# The Effect of Coconut Sugar in Aromatic Bran Production with Liquid Organic Rabbit-Feces Supplement on Lactic Acid Levels and Fleigh Value

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**DOI: https://doi.org/10.51244/IJRSI.2026.1307000119**

**Received: 20 July 2026; Accepted: 25 July 2026; Published: 31 July 2026**

## ABSTRACT

Rice bran is one of the most abundant and inexpensive feedstuffs in Indonesia; nonetheless, its utilization is constrained by relatively high contents of phytic acid and crude fiber, which hinder mineral absorption and reduce protein digestibility. Fermentation is an effective approach to improve its quality, and its effectiveness can be enhanced by combining a liquid starter with a source of soluble carbohydrate. Accordingly, this study was conducted to evaluate the effect of coconut sugar, as a substitute for molasses, in rice bran fermented with a liquid organic supplement (LOS) derived from rabbit feces, on lactic acid content, pH, dry matter loss, and Fleigh value. A completely randomized design was employed with three treatments and six replications, namely P0 (1 kg rice bran + 300 g rabbit-feces LOS, without coconut sugar), P1 (1 kg rice bran + 250 g LOS + 50 g coconut sugar), and P2 (1 kg rice bran + 225 g LOS + 75 g coconut sugar). The data were analyzed by analysis of variance, followed by Duncan's multiple range test where treatments differed significantly. The results revealed that coconut sugar significantly affected the lactic acid content, dry matter loss, and Fleigh value ($P<0.05$), whereas it did not significantly affect the pH ($P>0.05$). The highest lactic acid content and the lowest dry matter loss were obtained in P2 (2.74% and 2.01%, respectively), while the highest Fleigh value was recorded in P1 (200.19). The pH of all treatments remained within the optimal range (3.85 to 3.88), and all treatments produced Fleigh values above 85, which corresponds to very good fermentation quality. Considering all variables, P2 was regarded as the best treatment. It is therefore concluded that coconut sugar is a promising substitute for molasses as a soluble carbohydrate source in the production of fermented rice bran using rabbit-feces LOS.

**Keywords:** coconut sugar; fermented rice bran; Fleigh value; lactic acid; rabbit feces

## INTRODUCTION

Rice bran is a major by-product of rice milling and one of the most widely used feedstuffs in Indonesia, owing largely to its abundant availability and low cost (Rosani et al., 2024). It supplies considerable amounts of protein, fat, and metabolizable energy, and is therefore valued as an energy source in animal diets. Nonetheless, its utilization is limited by relatively high contents of phytic acid and crude fiber, which hinder mineral absorption and reduce protein digestibility (Herliani et al., 2014). Consequently, rice bran requires prior processing before it can be optimally utilized as feed.

One of the most effective and inexpensive methods to improve the quality of rice bran is fermentation. Through microbial activity, fermentation reduces anti-nutritional factors and crude fiber while simultaneously increasing nutrient availability and digestibility (Maulana et al., 2024). The resulting product, known as fermented rice bran or aromatic bran, is a probiotic-rich functional feed that is also more palatable, owing to the lactic acid and characteristic aroma produced during the process. In this regard, lactic acid bacteria (LAB) are primarily responsible, since they convert soluble sugars into lactic acid; the accumulated lactic acid subsequently lowers the pH and creates acidic conditions that suppress spoilage microorganisms and prolong storage life (Quinto et al., 2014).

Fermentation is commonly initiated using a liquid starter, such as rumen fluid or a commercial probiotic, together with molasses as a source of soluble carbohydrate. Both components can be replaced with materials that are more readily available at the local level, namely a liquid organic supplement (LOS) prepared from rabbit feces in place of the starter, and coconut sugar in place of molasses. Rabbit feces contain a large population of microorganisms, including LAB, as well as essential minerals that support microbial activity (Adi et al., 2020; Zhu et al., 2021). Coconut sugar, in turn, is a high-sucrose sweetener that is produced in large quantities in West Java. According to the Indonesian National Standard, good-quality coconut sugar contains a minimum of 77% sucrose (BSN, 1995), which is considerably higher than that of molasses and therefore makes it a promising energy source for LAB.

Because it provides a source of soluble carbohydrate, the addition of coconut sugar is expected to accelerate lactic acid formation, lower the pH, reduce dry matter loss, and increase the Fleigh value of fermented rice bran. Therefore, this study was conducted to evaluate the effect of coconut sugar on the lactic acid content, pH, dry matter loss, and Fleigh value of rice bran fermented with rabbit-feces LOS, and to determine the best ratio of coconut sugar to LOS.

