Research Article

The Use of Urea and Palmyra Sap (*Borassus flabellifer*) on the Characteristics and Nutrient Composition of Fermented Rice Bran

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Abstract | Rice bran is a viable feedstuff, but its use in non-ruminant feed is limited due to its high crude fiber content of 14-16%. One of the methods to reduce crude fiber is through anaerobic fermentation, which requires carbon from soluble carbohydrates such as palmyra sap and nitrogen from Non-Protein Nitrogen such as urea. This experiment aimed to evaluate the fermentation characteristics and nutrient content of rice bran fermented anaerobically for six days. This experiment used a Completely Randomized Design with four treatments and four replications. The treatments included rice bran without palmyra sap and urea (T0), rice bran with 2% urea (T1), rice bran with 10% palmyra sap (T2), and rice bran with 2% urea and 10% palmyra sap (T3). Urea was added at 2% of the rice bran weight, while palmyra sap was added at 10% of the dry matter of rice bran. Fermentation characteristics were measured by pH, NH3, and lactic acid levels, while nutrient content was measured by dry matter, crude protein content, crude fiber, calcium, phosphorus, NDF, ADF, and lignin. The data obtained were analyzed using Analysis of Variance followed by Duncan Multiple Range Test (DMRT). The result showed that addition of urea, palmyra sap and their mixture had a significant effect ($P<0,05$) on the value of pH, NH3 and lactic acid. Treatment T2 had the lowest values of pH and NH3 but had highest lactic acid. Additionally, the use of urea, palmyra sap and their mixture had a significant effect ($P<0.05$) on the value of dry matter, crude protein and crude fiber content of rice bran. Treatment T3 had the lowest dry matter and crude fiber content, but the highest crude protein content. The use of urea, palmyra sap and their mixture had a significant effect ($P<0.05$) on NDF, ADF and lignin. Treatment T3 resulted in the lowest content of NDF and ADF. In conclusion, the usage of 10% palmyra sap (T2) resulted in the lowest pH but the highest lactic acid production, while 2% urea and 10% palmyra sap (T3) had the highest crude protein and decreased the value of crude fiber, NDF and lignin of rice bran.

Keywords | Fermentation, Palmyra sap, Rice bran, Urea

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INTRODUCTION

Due to the high cost of feed ingredients, farmers often have to resort to using cheaper alternatives. Rice bran, a byproduct of rice milling and polishing, is one such cost-effective feed ingredient (Azrinnahar et al., 2021). During the rice milling process, 65% of the rice is produced while the remaining 35% consists of milled waste, comprising 23% husk, and 10% bran (Superianto et al., 2018).

The nutrient content in rice bran is 13-15% crude protein, 12-13% crude fat (Ahmad et al., 2019), metabolic energy of 2980 kcal/kg, Ca 0.07%, P 0.22%, and Mg 0.95% (Novita et al., 2017). Rice bran can be used as a concentrated mixed feed for ruminants and can be added up to 10% in poultry feed (Ahmad et al., 2019). However, the high crude fiber content of 14-16% limits its use for monogastric livestock (Ikhwanuddin et al., 2018). To improve the nutritional content, such as reducing crude fiber and increasing protein content, anaerobic fermentation can be applied (Ermalia et al., 2016). During anaerobic fermentation, lactic acid-producing microorganisms such as lactic acid bacteria, produce fiber-degrading enzymes such as cellulose and xylanase that degrade crude fibers, which results in the stretching of cellulose-lignin complex bonds and leads to a decrease in the levels of crude fiber (Wang et al., 2019). Moreover, the fermentation process can increase crude protein content due to the degradation of complex proteins into amino acids and microorganisms body proteins (Norrapoke et al., 2022).

Fermentation requires soluble carbohydrates as an energy source for optimal growth of microorganisms and to rapidly obtain an anaerobic condition. One potential source of soluble carbohydrates is palmyra sap. According to Naiola (2008), palmyra sap contains sugars in the form of 4% fructose, 36-78.7% sucrose, and 1.66-3.50% glucose. In addition to serving as a source of carbohydrates, palmyra sap can also provide microorganisms for the fermentation process. Several microorganisms have been successfully isolated from palmyra sap, including *Saccharomyces cerevisiae* (Theivendirarajah and Chrystopher, 1987; Humaidah et al., 2017), *Saccharomyces chevalieri*, and bacteria such as *Bacillus cereus, B. sphericus*, and *B. firmus* (Vengadaramana et al., 2016).

