were incubated for 21 days. Proximate analysis (dry ingredients, organic materials, crude fiber, and crude protein) was measured on the fermented rice straw to analyze the fermented rice straw quality (AOAC International, 2015). The dry matter digestibility and organic digestibility coefficients were analyzed *in vitro*, referred to Tilley and Terry (2006).

Data analysis

The nutrients of fermented rice straws were statistically analyzed by one-way ANOVA, followed by Duncan test with significant $P < 0.05$. Statistical analysis was conducted by SPSS software version 20.

RESULTS AND DISCUSSION

The nutrition composition of fermented rice straws

Various *Trichoderma viride* concentrations in 21 days rice straw fermentation impacted the quality of rice straw (Table 1). T1 (0.5% of *T. viride*) increased the organic materials and crude fiber significantly ($P < 0.05$). Besides that, T1 did not show significantly dry ingredients higher than T2 and T3. Muck et al. (2018) reported that the dry ingredients of rice straw fermented by local microorganisms ranged from 34.04% to 38.18%, lower than this study. Various effects of dry ingredients in fermented sources were caused by microorganism population. A higher population of microbes was effectively breakdown the substrate for energy production. Increased hydrolysis reaction of microorganisms decreased the dry ingredients of food stock. Furthermore, fermentation elevated the digestibility of raw fermented food (Jasin, 2014). According to Chen et al. (2019) statement, the decrease in silage dry matter is influenced by respiration and fermentation. Respiration will cause a lot of nutrient content to decompose so that it will reduce dry matter, while fermentation will produce lactic acid and water. Furthermore Surono et al. (2006) stated that the increase in the level of additive (*Trichoderma viride* inoculum) is thought to stimulate fermentation activity, causing H₂O production to also increase. The increase in water content in ensiling causes the dry matter content of the silage to decrease, causing an increase in dry matter loss.

The organic materials of fermented rice straws in Table 1 ranged from 80.03% to 82.50%. Similar to dry ingredients, T3 performed lowest organic materials, significantly ($P < 0.05$). Organic materials of fermented food were affected by lactic acid bacteria. Growth microorganisms metabolized carbohydrates and protein to organic materials (Zahra et al., 2020). It could also affect the nutrition value of fermented feed nutrition, especially the reduction of organic materials. Muck et al. (2018) described that chemical change of carbohydrate occurred during fermentation processes to generated energy. Surono et al. (2006) that in general it is known that lactic acid in ensilages is produced from organic components, especially carbides, thereby increasing the formation of lactic acid. Loss of organic matter in silage which mainly comes from carbohydrates, namely BETN with the main components of starch and sugar is used by bacteria to produce lactic acid. Carbohydrates were converted into alcohol, organic acid, water, and carbon dioxide. Daning and Karunia (2018), found that *Trichoderma* produced cellulase enzymes to hydrolyze cellulose and crystal, which caused the percentage of coarse fiber in the substrate.

The crude fiber showed the significantly high on the T1, followed by T2 and T3. The 1.5% dose of *T. viride* inoculum (T3) might have ideal performance to decompose rice straw and produced crude fiber. Fermented rice straw using *Trichoderma viride* for 21 days produced crude protein, ranged from 5.55% to 5.72%. Suningsih et al. (2019) identified the activity of proteolytic activity in the fermented raw food, which fermentation increased proteolytic activity and exhibited different value of crude protein. The amount of water-soluble N and water-soluble solid will be increased during fermentation. The increase of water-soluble N is caused by protease enzymes that decompose protein into water-soluble fragments. Then, microbes used the water-soluble fragments for their growth and contributed to the single cell protein, increasing microbes mass and substrate's coarse protein, respectively (Suningsih et al., 2019). The presence of *T. viride* inoculum is suspected as an energy source for lactic acid bacteria so that it can work optimally in fermentation where bacterial acid is a microbe that plays a role in the crude protein content of silage to ferment sugar into lactic acid where the bacteria are protein contributors of microbial origin. Furthermore, Santoso et al. (2013) stated that lactic acid bacteria have an important role in forage fermentation and affect the quality of the resulting silage. According to Afrianti (2022) results, fermentation improved the nutritional value and degraded raw food sources, indicating easier to digest.

Digestibility of fermented rice straws

Digestibility is the percentage of nutrients that were absorbed in the digestive tract. It was obtained from the difference of intake nutrients with the nutrients in feces. The dry matter digestibility coefficient of fermented rice straws ranged from 35.93% to 38.46% (Table 2).

