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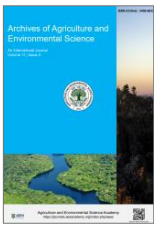

Original Research Article

# Effects of additive supplementation on the nutritional quality of rice bran fermented with rumen liquor for use as poultry feed

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## ABSTRACT

Rice bran (RB) is one of the most abundant agro-industrial by-products and used as an energy-rich ingredient in poultry diets. Its high fiber and some anti-nutritional substances limits nutrient digestibility and utilization by chickens, thereby reducing overall production efficiency. This study aimed to improve the nutritional quality of RB through fermentation. The RB was fermented for 48h using 50% rumen liquor (RL) supplemented with urea and molasses as an additive. The treatments (n=3) were; $T_0$: Unfermented RB, $T_1$: RB+RL, $T_2$: RB+RL+0.5% urea, $T_3$: RB+RL+1% urea, $T_4$: RB+RL+2% molasses, $T_5$: RB+RL+0.5% urea+2% molasses, and $T_6$: RB+RL+1% urea+2% molasses. Fresh and fermented RB was analyzed for nutritional composition. The pH decreased after fermentation in case of all the treatments. The crude protein (CP) increased linearly ($p<0.05$) from 13.45% in the control ($T_0$) to 15.97% in $T_6$, representing an 18.74% increase after fermentation, which was the highest value among the treatments. On the other hand, crude fiber (CF) decreased from 15.65% in the control ($T_0$) to 10.17% in $T_6$, representing 35.02% decrease. Other fiber components, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), cellulose and hemicellulose also decreased significantly ($p<0.05$) after fermentation. Ether extract (EE) showed significant ($p<0.05$) differences among treatments. Phytate phosphorus decreased and available phosphorus increased after fermentation with addition of highest levels of additive supplementation. The decrease in fiber components coupled with increase in CP and phosphorus availability may improve the nutritional value as well as feeding potential of fermented RB for poultry diets.



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## INTRODUCTION

Rice (*Oryza sativa* L.) is one of the world's most important staple food crops, with more than 110,000 varieties cultivated across over 100 countries (Fukagawa & Ziska, 2019; Mohidem *et al*., 2022). It serves as a primary food source for more than half of the global population, contributing approximately 23% of the world's dietary energy intake, with over 90% of people in Asian countries relying on rice as their staple food (Nidhishree *et al*., 2024). Global rice production reached 540.93 million metric tons (MMT) in 2024/25, of which Bangladesh contributed 36.6 MMT (USDA, 2025). The rice milling industry generates substantial quantities of agro-industrial by-products, including rice bran (RB), rice polish (RP), and de-oiled rice bran (DORB). These by-products can be effectively utilized as animal feed to reduce feed costs, improve resource efficiency, and enhance the sustainability of livestock production, with the potential to reduce its environmental impact by up to 20% (Clark & Tilman, 2017; Shah *et al*., 2025). Rice bran, which constitutes approximately 8–10% of the paddy grain weight (Heuzé & Tran, 2015) and it is a nutritionally valuable by-product containing high fiber and fat but low in crude protein (CP) (Devi *et al*., 2021). The RB also contain some bioactive compounds including phenolics, flavonoids, fatty acids, sterols and antioxidant including tocotrienols, tocopherols, $\gamma$-oryzanol and essential vitamins (Sen *et al*., 2020; Kalita *et al*., 2023;

Manzoor *et al.*, 2023).

Although RB contains several valuable nutrients, the inclusion of RB in poultry diet is limited because of its high fiber content and some anti-nutritional substances e.g. phytic acid, $\beta$-glucans and susceptibility to lipid rancidity immediately after milling (Bollinedi *et al.*, 2021; Chuang *et al.*, 2021; Qui & Linh, 2025). Phytic acid is considered as an anti-nutritional factor in poultry because it binds phosphorus and other important nutrients and decreases their bioavailability. All these factors reduce nutrient digestibility and mineral availability, particularly in case of poultry, thereby limiting the inclusion level of RB in poultry diets. In general, the proportion of phytate phosphorus varies from 60 to 80% of the total phosphorus in plant feed stuffs (Singh, 2008). Cereal by-products contain large amount of phytate-phosphorus and among the common cereal by-products, RB have the highest level of phytate-phosphorus content (Singh, 2008). Not only that, phytate may also inhibit proteolysis by altering the protein configuration of digestive enzymes and can produce protein-phytate complexes. Additionally, phytic acid reduces the bioavailability of phosphorus, calcium, magnesium, zinc and many other trace elements. Therefore, strategies are needed to overcome these limitations of RB and minimize its negative impacts on the performance of poultry. Among the different strategies, microbial fermentation is a viable bioprocess to lower crude fiber while upgrading crude protein and other nutrient bioavailability (Jannathulla *et al.*, 2017; Debi *et al.*, 2019, Liza *et al.*, 2022; Debi *et al.*, 2022a, 2022b, 2026).

