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# Effect of Banana Rhizome Microbial Levels on Fermented Rice Straw Quality

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**Abstract**
This study aimed to assess banana rhizome microbial as a fermentation starter and determine its optimal level for enhancing the chemical and physical properties of rice straw. The research design used a completely randomized design. Four treatments ($P_0$, $P_1$, $P_2$, $P_3$) were tested in two research phases: evaluating banana rhizome microbial quality through the Total Plate Count (TPC) and analyzing the physical and chemical properties of fermented rice straw. Among the treatments, $P_2$ (2 mL) exhibited the most favorable attributes as a fermentation starter, with a bacterial count of $4.7 \times 10^7$ CFU/mL. This concentration induced noticeable changes in rice straw, including a brownish-yellow color, sour smell, and slightly rough texture. Chemical analysis revealed significant improvements, with crude fiber content reaching 26.80% and crude protein content at 7.80%. These findings suggest that banana rhizome microbial communities, particularly at a concentration of 2 mL, hold promise as an effective fermentation starter for enhancing the nutritional quality of rice straw, potentially benefiting livestock nutrition and management practices. Future studies could explore broader applications and optimal conditions for utilizing banana rhizome microbes in agricultural fermentation processes.

## INTRODUCTION

Rice straw is a by-product of farming that includes the leaves and stems of harvested rice plants (Ilham et al., 2018; Permana et al., 2020). The abundant amount of rice straw makes it a commonly used alternative animal feed; however, using these wastes as feed for ruminant production resulted in lower animal production due to its lower protein and higher indigestible fiber content (Yanti and Yayato, 2017). Rice straw contains 84.22% dry matter, 4.60% crude protein, 28.86% crude fiber, 1.52% crude fat, and 50.80% extract material without nitrogen (Rasyid et al., 2022). High crude fiber content, including cellulose (23.05%), hemicellulose (19.09%), and lignin (22.93%), will be difficult for livestock to digest (Amin et al., 2015). Based on this, further processing is necessary to enhance the nutritional value of rice straw as an alternative feed for ruminants. One process that can be done is rice straw fermentation.

Kalsum et al., (2023) stated that fermentation is one of the feed processing technologies that involves microorganisms to degrade crude fiber and reduce lignin concentration, thereby increasing the quality of feed. The fermentation process is considered ideal for processing rice straw because it can break down anti-nutritional ingredients, such as those found in rice straw, to increase the availability of rice straw nutrients as animal feed (Lepongbulan et al., 2017). The fermentation process requires a starter as a source of microbes that will help break down the anti-nutritional ingredients. Local microorganisms from the banana rhizome serve as a starter for the fermentation process. Banana rhizome-based fermentation solution utilizes microorganisms derived from this plant source. This solution can be created from various carbohydrate-rich materials, such as molasses, coconut water, and the microorganism source itself, banana rhizome (Wardani et al., 2018).

Banana rhizome can be used as an ingredient for fermentation starters because it contains several microorganisms, such as *Bacillus sp., Aspergillus niger*, and cellulolytic microbes (Budiyani et al., 2016), that can play a role in decomposing organic materials, such as those found in rice straw. Khasanah et al., (2019) mentioned that banana rhizome microbial communities contain *Bacillus* as much as 3.05 x $10^2$ CFU/mL and cellulolytic bacteria as much as 6.65 x $10^5$ CFU/mL, where cellulolytic bacteria can help break down fibers in feed, especially lignin and hemicellulose.

*Bacillus sp* is a group of probiotic bacteria commonly used as probiotics and is capable of producing lactic acid and cellulase enzymes, which facilitate the composition of organic matter by breaking down fiber components in feed (Amin et al., 2015). Moreover, by producing extracellular ligninolytic enzymes, *Bacillus sp* oxidizes and degrades aromatic bonds in lignin, thereby breaking its polymer structure into simpler monomers. Similarly, *Aspergillus niger* degrades starch and cellulose by producing amylase and cellulase enzymes; these enzymes hydrolyze starch and cellulose in rice straw, there by contributing to relative reduction in lignin content and increase protein content (Supriyatna, 2017). In addition, *Aspergillus niger contains* nucleic acids that can serve as a high nitrogen source for single-cell protein (Suningsih et al., 2019). Finally, banana rhizomes provide a high carbohydrate content (66%), which can support the growth and activity of microorganisms (Patimah et al., 2020).

In this context, the study aimed to evaluate banana rhizome microbial as a fermentation starter and determine the optimal level to enhance the physical and chemical properties of rice straw.

## RESEARCH METHODOLOGY

### Material

The materials used to cultivate microbes from banana rhizome include banana rhizome, coconut water, and brown sugar. Tools utilized for this process include a chopper, scales, and a 5 L capacity gallon.