## MATERIALS AND METHODS

### Materials

The materials used in this study were rice bran obtained from a rice mill in Jatinangor, Sumedang, West Java; feces of local rabbits collected from a rabbitry in Jatinangor; coconut sugar obtained from Pangandaran, West Java; water; and distilled water.

### Preparation of Liquid Organic Supplement (LOS)

A total of 4.5 kg of fresh rabbit feces was mixed with 18 L of water at a feces-to-water ratio of 1:4 and homogenized, then filtered through a cloth strainer. Coconut sugar (200 g) was added and dissolved, and the mixture was filtered again through a plastic strainer. The filtrate was transferred into a jerry can fitted with a hose connected to a water-filled glass vessel to maintain anaerobic conditions, and fermented for 14 days in a cool place away from direct sunlight. The LOS was considered ready for use when it was clear brown in color, had a characteristic fermented aroma, produced slight foam, and had a low pH.

### Preparation of Fermented Rice Bran

Rice bran, coconut sugar, and LOS were mixed homogeneously according to each treatment, packed tightly into ziplock bags, and vacuum-sealed to remove air, then sealed with black tape to ensure anaerobic conditions. The bags were stored for 21 days, after which the fermented rice bran was analyzed. Successful fermentation was indicated by a light-brown color, a characteristic fermented aroma without rancidity, and the absence of visible yeast, mold, or fungal growth.

### Treatments and Experimental Design

A completely randomized design was used with three treatments and six replications, giving 18 experimental units. The treatments were: P0 = 1 kg rice bran + 300 g rabbit-feces LOS (without coconut sugar); P1 = 1 kg rice bran + 250 g LOS + 50 g coconut sugar; and P2 = 1 kg rice bran + 225 g LOS + 75 g coconut sugar. The total amount of additive was kept constant at 300 g across treatments, so that increasing coconut sugar was accompanied by a proportional decrease in LOS.

### Variables and Measurements

The pH was measured using a pH meter previously calibrated with buffer solutions at pH 4 and pH 7. Lactic acid content was determined by titration (Cappuccino & Sherman, 1991), based on the volume of NaOH required to neutralize the acid in the sample, and calculated as: lactic acid (%) = $(V \times N \times 9.008)$ / sample weight (g) × 100%, where V is the volume of NaOH used and N is the normality of NaOH. Dry matter loss

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VII July 2026

was calculated as (initial dry matter − final dry matter) / initial dry matter × 100% (Hernaman et al., 2007). The Fleigh value (FV) was calculated as FV = 220 + (2 × DM% − 15) − (40 × pH) (Idikut et al., 2009).

**Statistical Analysis**

Data were analyzed by analysis of variance based on the linear model $Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$, where $Y_{ij}$ is the observation, $\mu$ is the general mean, $\alpha_i$ is the effect of the i-th treatment, and $\varepsilon_{ij}$ is the random error. When treatments had a significant effect, the analysis was continued with Duncan's multiple range test. All analyses were performed using IBM SPSS Statistics 27.

## RESULTS AND DISCUSSION

### Lactic Acid Content

Lactic acid is the principal product of lactic acid bacteria (LAB) and one of the primary indicators of a successful fermentation, since its concentration reflects the intensity of LAB activity in converting sugars into organic acids. In general, a higher lactic acid content indicates a more favorable fermentation, because the acidic conditions it generates suppress spoilage microorganisms.

Table 1. Lactic acid content of fermented rice bran

| Treatment | Mean ± SD (%) | Notation |
|---|---|---|
| P0 | 2.39 ± 0.19 | a |
| P1 | 2.23 ± 0.10 | a |
| P2 | 2.74 ± 0.16 | b |

Note: P0 = 1 kg rice bran + 300 g rabbit-feces LOS; P1 = 1 kg rice bran + 250 g LOS + 50 g coconut sugar; P2 = 1 kg rice bran + 225 g LOS + 75 g coconut sugar. Means followed by different superscript letters differ significantly ($P<0.05$).