Among microbial fermenting substrates, rumen liquor is a rich source of fibrolytic and phytate-degrading enzymes, including $\beta$-endoglucanase, hemicellulase, phytase, maltogenic amylase, and xylanase, which are capable of degrading complex fiber components and phytic acid (Azrinnahar *et al.*, 2021; Debi *et al.*, 2019; 2026). Moreover, rumen microorganisms exhibit substantial phytase activity, facilitating the degradation of phytate phosphorus and enhancing its availability for absorption in ruminants (Haese *et al.*, 2014, 2017). Although previous studies demonstrated the effectiveness of fermentation of RB with rumen liquor (Debi *et al.*, 2019; 2022a), the method is relatively complex and less practical for large-scale or field-level application, particularly in developing countries. Additionally, proper microbial fermentation requires additional nutrient supplementation as an additive because RB is a by-product containing high fiber and low energy and protein that are not sufficient for maximum microbial activities. Among the available additives, urea and molasses are considered promising for enhancing the microbial fermentation process. Previous study reported that addition of 2% urea provides essential non-protein nitrogen (NPN) for microbial growth (Ahmad *et al.*, 2019). While molasses offers water-soluble carbohydrates (WSC) that fuel microbes, lowering pH and ensuring fermentation process (Baytok *et al.*, 2005; Wallace *et al.*, 2008).

Therefore, the present study evaluated a simplified one-step fermentation approach using rumen liquor supplemented with urea and molasses as an additional nutrient sources to improve the fermentation efficiency as well as the nutritional quality of rice bran. The specific objectives of this study were to increase protein content, reduce fiber fractions and phytate phosphorus, enhance available phosphorus, and ultimately improve the potential of fermented RB as a cost-effective poultry feed ingredient.

## MATERIALS AND METHODS

### Study area

The process of fermentation of RB was carried out at the Shahjalal Animal Nutrition Field Laboratory and all the nutritional parameters were conducted at the Animal Nutrition Laboratory, Department of Animal Nutrition, Bangladesh Agricultural University, Mymensingh. All procedures were conducted in accordance with appropriate safety measures and hazard management protocols approved by the Animal Welfare and Experimentation Ethics Committee, Bangladesh Agricultural University, Mymensingh-2202 {AWEEC/BAU/2026(2)/04(a)}.

### Collection of study materials (rice bran and rumen liquor)

Rice bran was collected from the local market (Shesh more, Bangladesh Agricultural University, Mymensingh, Bangladesh) and stored in clean, airtight polythene bags to prevent moisture absorption or contamination before fermentation. Rumen liquor was collected from three healthy cattle immediately after slaughter at Koshai Bari slaughterhouse, Mechua Bazar, Mymensingh. Rumen liquor was collected from a local slaughterhouse because this source provides a practical, readily available, and cost-effective means of obtaining rumen liquor. Collection from slaughtered animals also facilitates its potential application in large-scale commercial feed processing. Collection was made according to standard protocol to maintain appropriate environment for viability of the microbes in the collected rumen liquor (Shen *et al.*, 2017). After collection, the rumen liquor from three cattle mixed together and it was then filtered through a stainless-steel round strainer to remove any coarse feed particles. After that the collected rumen liquor was transferred into a preheated (39 °C) insulated flask to maintain anaerobic condition during transportation (Weiss *et al.*, 2017). Prior to rumen liquor collection, the collection flask was flushed with $CO_2$ gas to create an anaerobic environment and maintained at 39 °C to preserve microbial viability (Sechler *et al.*, 2017; Weiss *et al.*, 2017). Throughout the experiment, the collected rumen liquor's pH ranged from 6.7 to 7.4 and its temperature ranged from 37 °C to 39 °C. The methylene blue reduction test (MBRT) was performed to assess the viable anaerobic microbial activity in the collected rumen liquor (DePeters & George, 2014). In addition, physical characteristics, including color, odor, and consistency were evaluated. Fermentation was initiated only after the MBRT results and all physical observations confirmed that the collected RL met the acceptable quality criteria and contained sufficient viable anaerobic microorganisms.