For bacterial analysis, the materials include banana rhizome microbial extract, distilled water, and Plate Count Agar (PCA, Merck, 1.05463.0500, Germany). Equipment used for Total Plate Count (TPC) comprises Pyrex Erlenmeyer flasks, test tubes, petri dishes, watch glasses, measuring cups, beakers, yellow tips, micro pipettes, spatulas, magnetic stirrers, Memmert incubators, Maxi Mix II vortex mixers, Hirayama autoclaves, laminar flow cabinets (Aneka lab type H.F.079), aluminum foil, bunsen burners, cotton, tissue, knives, and Scout Pro analytical scales with 0.0001 accuracy.

Materials employed for fermenting rice straw include rice straw, rice bran, banana rhizome, microbial inoculum, water, urea, and molasses. Tools utilized for this process include scales, a chopper, buckets, plastic bags, rope, and syringes.

## METHOD

### Experimental Design

The research employed an experimental method with two Completely Randomized Designs (CRDs). The first study aims to analyze the quantity of bacteria contained within the banana rhizome microbial. The first experimental design has three treatments and five replications as follows:
P1 = 1 mL of banana rhizome microbial
P2 = 2 mL banana rhizome microbial
P3 = 3 mL banana rhizome microbial
The objective of the second experiment is to analyze the quality of fermented rice straw. The second experimental design has four treatments and five replications as follows:
P0 = Rice straw without banana rhizome microbial
P1 = Rice straw + 1 mL banana rhizome microbial
P2 = Rice straw + 2 mL banana rhizome microbial
P3 = Rice straw + 3 mL banana rhizome microbial
The research variables observed are the number of bacterial colonies of banana rhizome microbes, the chemical quality of fermented rice straw, and the physical quality of fermented rice straw.

### Cultivation of Banana Rhizome Microbial

The method for cultivating banana rhizome microbial follows the protocol established by Suningsih et al., (2019). Fermentation takes place over 15 days, with the gallon lid being opened every 2 days.

### Fermented Rice Straw

Fermentation of rice straw was performed using the method described by Suningsih et al., (2019). Initially, 1 kg of rice straw was chopped into pieces measuring 3-5 cm in length and aerated to reduce its moisture content. Subsequently, the straw was spread flat, and 5 g of rice bran was evenly sprinkled over it. A solution comprising 3 g of urea, 3 mL of molasses, and banana rhizome microbial inoculum (as per treatment specifications) was dissolved in 250 mL of water and then applied evenly to the straw, thoroughly mixed. The treated rice straw was subsequently packed into double-layered plastic bags, compacted to remove air, sealed with rope to maintain anaerobic conditions, and left to ferment for 21 days.

### Microbiological Assay

The bacterial count of the banana rhizome microbial was assessed using the Total Plate Count (TPC) method, in accordance with ISO 2718:2007 guidelines for microbiological analysis of food and animal feed ingredients, with a colony count range of 30-300 colonies. Bacterial numbers are reported as CFU/mL.

**Evaluation of Fermented Rice Straw Quality**

The evaluation of fermented rice straw quality included both chemical and physical assessments. Chemical quality was determined by measuring Crude Fiber (CF) and Crude Protein (CP) contents, following the methods outlined in SNI 01-2891-1992 and AOAC 2005, respectively.

Physical quality assessment involved observing the color, odor, and texture of the fermented rice straw, with evaluation criteria specified in Table 1. Data collection for the physical quality test involved 30 untrained panelists, in accordance with guidelines from SNI 01-2346-2006 for sensory testing procedures.

Table 1. The Criteria for Assessing The Physical Quality of Fermented Rice Straw

| Physical Quality | Score | Characteristics of Fermented Rice Straw |
|---|---|---|
| Color | 9,0-10,00 | Green |
| | 7,0-8,9 | Yellowish green |
| | 5,0-6,9 | Brownish yellow |
| | 0,0-4,9 | Blackish brown |
| Odor | 9,0-10,00 | Forage smell |
| | 7,0-8,9 | Pungent smell |
| | 5,0-6,9 | Sour smell |
| | 0,0-4,9 | Musty smell |
| Texture | 9,0-10,00 | Very soft |
| | 7,0-8,9 | Soft |
| | 5,0-6,9 | Slightly rough |
| | 0,0-4,9 | Rough and brittle |

(Suningsih et al., 2019)

**Data analysis**

The data were analyzed using ANOVA (Analysis of Variance) in SPSS 20 software with a significance of $P < 0.05$, followed by post-hoc testing using Duncan's multiple range test.

## RESULTS AND DISCUSSION

**Total Bacterial Count of Banana Rhizome Microbial**

Evaluating the quality of rice straw fermentation using banana rhizome microbial as a starter is essential. One method to assess this quality is through TPC analysis. However, TPC serves only as an indicator of bacterial quantity within the banana rhizome microbial community. It does not provide insights into the specific types, activities, or functions of the bacteria present. The TPC test results for banana rhizome microbial in each treatment are detailed in Table 2.

