The effect of fermentation without or with lactic acid bacteria and storage time on the phytic acid, in vitro dry matter digestibility, and nutrient contents of rice bran

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Abstract: Storage of rice bran (RB) at anaerobic conditions is the potential way to reduce phytic acid (PA) and stabilize the nutrient contents. This study was aimed to examine the effect of fermentation without or with lactic acid bacteria (LAB) and storage time on the PA, in vitro dry matter digestibility, and nutrient content of RB. A factorial completely randomized design was used in this study. The first factor was the fermentation method, RB with no fermentation (M0), with fermentation without LAB (M1), with fermentation plus LAB (M2). The second factor was storage time, namely 0 (S1), 5 (S2), 10 (S3), and 15 (S4) weeks. Each unit was repeated 4 times. Parameters measured include phytic acid (PA), in vitro dry matter digestibility (IVDMD), crude protein (CP), and crude fiber (CF) contents. A two-way analysis of variance was employed to determine the significant effect of the treatment. For PA the interaction between method and storage time and the main effects were all significant (P<0.01). For IVDMD, only the fermentation method affected significantly (P<0.05), with M0 as the lowest and M2 as the highest at all storage time. However, the CP content of RB was not affected significantly (P>0.05) by treatment. In addition, the CF content of rice bran was not significantly (P>0.05) affected by an interaction between method and storage time but significantly reduced (P<0.01) by the method of fermentation. It was concluded that fermentation with the addition of LAB anaerobically decreased anti-nutrient content, increased the feeding and nutritive value of RB.

1. Introduction
The use of concentrate feed for livestock feed increased from year to year to boost animal productivity. Among the concentrate feed, rice (Oryza sativa) bran is the one which is commonly used by producers or farmers in Indonesia due to its availability, nutrient content, and price. Rice bran is a rice processing by-product which accounts for a lot of waste material every year contained by several nutrients and bioactive substances (1). Rice production in Indonesia in 2018, for example was 56.54 million tons [2] which can produce around 5 million rice bran. Rachmat [3] noted that rice contained about 8-10% rice bran. This data showed the potential of rice bran for animal feed in this country.

The use of rice bran as animal feed is often limited by the nutrient instability of the bran during storage. This nutrient instability is mainly due to the presence of the lipase enzyme in the bran. Furthermore, rice bran also contains a peroxidase enzyme which can cause oxidative damage or rancidity to the oil components in the bran [4,5]. Another problem that exists in the use of rice bran as animal feed is the high content of CF, low content of CP and antinutritional factor such as PA (6). Phytic acid will form insoluble salts when the PA binds to phosphorus and other minerals so that these minerals
cannot be absorbed by the intestine [7]. In addition, PA can bind to metal ions such as P, Ca, Mg, Zn as well as positive proteins such as terminal amino groups at pH below its isoelectric point. The formation of insoluble phytate-mineral or protein phytate compounds can cause a decrease in mineral availability and protein nutritional value [8,9]. To overcome the nutrient deterioration effect during storage and PA problems in rice bran, anaerobic fermentation technology can be applied (10). Anaerobic fermentation processing into silage is a strategy to preserve feed (11). This happens because there is an inhibition of the enzymatic reactions contained in feed ingredients such as rice bran in the silage-making process.

Acid conditions that appear in the process of making silage are thought to be able to hydrolyze PA in rice bran. Related to this, anaerobic fermentation technology can be used to reduce PA levels and to preserve the bran. Fermentation also could also affect the digestibility and nutrient content of the rice bran. This study was done to examine the effect of fermentation without or with lactic acid bacteria and storage time on the phytic acid, in vitro dry matter digestibility, and nutrient contents of rice bran.

2. Materials and methods

2.1 Site and time

This experiment was conducted at Animal Nutrition Laboratory, Department of Animal Science, and Chemistry Laboratory, Department of Chemistry, Tadulako University, Palu, Central Sulawesi, from June to September 2020.

2.2 Materials

Rice bran used in this experiment was obtained from five different rice mills in Sigi District Central Sulawesi. Lactic acid bacteria contained *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Lactobacillus acidophilus* was also used as an additive. The LAB was cultured with mann rhogose shapeagar (MRS)-broth at Animal Nutrition Laboratory, Tadulako University.