### Procedure of fermentation of rice bran with rumen liquor supplemented with urea and molasses

Rice bran was fermented using 50% diluted rumen liquor (DRL) with addition of different combinations of urea and molasses to improve the fermentation condition as well as nutritional value of fermented bran. At the initial stage of the experiment, rice bran, urea and molasses were collected from the market and weighed before fermentation day and rumen liquor that had been collected on the day of fermentation. The treatments are as follows:

$T_0$. Fresh Unfermented rice bran (Controlled)

$T_1$. Fermented rice bran (with 50% rumen liquor)

$T_2$. Fermented rice bran (with 50% rumen liquor + 0.5% urea)

$T_3$. Fermented rice bran (with 50% rumen liquor + 1% urea)

$T_4$. Fermented rice bran (with 50% rumen liquor + 2% molasses)

$T_5$. Fermented rice bran (with 50% rumen liquor + 0.5% urea + 2% molasses)

$T_6$. Fermented rice bran (with 50% rumen liquor + 1% urea + 2% molasses)

On the day of fermentation, pre-weighed RB was transferred into a clean mixing bowl. Rumen liquor was added to the RB at a ratio of 2:1 for RB and RL, respectively. For treatments $T_2$-$T_6$, urea and molasses were incorporated according to the experimental design. To facilitate uniform mixing, lukewarm water (50%) was added to the mixture. The mixture was mixed thoroughly by hand to ensure the additives and rumen liquor were evenly distributed. The initial pH measurement was carried out immediately after mixing. After that, the mixture was transferred into airtight polythene bags according to the experimental design. Air was manually expelled with addition of $CO_2$ gas to maintain an anaerobic environment, and the bags were hermetically sealed. Then the mixture was kept and allowed to ferment for 48h. For maintaining optimal fermentation temperature of 37-39 °C, the bags were kept at room temperature and occasionally provide sunlight or an electric bulb to provide supplemental heat. Therefore, following the 48h fermentation period, the bags were opened. Immediately after opening the bag, post-fermentation pH was measured. Then the fermented RB was spread evenly onto trays and subjected to sun-drying to reduce moisture content and stop the fermentation process prior to further nutritional analysis. All experimental treatments were conducted with three independent biological replicates (n=3), each performed in separate container. For nutritional analysis, samples from each replicate were analyzed in duplicate to ensure analytical accuracy. The flow chart of the fermentation process can also be shown in Figure 1.