The lactic acid content of the fermented rice bran ranged from 2.23% to 2.74% (Table 1), with the lowest value recorded in P1 and the highest in P2, the treatment containing the largest amount of coconut sugar. Analysis of variance confirmed that coconut sugar exerted a significant effect on the lactic acid content ($P<0.05$), and the eta-squared value of 0.685 indicates that approximately 68.5% of the variation was attributable to the treatment. According to Duncan's test, P0 and P1 belonged to the same subset (notation a), whereas P2 stood alone with the highest content (notation b). In other words, the addition of 50 g of coconut sugar (P1) was not sufficient to raise the lactic acid content significantly above the control, whereas 75 g (P2) was. These findings are consistent with previous reports that a greater availability of soluble carbohydrate results in a higher lactic acid content; studies employing LOS from rabbit feces with molasses, sheep feces, cow feces, and horse feces likewise obtained their highest lactic acid contents in the treatments with the greatest substrate and starter availability (Audhya et al., 2025; Audina et al., 2025; Mahardika et al., 2025; Suryadi et al., 2024). The differences in absolute values among these studies are attributable to differences in titration, dilution, and extraction methods rather than to the success of the fermentation itself.

The high lactic acid content in P2 was primarily attributable to the role of coconut sugar as a readily available carbohydrate source for LAB. Coconut sugar is dominated by sucrose (83.18% to 90.50%), together with glucose (9.40% to 11.45%) and fructose (2.89% to 3.69%) (Saraiva et al., 2023), and therefore contains far more sugar than molasses, which holds approximately 38.10% sucrose (Khairul et al., 2022). LAB synthesize lactic acid from hexoses, namely glucose and fructose, through glycolysis (Abedi & Hashemi, 2020), and homofermentative LAB convert one molecule of hexose into two molecules of lactic acid. Hexoses are utilized most rapidly because they can be fermented directly, and among them glucose is the sugar preferentially taken up by LAB (Chen et al., 2018). Sucrose, being a disaccharide, cannot be fermented directly and must first be hydrolyzed by invertase into glucose and fructose (Gänzle, 2015). Consequently, coconut sugar provides a substrate that is readily utilized both directly, through its free reducing sugars, and after the hydrolysis of sucrose. The larger dose in P2 therefore supplied the bacteria with more of this substrate, so that a greater amount of lactic acid accumulated (Akbay, 2025).

The type and number of microorganisms carried by the rabbit-feces LOS also influenced lactic acid production. Rabbit feces contain abundant beneficial LAB, such as Lactobacillus, Enterococcus faecium, and Ligilactobacillus animalis, which can be isolated from healthy animals (Li et al., 2024; Pogány Simonová et al., 2020); most of these are homofermentative and are known to produce lactic acid as their main fermentation product (Stephen & Saleh, 2023). Although P0 received the largest amount of LOS, its lactic acid content did not exceed that of P2. This suggests that, in the present study, the availability of fermentable substrate from coconut sugar was more decisive than the size of the LAB inoculum, since the LOS already carried a sufficient LAB population in all treatments. The condition of the feces was likewise important; in order to provide a viable inoculum and to avoid pathogens, the feces should be obtained from healthy rabbits and used fresh rather than dried or stored for a long period.

Fermentation conditions also had a further influence. According to Stephen and Saleh (2023), pH, temperature, fermentation duration, and the availability of nutrients such as amino acids, peptides, and vitamins all affect LAB growth, while the anaerobic condition created by airtight packaging favors lactic acid formation. The 21-day anaerobic fermentation applied in the present study is therefore assumed to have provided the LAB with sufficient time and a suitable environment to convert coconut sugar into lactic acid, which explains why P2, with the largest amount of coconut sugar, produced the highest lactic acid content.

**pH**

The pH is an important indicator of fermentation success. It varies inversely with the amount of organic acid produced, so that a decline in pH signals active LAB, while a low pH keeps the material sufficiently acidic to inhibit pathogens during storage.

Table 2. Final pH of fermented rice bran

| Treatment | Mean ± SD | Notation |
|---|---|---|
| P0 | 3.88 ± 0.01 | a |
| P1 | 3.85 ± 0.04 | a |
| P2 | 3.86 ± 0.04 | a |

Note: P0 = 1 kg rice bran + 300 g rabbit-feces LOS; P1 = 1 kg rice bran + 250 g LOS + 50 g coconut sugar; P2 = 1 kg rice bran + 225 g LOS + 75 g coconut sugar. The same superscript letters indicate no significant difference (P>0.05).