Table 2. Total Bacteria of Banana Rhizome Microbial

| Treatment | Total Bacterial (CFU/mL) |
|---|---|
| P1 | $1.4 \times 10^{6ns}$ |
| P2 | $4.7 \times 10^{7ns}$ |
| P3 | $4.2 \times 10^{8ns}$ |

Remarks: ns (not significant) indicates a non-significant effect ($P > 0.05$).

The data presented in Table 2 indicate that the concentration of banana rhizome microbial solution did not have a significant effect on the total bacterial count ($P > 0.05$). Specifically, the bacterial counts were $1.4 \times 10^6$ CFU/mL for treatment P1, $4.7 \times 10^7$ CFU/mL for treatment P2, and $4.2 \times 10^8$ CFU/mL for treatment P3. These results demonstrate an increase in the microbial population of the banana rhizome solution with higher concentrations tested.

The research conducted by Yunilas et al., (2024) indicated that the peak of microbial population growth occurred on day 14 of fermentation, with a total bacterial count of $1 \times 10^6$ CFU/mL. Under these conditions, local microorganisms can be declared qualified for use as a starter. According to the research results by Fitria et al., (2023), the total number of bacteria in local microorganisms with potential as feed fermentation starters to improve the quality of low-grade feed was $1 \times 10^7$ CFU/mL. Based on the source of the discussion, the research carried out indicates that the number of bacteria in the banana rhizome microbial community in all treatments meets the criteria for use as a feed fermentation starter.

**Physical Quality of Fermented Rice Straw**

An assessment of the physical quality of fermented rice straw during the fermentation process, including color, odor, and texture, is necessary because it can serve as an indicator of the effectiveness of the fermentation process that has been carried out. The following are the results of the physical quality of fermented rice straw with the addition of different levels of banana rhizome microbial (Table 3).

Table 3. The Physical Quality of Fermented Rice Straw

| Treatment | Color | Odor | Texture |
|---|---|---|---|
| P0 | $6.5 \pm 0.23^{ab}$ | $6.1 \pm 0.23^{ns}$ | $6.0 \pm 0.14^{ns}$ |
| P1 | $6.7 \pm 0.13^{b}$ | $6.5 \pm 0.33^{ns}$ | $6.3 \pm 0.35^{ns}$ |
| P2 | $6.2 \pm 0.33^{a}$ | $6.7 \pm 0.81^{ns}$ | $6.4 \pm 0.47^{ns}$ |
| P3 | $6.2 \pm 0.18^{a}$ | $6.4 \pm 0.11^{ns}$ | $6.6 \pm 0.29^{ns}$ |

Remarks:

$^{a,\,b)}$ Different superscripts within the same column indicate a significant difference ($P < 0.05$).

$^{ns}$ (not significant) indicates a non-significant effect ($P > 0.05$)

**Color**

The data in Table 3 show that color was significantly affected by the treatment ($P < 0.05$). Based on the color assessment criteria (Table 1), all treatments exhibited color scores ranging from 6.2-6.7, which fall within the brownish-yellow category (score 5.0-6.9). Treatment P0 was not significantly different from P1, P2, and P3, while P1 showed a significantly higher color score than P2 dan P3. The brownish-yellow color observed across all treatments indicates a clear shift from the initial green color of unfermented rice straw. This color change is consistent with the findings of Oladosu et al., (2016), who reported that fermented rice straw turns brownish due to heat generation during fermentation, leading to pigment degradation. The observed color characteristics, therefore, reflect the fermentation process and are commonly associated with changes in nutritional quality. Similar color characteristics were also reported by Sulistyo et al., (2019), in rice straw fermented for 21 days.

**Odor**

The odor aspect showed that there was no significant effect of the level of banana rhizome microbial on the odor of fermented rice straw (P>0.05). The odor in all treatments is a sour smell, susceptible to change from the initial condition of rice straw without fermentation, which initially smelled like forage. According to Suningsih et al., (2019) changes in the odor of rice straw after the fermentation process are due to the process of breaking down carbohydrates (cellulose and hemicellulose) into organic acids. Research results by Suningsih et al., (2019) also showed that one of the characteristics of good rice straw fermentation is its sour smell. The same phenomenon is also demonstrated by the research of Oladosu et al., (2016), which states that the sour smell from the fermentation of rice straw is a result of yeast fermentation, resulting in high ethanol levels.