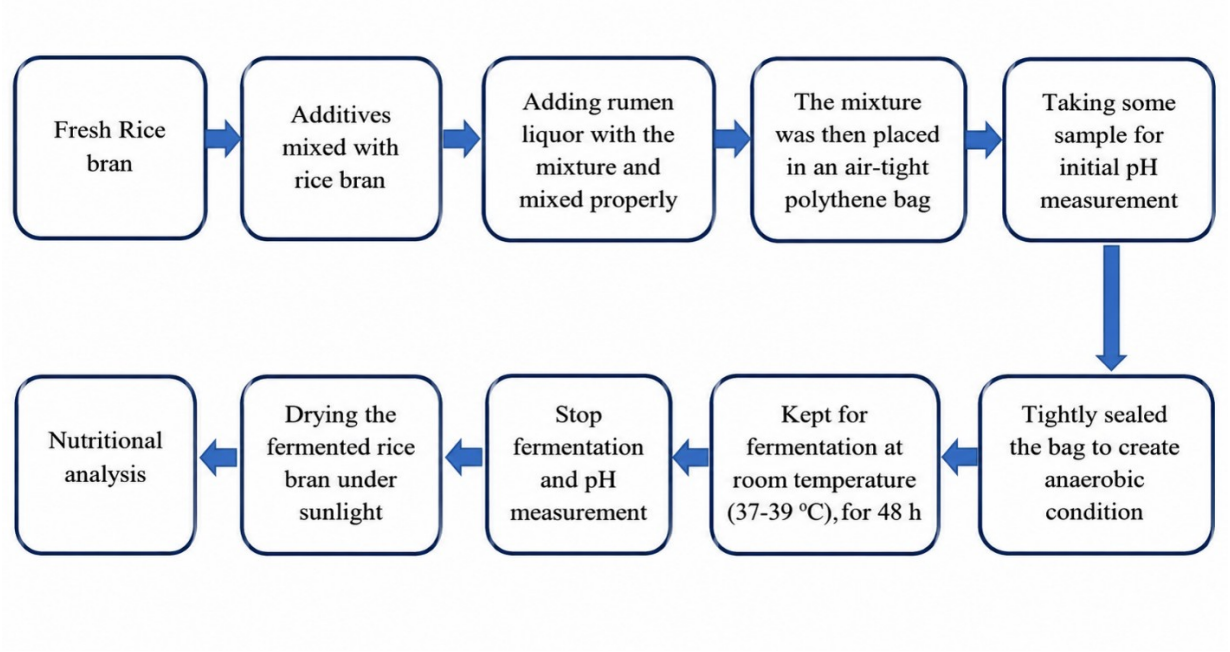


**Figure 1.** *Flow chart of fermentation of rice bran with rumen liquor and additives in a controlled environment.*

## Measurement of pH value

The pH value was recorded using a digital pH meter (NINGBO PHS-25 Digital Bench-Top pH Meter, CE ISO, Zhejiang, China) both before and after fermentation of 48h.

## Nutritional analysis

All fermented and unfermented RB were examined for crude protein (CP), ash, ether extract (EE), crude fiber (CF), and Van Soest fiber, acid detergent fiber (ADF), neutral detergent fiber (NDF) as per the method of AOAC (2005). All samples were measured for two times and the mean values were calculated. Celluloses and hemicelluloses were calculated from the difference of acid detergent fiber (ADF)-acid detergent lignin (ADL) and NDF-ADF respectively (Lopez *et al.*, 2016) to know how much the fiber content actually decreased during the two-step fermentation process. Nutrient content changes were shown as percentages (%).

## Determination of total phosphorus

Total phosphorus was determined by acid digestion followed by colorimetric analysis. Briefly, 100 mg of oven-dried sample was digested with concentrated $H_2SO_4$ and $H_2O_2$ at $\leq$200 °C until a clear digest was obtained. After cooling, the digest was diluted to 100 mL with deionized water. For color development, 1 mL of the digest was mixed with distilled water and a freshly prepared reaction solution containing 6 N $H_2SO_4$, 2.5% ammonium molybdate, and 10% ascorbic acid (2:1:1:1, v/v). The mixture was incubated at 37 °C for 1h, diluted with deionized water, and the absorbance was measured at 660 nm using a spectrophotometer (Debi *et al.*, 2026).

## Determination of phytate phosphorus

Phytate phosphorus was determined by acid extraction followed by iron precipitation. Briefly, 100 mg of sample was extracted overnight (16h) with 0.4 N HCl containing 10% $Na_2SO_4$ at room temperature under continuous shaking. The extract

was centrifuged and filtered, after which the filtrate was reacted with an $FeCl_3$ reagent, heated at 90–100 °C for 30 min, cooled, and centrifuged. The resulting precipitate was washed with 0.2 N HCl, dried, and subjected to total phosphorus determination. The phosphorus content of the final precipitate was considered as phytate phosphorus (Debi *et al.*, 2026).

### Preparation of standard calibration curve

A phosphorus standard curve was prepared using $KH_2PO_4$. A primary standard solution was prepared and diluted to obtain a secondary standard containing 2 mg P/L. Aliquots of the secondary standard were reacted with sulphomolybdic acid and stannous chloride, diluted to volume, and used to prepare standard solutions containing 0.1–0.6 mg P/L. The absorbance of the standards was measured, and a calibration curve was generated in Microsoft Excel using the known phosphorus concentrations (Debi *et al.*, 2026).