The final pH remained within a narrow range from 3.85 to 3.88 (Table 2), namely P1 (3.85), P2 (3.86), and P0 (3.88). It had declined from an initial value of approximately 5.7 to 5.9, which confirms that active acidification had taken place. All final values were low and fell within the optimal range for good fermentation, generally below 4.2 (Carvalho et al., 2021). Indeed, the pH obtained here was more acidic than in several earlier studies: molasses-based rabbit-feces LOS reached 3.99 (Audhya et al., 2025), sheep-feces LOS 4.75 (Mahardika et al., 2025), and tauco LOS 4.75 (Awaliyah et al., 2025), while on elephant grass silage the pH likewise decreased as the sugar dose increased (Sadarman et al., 2025). The fact that even the control P0, which contained no coconut sugar, attained such a low pH demonstrates that the rabbit-feces LOS by itself was sufficient to drive a strongly acidic fermentation. Analysis of variance revealed no significant effect of treatment on final pH (P>0.05), and the eta-squared value of only 0.113 supports this finding; all treatments formed a single homogeneous group, and Duncan's test was therefore not applied.

A relatively low lactic acid content (up to 2.74%) may nevertheless coincide with a very low pH (3.85 to 3.88), because the amount of acid and the pH do not vary in direct proportion. Only the fraction of acid that dissociates into free hydrogen ions determines the pH, and since the pH scale is logarithmic, even a modest amount of lactic acid is sufficient to reduce the pH to a strongly acidic range. The low lactic acid value is therefore not an indication of failed fermentation; rather, it demonstrates that the acid formed was sufficient to establish favorable acidic conditions (Tyl & Sadler, 2017; Kung et al., 2018). Furthermore, once the pH has reached a low level, any further reduction becomes increasingly difficult and requires a disproportionately larger amount of acid. In addition, minerals present in the coconut sugar, rabbit-feces LOS, and rice bran exert a buffering effect that resists a further decline in pH. Because all treatments had already attained a low pH through the activity of the LAB in the LOS, and because the differences in lactic acid content among the treatments were small, the addition of coconut sugar in P1 and P2 did not produce a significant difference in pH.

**Dry Matter Loss**

Dry matter loss represents the breakdown of organic matter by microorganisms. During fermentation, dry matter is degraded and utilized as an energy source, and under anaerobic conditions this loss results from the microbial degradation of the substrate accompanied by the release of water (Hernaman et al., 2007). A lower dry matter loss therefore indicates a better fermentation, since fewer nutrients are lost during the process.

Table 3. Dry matter loss of fermented rice bran

| Treatment | Mean ± SD (%) | Notation |
|---|---|---|
| P0 | 3.72 ± 0.68 | b |
| P1 | 2.30 ± 0.83 | a |
| P2 | 2.01 ± 0.84 | a |

Note: P0 = 1 kg rice bran + 300 g rabbit-feces LOS; P1 = 1 kg rice bran + 250 g LOS + 50 g coconut sugar; P2 = 1 kg rice bran + 225 g LOS + 75 g coconut sugar. Means followed by different superscript letters differ significantly ($P<0.05$).

Dry matter loss ranged from 2.01% to 3.72% (Table 3). It was highest in the control P0 (3.72%), followed by P1 (2.30%), and lowest in P2 (2.01%), with every value remaining well below the 10% threshold considered acceptable for good fermentation (Borreani et al., 2018). Analysis of variance revealed a significant effect of treatment ($P<0.05$), with an eta-squared value of 0.510. According to Duncan's test, P0 stood alone with the highest loss (notation b), whereas P1 and P2 shared a lower subset (notation a) and did not differ from each other. The addition of coconut sugar, whether at 50 g or 75 g, therefore retained significantly more dry matter than the control. These values are comparable with earlier reports, namely 1.14% to 1.87% for molasses-based rabbit-feces LOS (Audhya et al., 2025), 1.15% to 2.80% for tauco LOS (Awaliyah et al., 2025), and −1.10% to 0.02% for sheep-feces LOS (Mahardika et al., 2025), all of which were far below 10%.

The extent of dry matter loss is determined by two factors acting together, namely the type of microorganism that dominates the fermentation and the type of substrate being fermented (Borreani et al., 2018; Wróbel et al., 2023). The loss occurs as microorganisms break down the substrate and release water, carbon dioxide, and heat through respiration and proteolysis in the early stage of fermentation (Suryani & Hernaman, 2017), and its magnitude depends on the pathway that prevails. Homofermentative LAB convert hexoses into lactic acid without producing gas, so that only about 0.7% of gross energy is lost, whereas heterofermentative fermentation is considerably more costly, causing losses of up to approximately 24% of dry matter through the formation of $CO_2$ and ethanol (Wróbel et al., 2023). The homofermentative pathway is thus the most efficient for conserving dry matter.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VII July 2026