The use of palmyra sap in fermentation has been studied by several researchers. Helda and Sabuna (2012) found that using 15% palmyra sap in the fermentation of goat and chicken feces increased the crude protein content and reduced the crude fiber content from 13.7% to 15.8% and from 8.01% to 6.96%, respectively. Similarly, Koni et al. (2021), reported that the crude fiber content of banana peels decreased from 18.7% to 11.6% after 6 days of fermentation with 20% palmyra sap. In addition to carbon sources, the fermentation process requires protein sources, such as non-protein nitrogen, which can be used by microorganisms. Better fermentation results can be achieved with the addition of 2% urea (Ahmad et al., 2019). According to Suryani et al. (2017), urea, as a non-protein nitrogen source, is a macronutrient for the growth of microorganisms. Febrina et al. (2020) reported that adding 5% urea to palmyra midrib fermentation resulted in a lignin content of 21.6%.

Based on the explanation above, a study was conducted to examine the effect of adding palmyra sap, urea and their mixture to ferment rice bran on the quality of fermentation (pH, lactic acid content, and ammonia) and nutritional content (crude protein, crude fiber, fat, calcium, phosphorus, and fiber fraction).

MATERIALS AND METHODS

Rice bran fermentation

Rice bran was obtained from rice mills, and palmyra sap was obtained from palmyra tappers, while urea was purchased from agricultural shops. The rice bran was cleaned to remove any foreign matter, and its nutrient content was analyzed before application (as shown in Table 1). The fermented materials were made under a 40% moisture condition. The rice bran was mixed according to the treatment and then placed in a plastic jar, compacted, and tightly covered, and the surface of the jar was insulated to ensure complete airtightness. These materials were stored for six days in a room that was not exposed to direct sunlight, at a room temperature of 27-32°C. Each treatment had Four replications. After fermentation, samples were taken for pH, lactic acid, and NH3 content analysis. The fermented bran was then dried in an oven at 60°C for 48 hours to stop the fermentation process. The dried fermented rice bran was then ground.

Table 1: Proximate and Van Soest analysis of rice bran (% dry matter).

Nutrient content	% DM
Dry matter	89.41
Total ash	13.13
Crude protein	8.69
Crude fiber	29.43
Ether extract	7.9
NDF	59.8
ADF	36.34
Lignin	10.97

DM: dry matter, NDF: neutral detergent fiber, ADF: Acid detergent fiber.

Research design

The experimental design used in this study was a completely randomized design with four treatments and four replications. The treatments included T0, which consisted of rice bran without palmyra sap and urea; T1, which included rice bran with 2% urea; T2, which included rice bran with 10% palmyra sap; and T3, which included rice bran with 2% urea and 10% palmyra sap.

Measurement of pH, NH_3 and lactic acid

The pH value of the bran sample was measured following the procedure described by (Bernardes et al., 2019): 1 gram of sample was added to 10 mL of distilled water and stirred until homogenized. The pH value was then measured using a pH meter. NH3 and lactic acid levels were determined using the methods of Chaney and Marbach (1962) and Barker and Summerson (1941), respectively.

Nutrient analysis

A 10% sample of the fermented bran was taken to analyze dry matter, crude protein, crude fiber, calcium, and phosphorus levels using the AOAC (2005) method. Fiber fraction analysis, including neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin, was conducted according to the procedure outlined by Van Soest et al. (1991).

Data analysis

The data obtained were analyzed for variance. If there were any significant differences among the treatments, Duncan's multiple range test was performed using the Statistical Program for Social Science (SPSS) software version 20.