**Texture**

The texture aspect revealed that there was no significant effect of the level of banana rhizome microbial on the texture of fermented rice straw (P > 0.05). The texture in all treatments exhibits a slightly rough vulnerability. At first observation, the vulnerability appears to have no difference in texture between fermented rice straw and unfermented rice straw. However, the slightly rough texture of the fermented rice straw indicates a much softer condition compared to the unfermented rice straw. The change in texture of fermented rice straw is due to the activity of the cellulase enzyme, which hydrolyzes cellulose into glucose, thereby reducing the crude fiber content. The decrease in crude fiber content results in a change to the texture of the rice straw, making it smoother (Nuraya et al., 2016). A slightly rough texture was also produced due to the heating during storage, which leads to protein degradation and a reduction in metabolizable energy (Oladosu et al., 2016).

**Chemical Quality of Fermented Rice Straw**

Crude fiber (CF) and crude protein (CP) are critical components in ruminant feed because they can affect the health and the productivity of livestock. CF is defined as the fraction of carbohydrates that are insoluble in alkaline and dilute acids after boiling for 30 minutes (Agustono et al., 2017), when CP is interpreted as all nitrogen-containing substances, including non-protein nitrogen such as urea (Rosa, 2022).

Based on the proximate test conducted, the results of CF and CP in fermented rice straw are shown in Table 4.

Table 4. The Chemical Quality of Fermented Rice Straw

| Treatment | Parameters | |
|---|---|---|
| | **CF (%)** | **CP (%)** |
| P0 | $28.63 \pm 0.83^{ab}$ | $8.53 \pm 0.89^{b}$ |
| P1 | $26.82 \pm 1.27^{a}$ | $7.88 \pm 0.62^{b}$ |
| P2 | $26.80 \pm 0.57^{a}$ | $7.80 \pm 0.88^{b}$ |
| P3 | $29.25 \pm 2.66^{b}$ | $6.53 \pm 0.65^{a}$ |

Remarks: $^{a,\,b)}$ Different superscripts within the same column indicate a significant difference (P < 0.05).

**Crude Fiber (CF)**

Table 4 presents that the difference in the level of application of banana rhizome microbial shows a significant effect on the CF content of fermented rice straw ($P<0.05$), with the highest CF content in P3 and the lowest in P2. The P3 was significantly different from the P1 and P2, and not significantly different from the P0.

The decrease of CF content in P1 and P2 occurred due to the addition of banana rhizome microbial, in which it contains microorganisms such as *Aspergillus niger*, *Bacillus sp.,* and cellulolytic microbes that produce cellulase, protoase, and amylase enzyme so that they can break down structural carbohydrate bonds in rice straw into glucose, amino acids, and organic acids (Wahyono et al., 2018). The breakdown of structural carbohydrates in rice straw can cause a decrease in CF content of fermented rice straw because CF reflects the content of cellulose, hemicellulose, and lignin in feed ingredients. If structural carbohydrates are reduced, CF will also be reduced. Furthermore, the addition of urea in each treatment increases the nitrogen content of rice straw, which is used for the growth of microorganisms producing cellulase, protease, and amylase (Agustin, 2022).

Unlike P1 and P2, P3 experienced an augmentation in CF content. This resulted from the elevated dosage of banana rhizome microbes utilized in P3. The use of an excessively high dose of banana rhizome microbial can result in an excessive fermentation process, which can raise the amount of CF in fermented rice straw (Lestari et al., 2014). A further sign is the insufficient supply of nitrogen necessary for microbial growth, which hinders effective degradation of CF content.

**Crude Protein (CP)**

The data in Table 4 show that the level of banana rhizome microbial inoculum had a significant effect on the crude protein (CP) content of fermented rice straw ($P < 0.05$), with the highest CP content observed in P0 and the lowest in P3. Treatment P3 was significantly different from P0, P1, and P2.

In the P0, the CP content was the highest due to the addition of urea as a nitrigen source, which can increase CP content (Amin et al., 2015). In contrast P1, P2, and P3 showed a decrease in CP content. This decrease may be attributed to several factors. First, the urea dose may have been insufficient to provide adequate nitrogen for microorganisms, thereby limiting their contribution to CP enhancement (Hasan and Yanuarianto, 2015). Second, microorganisms in the banana rhizome-based inoculum may have utilized nitrogen from urea for their own growth, reducing nitrogen availability for protein synthesis in the substrate (Suningsih et al., 2019). Third, CP is estimated based on nitrogen content in rice straw tissue; therefore, increased microbial nitrogen utilization in P1, P2, and P3 may result in lower CP values detected in proximate analysis (Amin et al., 2015).

**CONCLUSIONS AND SUGGESTIONS**

Banana rhizome microbial as much as 2 mL in P2 has the most optimal quality to be used as a fermentation starter with a total number of bacteria as much as 4.7x107 CFU/mL, giving physical changes in rice straw to brownish yellow color, sour smell, and slightly rough texture with CF content of 26.80% and CP 7.80%. However, further research is needed regarding the cause of the decrease in CP content in rice straw fermentation using banana rhizome microbial.

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