Accordingly, the lower dry matter loss in P1 and P2 is consistent with this explanation and can be traced to coconut sugar being a readily fermentable carbohydrate. Its abundant soluble sugars, comprising approximately 80% to 90% sucrose together with free glucose and fructose (Saraiva et al., 2023; Wrage et al., 2019), prompted the LAB to utilize the coconut sugar first and to produce lactic acid rapidly, so that acidic conditions were established sooner and the microorganisms responsible for degrading dry matter ceased their activity earlier. Moreover, the rapid decline in pH suppressed undesirable microorganisms, such as enterobacteria, clostridia, and yeasts, that cause nutrient loss, which is in agreement with Peng et al. (2024), who reported that the addition of LAB or molasses during fermentation reduces dry matter loss and increases soluble carbohydrate. In the control P0, by contrast, which received no coconut sugar, the microorganisms relied more heavily on the dry matter of the substrate itself as an energy source, so that degradation proceeded for longer and a greater amount of dry matter was lost.

**Fleigh Value**

The Fleigh value is an index used to assess the overall quality of a fermentation on the basis of a combination of pH and dry matter content. A lower pH and a higher dry matter content yield a higher Fleigh value, which corresponds to a better fermentation.

Table 4. Fleigh value of fermented rice bran

| Treatment | Mean ± SD | Notation |
|---|---|---|
| P0 | 194.11 ± 1.03 | a |
| P1 | 200.19 ± 1.49 | c |
| P2 | 196.13 ± 1.70 | b |

Note: P0 = 1 kg rice bran + 300 g rabbit-feces LOS; P1 = 1 kg rice bran + 250 g LOS + 50 g coconut sugar; P2 = 1 kg rice bran + 225 g LOS + 75 g coconut sugar. Means followed by different superscript letters differ significantly ($P<0.05$).

The Fleigh value ranged from 194.11 to 200.19 (Table 4), being highest in P1 (200.19), followed by P2 (196.13), and lowest in P0 (194.11). According to the classification of Killic (1984), every treatment exceeded 85 and was therefore rated as very good quality. These values are close to the approximately 199 obtained by Audhya et al. (2025) with molasses-based rabbit-feces LOS, higher than the 168.72 reported for tauco LOS (Awaliyah et al., 2025), and lower than the high value of 204.46 recorded for horse-feces LOS (Audina et al., 2025). The same pattern, in which a larger amount of sugar yields a higher Fleigh value, has also been observed in elephant grass silage, where the highest value was obtained with the largest dose of commercial syrup as a glucose source (Sadarman et al., 2025). Analysis of variance revealed a significant effect of treatment ($P<0.05$), and according to Duncan's test all three treatments differed significantly, namely P1 (notation c), P2 (notation b), and P0 (notation a). This ranking differs from those of lactic acid content and dry matter loss, in which P2 proved to be the best treatment.

The reason for this difference lies in the manner in which the Fleigh value is calculated, that is, $FV = 220 + (2 \times DM\% - 15) - (40 \times pH)$ (Idikut et al., 2009; Septian et al., 2024), so that it is governed jointly by dry matter content and pH. P1 attained the highest Fleigh value because it combined a high final dry matter content with a relatively low pH. Both coconut-sugar treatments (P1 and P2) were higher than the control, which is to be expected, since a readily fermentable sugar improves both components of the Fleigh value simultaneously by retaining more dry matter and lowering the pH (Sadarman et al., 2025). The very high absolute values obtained here are a consequence of the considerably higher dry matter content of rice bran compared with forage silage; they should therefore be interpreted as indicators of a successful fermentation rather than compared directly with the Fleigh thresholds established for forage silage.

Taken as a whole, P2 was superior in lactic acid content and dry matter loss, P1 was superior in Fleigh value, and every treatment maintained its pH within the optimal range. The addition of coconut sugar to rice bran fermented with rabbit-feces LOS is therefore capable of producing a fermentation of very good quality.

## CONCLUSION

The addition of coconut sugar to rice bran fermented with rabbit-feces LOS significantly affected the lactic acid content, dry matter loss, and Fleigh value, but did not significantly affect the pH. Considering all of the variables together, the best ratio was obtained in P2 (1 kg rice bran + 225 g rabbit-feces LOS + 75 g coconut sugar), which produced the highest lactic acid content (2.74%) and the lowest dry matter loss (2.01%), together with a pH (3.86) and a Fleigh value (196.13) that were both rated as very good; P1, meanwhile, produced the highest Fleigh value (200.19). The pH of all treatments remained within the optimal range (3.85 to 3.88), and all treatments were rated as very good quality. It is therefore concluded that coconut sugar is a promising substitute for molasses as a soluble carbohydrate source in the production of fermented rice bran using rabbit-feces LOS.

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