RESULTS AND DISCUSSION

Fermentation characteristics

Table 2 presents the fermentation characteristics of fermented rice bran with different additives, such as urea, palmyra sap, and an admixture, including pH, lactic acid, and NH3 values. The results demonstrated that the addition of these additives had a significant effect ($p<0.01$) on the pH value. Urea (T1 and T3 treatments) resulted in a higher pH value compared to other treatments due to its nitrogen content, which inhibited a decrease in pH value. According to Wilkinson et al. (1976), high protein content in silage raw material increases buffer capacity and makes it difficult for the pH to decrease and establish an acidic condition. In contrast, palmyra sap (T2 treatment) resulted in a lower pH value due to its carbohydrate content, which provided energy for lactic acid bacteria (LAB) to grow rapidly, leading to a decline in pH value from high lactic acid production. Despal et al. (2011) reported that high levels of water-soluble carbohydrates provided dissolved carbohydrates needed by LAB to grow, and the fermentation of the substrate produced organic acids that caused the pH to drop. In the treatment with a mixture of urea and palmyra sap (T3), the pH value ranged within the pH values of T1 and T2, suggesting that the buffering capacity of urea may have been neutralized by the presence of 10% palmyra sap, which supported microbial growth to produce organic acids and decrease the pH. Azrinnahar et al. (2021) reported a decrease in pH value ranging from 5.1-5.2 in fermented bran added with 2% urea after two days, while Kang et al. (2018) found that the pH value of cassava leaf silage added with 0.5-1% urea (4.6-4.7) was higher than that without urea (3.4).

The addition of urea in the treatment resulted in high NH3 levels, likely due to the nitrogen content in urea being utilized by microorganisms during the fermentation process. Ramadhan et al. (2019) reported that urea contained 46% nitrogen, which could explain the high NH3 levels observed. High levels of nitrogen increase buffering capacity, which can inhibit pH decrease and increase proteolysis. Similar results were found in silage using legume greens, which are also rich in protein. Kurniawan and Chistie (2020) found that higher use of Indigofera zolingeriana in sorghum silage resulted in a higher pH, likely due to the plant's high levels of nitrogen or crude protein (27.67% DM).

Table 2: pH, lactic acid and NH_3 of fermented rice bran.

Parameters	Treatment			
	T0	T1	T2	T3
pH	4.50±0.29[b]	5.34±0.33[c]	3.91±0.05[a]	4.09±0.10[ab]
NH_3 (mg / 100g)	37.94±1.85[a]	64.98±2.23[c]	34.82±4.08[a]	57.73±1.92[b]
Lactic acid (mg/100g)	84.81±1.49[b]	77.21±0.93[a]	116.94±0.58[d]	111.29±0.73[c]

[a,b,c] significantly difference in a row ($p < 0.05$). T0: rice bran without palmyra sap and urea; T1: rice bran with 2% urea, T2: rice bran with 10% palmyra sap, T3: rice bran with 2% urea and 10% palmyra sap.

Table 3: Nutrient composition of fermented rice bran.

Parameters	Treatment			
	T0	T1	T2	T3
DM (%)	55.48±0.69[b]	56.07±2.29[b]	54.56±0.49[b]	47.60±3.19[a]
CP (% DM)	8.96±0.49[a]	9.21±0.51[a]	8.93±0.43[a]	10.58±0.15[b]
CF (% DM)	28.14±0.50[b]	27.30±0.55[b]	24.05±1.34[a]	24.59±0.73[a]
NDF (% DM)	56.53±1.18[c]	54.92±0.77[b]	42.51±0.48[a]	41.64±0.65[a]
ADF (% DM)	36.82±0.05[d]	30.81±0.72[b]	31.67±0.39[c]	28.19±0.08[a]
Lignin (% DM)	9.64±0.27[b]	7.89±0.59[a]	7.92±0.11[a]	8.07±0.19[a]

DM: Dry Matter, CP: Crude Protein, CF: Crude Fiber, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, [a,b,c,d] significantly difference in a row ($p < 0.05$). T0: rice bran without palmyra sap and urea; T1: rice bran with 2% urea, T2: rice bran with 10% palmyra sap, T3: rice bran with 2% urea and 10% palmyra sap.

A successful fermentation process requires anaerobic conditions, low pH values, and high lactic acid production for the lactic acid bacteria (LAB) to flourish (Norrapoke et al., 2022). The use of urea, palmyra sap, and their mixture significantly affected ($p < 0.01$) the lactic acid levels in fermented rice bran in this study. The treatment with palmyra sap resulted in high lactic acid production due to the presence of soluble carbohydrates in the sap. These carbohydrates provided a readily available energy source for the microorganisms to grow rapidly and produce organic acids that caused a decrease in pH. Palmyra sap contains 10-16% sugar in the form of sucrose (Vengadaramana et al., 2016). Previous research has shown that the use of a soluble carbohydrate source in silage fermentation by LAB leads to high levels of lactic acid production (Despal et al., 2011; Utomo et al., 2013; Chalisty et al., 2017).