### Determination of available phosphorus

Total phosphorus in feed comprises both organic and inorganic forms, with phytate phosphorus representing the major organic fraction. In this study, available phosphorus (non-phytate phosphorus) was estimated as the difference between total phosphorus and phytate phosphorus, assuming that the non-phytate fraction represents the bioavailable phosphorus for poultry (Debi *et al.*, 2026).

Available phosphorus = Total phosphorus − Phytate phosphorus

### Statistical analysis

Data on pH, dry matter (DM), crude protein (CP), crude rice bran were subjected to analysis of variance (ANOVA) using IBM SPSS 20 (IBM SPSS statistics for Windows, 2012 IBM Crop, New York, USA). Differences among treatments means were determined using Tukey's multiple comparison tests. The results are presented as mean ± standard error of the mean (SEM), and differences were considered statistically significant at $p<0.05$

## RESULTS AND DISCUSSION

### pH levels during fermentation

The pH of RB before and after fermentation with different treatments are presented in Figure 2. Fermentation significantly ($p<0.05$) reduced the pH of all treatments compared with the fresh RB ($T_0$). After fermentation, there was a significant difference found in case of pH content of RB in response to different levels of additive supplementation. After fermentation, the greatest reduction in pH (35%) was observed in case of treatment $T_3$ that was supplemented with 1% urea containing more nitrogen compared to fresh RB. The pH reduced 19%, 35%, 31%, 21% 32% in response to 0.5% urea, 1% urea, 2% molasses, 0.5% urea + 2% molasses and 1% urea + 2% molasses supplementation during fermentation, respectively. More urea supplementation exhibited a greater decline in pH, indicating enhanced microbial activity and efficient fermentation that might improve the nutritional quality of RB after fermentation. This decrease in pH can be attributed to the production of different kinds of organic acids during microbial fermentation.

Maintaining the appropriate environment during fermentation is crucial for optimizing microbial activity, with pH being a key factor among that (Santra *et al.*, 2003). In this study, fermentation method supplemented with additional additive was adopted for improving the fermentation condition as well as better fermented products. The rumen's anaerobic condition, within a pH range of 6.0 to 7.0, supports various bacteria that dominate its microbial population (Pitta *et al.*, 2010). The pH is a key factor for influencing ruminal process for degrading fiber components (Santra *et al.*, 2003) and pH lower than appropriate range reduce the efficiency of fiber degradation during fermentation (Dijkstra *et al.*, 2012). In case of live animal, rumen pH is maintained by a variety of variables, including salivation, the formation and absorption of VFA, kinds and quantity of the feed consumed and the exchange of buffers through the epithelium of the rumen (Aschenbach *et al.*, 2011). In the present study the pH decreased significantly after fermentation. This might be due to the fermentative action of rumen microorganisms converting complex CHO to simple sugar molecules which decreases pH with time changes which agrees with (Liza *et al.*, 2022; Debi *et al.*, 2019; 2022a; 2024; Debi & Ivy, 2024). Due to fermentation of feed in the rumen, pH changes continuously. Previous study revealed that, the pH decreases might be due to the generation of different volatile fatty acids (VFAs) along with lactic acid during fermentation (Dijkstra *et al.*, 2012; Aschenbach *et al.*, 2011) and further accumulation of these acids (Chibisa *et al.*, 2016). Due to their weak acidic nature, these organic acids dissociate rapidly, releasing protons and consequently lowering pH. As the present fermentation system is in-vitro and absorption are not possible, the produced acids were accumulated in the fermentation chamber. Therefore, pH reduced more with addition of molasses and with addition of urea and molasses together. Water-soluble carbohydrates promote the growth of rumen bacteria, resulting in increased production of organic acids that lower the pH. In this study, molasses served as a source of water-soluble carbohydrate (WSC), while urea supplied non-protein nitrogen required for microbial growth. Molasses stimulates ruminal bacteria to produce lactic acid, thereby lowering pH and improving fermentation quality (Baytok *et al.*, 2005; Wallace *et al.*, 2008).