The variance analysis showed that fermentation with *Trichoderma viride* to rice straws was significantly different ($P < 0.05$). The Duncan test showed that T1 was not different with T2, but showed significantly different results with T3. The 1.5% of *T. viride* inoculum increased the activity of decomposing microbes and produced higher energy. With so many decomposing microorganisms, it functions to digest crude fiber, namely as a digester of cellulose as well as hemicellulose and starch.

Energy source stimulates microbes to degrade crude protein (Owens and Basalan, 2016). Jasin (2014) explained that Volatile fatty acids (VFAs) are important for microorganisms' growth that digest the crude fiber in the rumen and be the source of carbon chain for protein synthesis. Ouyang et al. (2019) reported that high digestibility in ruminants showed high digested-nutrition by rumen's microbes. The increase of rumen's metabolites will affect the increase of growth followed by livestock weight gain as reported by Cantalapiedra-Hijar et al. (2018) and Mwangi et al. (2022).

Digestibility of organic matter in ruminants' intestines includes carbohydrates, protein, fat, and vitamin. This indicator also relates to the dry matter digestibility coefficient because most of the dry matter in feed consisted of organic and inorganic material. According to the study, fermented rice straws' organic matter digestibility coefficient ranged from 56.88% to 61.05%. However, in the variance analysis, the fermentation with *T. viride* inoculum showed insignificant difference ($P < 0.05$). Similarly, dry matter digestibility coefficient, the high level of *T. viride* inoculum resulted in a high organic matter digestibility coefficient. From this result, it could be assumed that the high microorganism level was directly proportional to the digestibility coefficient. The high percentage of organic matter digestibility coefficients was caused by cellulose's complete digest (Nahak et al., 2019; Xia et al., 2017). Daning and Karunia (2018) explained that *Trichoderma* could convert the organic materials in the feed, causing high organic matter digestibility coefficient.

Table 1 - Nutrition contents of fermented rice straws

Treatment	T1	T2	T3
Nutrition (%)			
Dry ingredients	80.98 ± 1.16 ^a	80.41 ± 2.29 ^a	80.02 ± 2.21 ^a
Organic materials	82.50 ± 0.34 ^a	81.19 ± 0.19 ^b	80.03 ± 0.06 ^c
Crude fiber	32.88 ± 0.45 ^a	32.35 ± 0.04 ^{ab}	31.68 ± 0.35 ^b
Crude protein	5.55 ± 0.35 ^a	5.70 ± 0.36 ^a	5.72 ± 0.49 ^a

T1=rice straw + 0.5% *Trichoderma viride* inoculum; T2=rice straw + 1,% *T. viride* inoculum; T3= rice straw + 1.5% *T. viride* inoculum; Mean ± standard error; Different superscripts in the same row represented significantly different ($P < 0.05$).

Table 2 - Percentage of digestibility of fermented rice straws

Treatment	T1	T2	T3
Digestibility coefficient (%)			
Dry matter digestibility coefficient	35.93 ± 0.78 ^a	36.69 ± 1.00 ^{ab}	38.46 ± 1.37 ^b
Organic matter digestibility coefficient	56.88 ± 1.79 ^a	59.08 ± 1.82 ^a	61.05 ± 2.56 ^a

T1=rice straw + 0.5% *Trichoderma viride* inoculum; T2=rice straw + 1,% *T. viride* inoculum; T3= rice straw + 1.5% *T. viride* inoculum; Mean ± standard error; Different superscripts in the same row showed significantly different effects ($P < 0.05$).

CONCLUSION

Fermentation of rice straws using 1.5% *Trichoderma viride* inoculum showed the better nutritional contents , with the score of dry ingredients of 80.02%, organic materials 80.03%, coarse fiber 31.68%, crude protein 5.72%, dry matter digestibility coefficient 38.46%, and organic matter digestibility coefficient 61.05%. *Trichoderma viride* inoculum can be used in fermentation of fibrous feedstuffs in alternative ways.

DECLARATION

Author's contribution

B. MUWAKHID and U. KALSUM designed the study, and manuscript writing; RIFA'I collected samples and data, H.Y. SIKONE performed data analysis and manuscript writing. All authors drafted, revised and approved the final manuscript.

Conflict of Interests

The author has no possible conflicts of interest in this paper's research, authorship, or publishing.

Acknowledgement

The authors thank the support from the Institute of Research and Community Services University of Islam Malang (LPMM).

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