Nutritional composition

The use of palmyra sap in the fermentation of rice bran resulted in a decrease in dry matter content in the resulting bran silage. This was due to the liquid state and high-water content of the palmyra sap, which was measured at 85.2% (Naiola, 2008). However, when compared to the dry matter content of rice bran before fermentation, which was measured at 89.41% (Table 1), the moisture content of the silage was still lower. This can be attributed to the high-water content of the rice bran before fermentation, which was around 40%. After six days of fermentation, there was a noticeable decrease in dry matter content caused by the metabolic process of microorganisms that utilize dry matter to produce organic acids, CO_2, and H_2O. An increase in rumen fluid used in rice bran fermentation caused a decrease in dry matter content of 0.92-1.15%, which is attributed to the activity of microorganisms that produce organic acids, N-ammonia, CO_2, and heat (Despal et al., 2011; Ermalia et al., 2016).

The crude protein content of rice bran significantly increased ($p < 0.05$) in the treatment that used a mixture of palmyra sap and urea. This increase was due to the contribution of nitrogen from urea, as well as the decrease in pH that inhibited protein-degrading microorganisms. These microorganisms cannot survive under low pH conditions, which prevents the reduction of rice bran protein. Santoso et al. (2009) reported that protein breakdown into amino acids and ammonia is mainly due to the action of proteolytic clostridia, which thrive at a pH range of 6-8.5. In the treatment using urea, an increase in protein levels was caused by microorganisms that could use non-protein nitrogen, such as urea, to form protein-related cell structures. This process increased the protein content of rice bran. Kang et al. (2018) reported that lactic acid bacteria in the fermentation process can use nitrogen from non-protein sources. The highest crude protein content was observed in the treatment that used 10% palmyra sap and 2% urea.

The treatment using palmyra sap significantly decreased ($p<0.01$) the crude fiber content of rice bran, likely due to the presence of lactic acid bacteria in the sap that produce enzymes capable of degrading crude fiber. Das and Tamang (2021) reported the isolation of *Leuconostoc, Lactobacillus, Gluconoacetobacter, Enterobacter, Streptococcus*, and *Lactococci* from palmyra sap, which are microorganisms that can degrade fiber fractions like NDF and ADF.

The use of urea, palmyra sap, and the admixture significantly reduced the levels of NDF, ADF, and lignin ($p<0.05$) in fermented rice bran, possibly due to the microorganisms present in the palmyra sap. Notably, the NDF content of the treatment using sap was lower than that of the other treatments, indicating that the soluble carbohydrates present in palmyra sap promote the growth of microorganisms that produce enzymes capable of degrading crude fiber. The addition of additives to the fermentation process has been shown to increase the reduction of crude fiber, as reported by Lukkananukool et al. (2019). Fermentation of rice bran in this experiment also reduced the levels of NDF, ADF, and lignin compared to unfermented bran (Table 1) by the production of extracellular enzymes such as cellulase and xylanase by microorganisms. Ermalia et al. (2016) found that fermenting rice bran with 30% rumen fluid and incubating it for 3 days resulted in decreased levels of NDF and ADF, which enhances the digestibility of rice bran and makes it a more favorable ingredient for monogastric animal feed.

CONCLUSIONS AND RECOMMENDATIONS

The use of 10% palmyra sap had the lowest pH and highest lactic acid production. The use of 2% urea and 10% palmyra sap had highest crude protein, also can decrease the value of crude fiber, NDF and lignin of rice bran.

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NOVELTY STATEMENT

This research is the first to utilize a combination of local and inexpensive additives, based on dry matter of rice bran as agricultural waste. The results offer a cost-effective solution to improve the nutritional content, reduce fiber content, and transform rice bran into a valuable source of protein and energy for livestock. By promoting the use of locally sourced and renewable ingredients, this approach not only reduces waste but also benefits both the environment and local communities. To our knowledge, this is the first study to utilize this innovative approach, which has the potential to revolutionize the utilization of agricultural waste for livestock feed.

AUTHOR'S CONTRIBUTION

Theresia Nur Indah Koni contributed to creating the research ideas, designing experiments, analyzing the data, and writing this article. Tri Anggarini Yuniwati Foenay and Stormy Vertygo contributed to analyzing the data and check the written paper.

Conflict of interest

The authors have declared no conflict of interest.

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