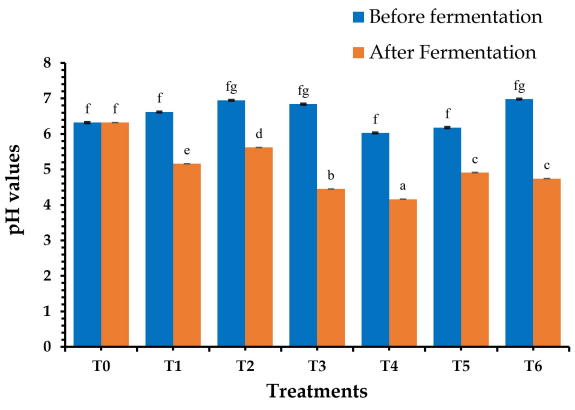


**Figure 2.** *Effects of additive supplementation on pH values of rice bran fermented with rumen liquor.*

## Proximate component of rice bran

### Dry matter, ash, crude protein, ether extract and nitrogen free extract

The dry matter content of fresh and fermented RB is presented in Table 1. Fermentation caused a slight increase in DM (91-93%) corresponding to an increase of approximately 1.76%-2.80%. However, DM remained relatively stable, indicating that fermentation did not significantly affect ($p<0.05$) the overall dry matter content of RB. The ash content differed significantly ($p<0.05$) among treatments (Table 1), with the highest value observed in $T_6$ (14.53%) and lowest value was observed in $T_1$ (13.52%) compared to fresh RB (12.23%). There was an 19% increase of ash content in $T_6$ compared than the fresh RB. Crude protein (CP) content increased significantly ($p<0.05$) following fermentation, particularly in treatments supplemented with urea and molasses (Table 1). The highest CP content was observed in $T_6$ (15.97%) containing 1% urea and 2% molasses followed by $T_3$, $T_5$, $T_2$, $T_4$ and $T_1$ compared to $T_0$ (13.45%), indicating that the supplementation of urea and combined supplementation of urea and molasses effectively enhanced microbial protein synthesis during fermentation that further increased the protein content of the bran. Ether extract (EE) content differed significantly ($p<0.05$) among the treatments (Table 1). The highest EE content was found in case of $T_6$ indicating that combined supplementation of urea and molasses during fermentation increased the microbial proliferation that further increased the lipid composition of RB. In contrast, nitrogen-free extract (NFE) showed only minor variation among treatments, stating that fermentation and additive supplementation had little effect on this parameter.

Recently, fermentation technology has attracted considerable attention as a promising strategy for improving the nutritional quality of rice bran (Manlapig & Matsui, 2025). Fermentation promotes microbial degradation of complex feed components, leading to favorable modifications in nutrient composition and enhanced nutrient availability (Heo *et al*., 2022; Su *et al*., 2022; Islam *et al*., 2022). In agreement with previous reports, the present study showed that fermentation enhanced the overall nutritional quality of rice bran, making it a more valuable and nutritionally efficient ingredient for poultry diets. The increase in CP content can be attributed to the availability of non-protein nitrogen from urea and readily fermentable carbohydrates from molasses supplemented as an additive, which promoted microbial growth that might improve the protein synthesis process. In addition, the acidic conditions created during fermentation inhibited proteolytic microorganisms, thereby reducing protein degradation (Yunus *et al*., 2015; Supriyati *et al*., 2015; Kang *et al*., 2018).

**Table 1.** Effects of additive supplementation on proximate composition of rice bran fermented with rumen liquor for use as poultry feed.

| Variables (%) | $T_0$ | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ | P-value |
|---|---|---|---|---|---|---|---|---|
| DM | 91.00a ±0.06 | 93.23c ±0.01 | 93.51c ±0.01 | 93.21c ±0.01 | 92.79b ±0.06 | 92.60b ±0.06 | 92.79b ±0.03 | <0.001 |
| Ash | 12.23a ±0.01 | 13.52b ±0.00 | 13.97c ±0.01 | 14.05c ±0.03 | 13.59b ±0.00 | 13.10ab ±0.06 | 14.53d ±0.01 | <0.001 |
| CP | 13.45b ±0.10 | 13.57a ±0.01 | 14.65b ±0.01 | 15.76c ±0.01 | 13.95ab ±0.01 | 14.81 cd ±0.20 | 15.97d ±0.01 | <0.001 |
| EE | 14.64a ±0.02 | 15.87bc ±0.01 | 15.12a ±0.01 | 15.65bc ±0.05 | 15.99bc ±0.01 | 15.91cd ±0.01 | 16.10cd ±0.10 | <0.001 |
| NFE | 44.03ab ±0.00 | 43.06a ±0.01 | 43.16b ±0.13 | 42.36a ±0.08 | 43.32b ±0.00 | 44.96b ±0.20 | 43.23ab ±0.07 | <0.001 |