2.3 Methods

Rice bran was weighed as much as 48 kg then divided into 48 parts with 1 kg for each treatment. The rice bran then allocated randomly to the treatment combination (fermentation method M0, M1, and M2) and storage time (0, 5, 10, and 15 weeks). The rice bran used for M1 and M2 treatments was processed into silage with a fermentation time according to the treatment at the anaerobic condition. Water was added to the silage with the ratio of moisture content and dry matter used in making silage was 1: 1 (w/w). After that, the LAB was sprayed on rice bran for treatment M2.

2.3.1 Experimental design and procedures. A factorial completely randomized design with 2 factors and 4 replications were used in this experiment. Factor 1: M0 = rice bran without treatment (control) M1 = rice bran that was made silage without the addition of lactic acid bacteria M2 = rice bran made by silage added by lactic acid bacteria Factor 2: S1 = storage time 0 weeks S2 = storage time 5 weeks, S3 = storage time 10 weeks, and S4 = storage time 15 weeks. Parameters measured include PA content, IVDMD, CP and CF content of rice bran.

2.3.2 Feed and statistical analysis. Samples of rice bran for each treatment were ground using a grinder (1-mm screen) for analyses of DM, CP, CF according to [12] In vitro dry matter digestibility of rice bran was determined using the method of [13]

3. Results and discussion

The content of PA, DM, CP, and CF of rice bran was 8.52, 86.63, 14.88, and 27.67%, respectively. The effect of fermentation without or with the addition of LAB and storage time on PA, IVDMD, CP and CF contents of rice bran is presented in Tables 1, 2, 3, and 4, respectively. Concerning PA content, both the interaction between fermentation without or with LAB and storage time and the main effects were significant (P<0.01). In each fermentation treatment, increasing storage time decreased significantly (P<0.05) PA content of rice bran. Fermentation without or with LAB in all storage time decreased
significantly (P<0.05) PA content of rice bran but no significant difference (P>0.05) between M1 and M2 (Table 1). Table 1 shows that increasing the time of storage was associated with a linear PA content of rice bran. This decrease could be associated with decreasing pH content in the silage. At the storage time of 0, 5, 10, and 15 weeks the average pH were 5.4, 5.0, 4.7, and 4.4, respectively. With a lower pH, more PA bonds are broken because PA is unstable at low pH. Acidic conditions created in anaerobic conditions will have an effect on reducing the composition of pH. Lowering pH during storage in the form of silage could be associated with the presence of phytic acid (PA) enzymes found in rice bran. This enzyme broke the phytic bond, which leads to a decrease in PA in the bran. Lendrawati [14] noted that the optimum pH of phytase enzyme activity in rice bran is 4.5. The acidic conditions of silage storage in an anaerobic condition resulted in a decrease in the PA content.

**Table 1.** The effect of fermentation without or with the addition of lactic acid bacteria and storage time on phytic acid of rice bran

| Fermentation method | Storage time (weeks) | 0       | 5       | 10      | 15      |
|---------------------|----------------------|---------|---------|---------|---------|
|                     |                      | M0      | M1      | M2      |         |
|                     |                      | 8.39±0.12^A | 6.51±0.11^B | 6.19±0.28^B |         |
|                     |                      | 7.10±0.10^B | 5.30±0.12^C | 4.55±0.15^C |         |
|                     |                      | 5.28±0.05^C | 3.45±0.06^D | 3.27±0.05^D | 3.11±0.08^D |
|                     |                      | 3.16±0.04^E | 2.16±0.04^E | 2.13±0.07^E |         |

Means with different superscripts are significantly different (P<0.05). M0 = rice bran without treatment (control) M1 = rice bran that was made silage without the addition of lactic acid bacteria M2 = rice bran made by silage added by lactic acid bacteria

Table 2 shows the interaction between the fermentation method and storage time on IVDMD of rice bran. The interaction between the fermentation method and storage time and the effect of storage time per se was not significant (P>0.05) with respect to IVDMD. However, the fermentation method as the main impact did significantly (P<0.01) affect the IVDMD of rice bran. Fermentation significantly (P<0.01) increased IVDMD of rice bran which was stored at 0, 5, 10, and 15 weeks. The highest IVDMD value was achieved by treatment M2, and the lowest was at treatment M0. Increasing IVDMD of rice bran as a result of fermentation could be associated with a reduction in the decrease of CF content (Table 4). This current finding was supported by a previous study [15], who reported an increase in IVDMD of rice bran from 34.72% to 40.37% with fermentation plus 0.4 *Saccharomyces* spp. Increasing IVDMD in this study is not in agreement with the finding of [16] who recorded no change in digestibility of Cadamba leave silage with the addition of LAB. The different materials for fermentation could result in a different response to the treatment.

**Table 2.** The effect of fermentation without or with the addition of lactic acid bacteria and storage time on in vitro dry matter digestibility of rice bran

| Fermentation method | Storage time (weeks) | 0       | 5       | 10      | 15      |
|---------------------|----------------------|---------|---------|---------|---------|
|                     |                      | M0      | M1      | M2      |         |
|                     |                      | 62.35±0.17^C | 65.49±0.46^B | 68.46±0.35^A |         |
|                     |                      | 63.20±0.26^C | 66.97±0.52^B | 70.28±0.64^A |         |
|                     |                      | 62.09±0.58^C | 65.47±0.30^B | 68.33±0.37^A | 62.14±0.51^C |
|                     |                      | 62.09±0.58^C | 65.47±0.30^B | 68.33±0.37^A | 68.29±0.36^A |

Means with different superscripts are significantly different (P<0.05). M0 = rice bran without treatment (control) M1 = rice bran that was made silage without the addition of lactic acid bacteria M2 = rice bran made by silage added by lactic acid bacteria

Neither the main effects nor the interaction between them was significantly affected (P>0.05) the CP content of rice bran (Table 3) and the overall mean value was 10.73%. No increase in CP content due to rice bran fermentation was also reported by other study [17]. Lack of additional nitrogen to the fermented rice bran was the reason for the non-significant effect. Other study [18] on the other hand, showed an increase of CP content of rice bran with fermentation. In this study [18] indicated that the addition of 15 ml of effective microorganism plus 250 g of palm sugar increased CP content of rice bran...
from 6.38% to 7.44%. This was due to additional nitrogen from effective microorganisms that grow on the fermented rice bran.

### Table 3. The effect of fermentation without or with the addition of lactic acid bacteria and storage time on crude protein of rice bran

| Fermentation method | Storage time (weeks) | 0      | 5      | 10     | 15     |
|---------------------|----------------------|--------|--------|--------|--------|
|                     |                      | 10.64±0.60 | 10.52±0.08 | 10.34±0.32 | 9.48±0.32 |
| M0                  |                      | 10.61±0.53 | 10.86±0.23 | 11.75±0.43 | 11.14±0.62 |
| M1                  |                      | 10.34±0.54 | 11.13±1.04 | 11.09±0.49 | 10.48±0.31 |

Means with different superscripts are significantly different (P<0.05). M0 = rice bran without treatment (control) M1 = rice bran that was made silage without the addition of lactic acid bacteria M2 = rice bran made by silage added by lactic acid bacteria

The interaction between treatments and storage time was non-significant (P>0.05) for CF of rice bran. However, fermentation without and with LAB as the main effect significantly (P<0.05) reduced CF content of rice bran (Table 4). In all storage time, the highest CF content was found on treatment M0, and the lowest was M2. This finding is in agreement with previous studies [18, 19]. Nuraini [18] reported a significant decrease in the fiber component of Cadamba leaves silage with LAB addition. Supriyati [19] documented a significant decrease in CF of rice bran after fermented using Bacillus amyloliquefaciens and Humic substances. It could be associated with an enzyme that enables to degrade fiber components.

### Table 4. The effect of fermentation without or with addition of lactic acid bacteria and storage time on crude fibre of rice bran

| Fermentation methods | Storage time (weeks) | 0      | 5      | 10     | 15     |
|----------------------|----------------------|--------|--------|--------|--------|
|                      |                      | 27.63±0.31A  | 26.75±0.78A  | 26.99±0.58A  | 26.20±0.51A  |
| M0                   |                      | 25.44±0.36B  | 26.09±0.19A  | 25.83±0.43B  | 24.72±0.17B  |
| M1                   |                      | 24.13±0.33C  | 23.77±0.57C  | 24.40±0.55C  | 23.85±0.34C  |

Means with different superscripts are significantly different (P<0.05). M0 = rice bran without treatment (control) M1 = rice bran that was made silage without the addition of lactic acid bacteria M2 = rice bran made by silage added by lactic acid bacteria

### 4. Conclusions

It was concluded that fermentation with or without the addition of LAB anaerobically for 15 weeks decreased PA content, while the addition of LAB per se increased IVDMD and decreased CF content of rice bran. It increased the feeding and nutritive value of rice bran which indicated preservation of rice bran successfully.

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