Values are expressed as mean ± standard error (SE) of mean (n=3). Means within the same row bearing different superscripts differ significantly ($p<0.05$) as determined by Tukey's HSD test. DM= Dry matter; Ash= Total mineral content; CP= Crude protein; EE= Ether extract; NFE= Nitrogen free extract. $T_0$ = Fresh rice bran; $T_1$ = Fermented rice bran with rumen liquor; $T_2$= Fermented rice bran with rumen liquor+0.5% urea; $T_3$= Fermented rice bran with Rumen liquor+1% urea; $T_4$ = Fermented rice bran with rumen liquor+2% molasses; $T_5$ = Fermented rice bran with rumen liquor+0.5% urea+2% molasses; $T_6$ = Fermented rice bran with rumen liquor+1% urea+2% molasses.

Rumen microorganisms can enhance CP content by producing extracellular enzymes that degrade complex feed components, thereby improving nutrient digestibility and increasing microbial protein synthesis (Ardiansyah & Hartinah, 2018). Several studies have reported increased CP levels following fermentation of different feed ingredients (Supriyati *et al*., 2015; Jazi *et al*., 2017). Similarly, previous research has demonstrated that fermentation increases the proportion of CP and EE in RB and other agro-industrial by-products (Alam *et al*., 2023; Manlapig & Matsui, 2025). Fermentation of RB and various agro-industrial by-products has been shown to improve CP content (Ullah *et al*., 2021; Shuvo *et al*., 2022). Similarly, previous studies have demonstrated that rumen liquor fermentation significantly increases the CP content of bran (Debi *et al*., 2022b; 2024; Debi & Ivy, 2024). These studies are agreement with our present study and these findings suggest that rumen liquor fermentation is an effective strategy for enhancing the protein value of bran, thereby improving its nutritional quality and potential as a poultry feed ingredient.

In this study, we found that, the fermentation has significant effect ($p<0.05$) on changing the value of EE in the fermented brans compared to fresh bran. Islam *et al*. (2026) stated that fermentation of RB with yeast significantly ($p<0.05$) increased CP, EE and decreased NFE. The EE content increased in fermented RB with highest additive supplementation compared to unfermented bran. In the present research, ash content significantly increased ($p<0.05$) following fermentation and highest value was found with highest levels of additive supplementation ($p>0.05$), which is in line with the findings reported by Debi *et al*. (2019). Terefe *et al*. (2022) showed that ash content increased from 6.76% to 7.03% and 7.44% after 12h and 24h fermentation of cassava leaf with Lactobacillus plantarum. Nitrogen free extract (NFE) remained unchanged

after fermentation (p>0.05). This study, however, did not observe significant effects of fermentation on NFE values. Fermented RB could serve as a cost-effective feed ingredient by enhancing its protein content and overall nutritional value for poultry production.

### Fiber components of rice bran (CF, ADF, NDF, ADL, cellulose and hemicellulose)

Fermentation significantly (p<0.05) reduced fiber fractions of RB after fermentation of 48h and data are presented in Table 2. Crude fiber (CF) decreased from 15.65% in $T_0$ to 10.17% in $T_6$, showing the highest reduction (35%) among all the treatments. Similarly, neutral detergent fiber (NDF) decreased significantly (p<0.05) across treatments, with the highest reduction (33%) observed in $T_6$ compared with the control and other treatments. Fermentation also had significant (p<0.05) effect on acid detergent fiber (ADF) content of RB before and after fermentation. The ADF content decreased from 18.46% in $T_0$ to 14.77% in $T_6$, representing a 20% reduction. Cellulose and hemicellulose contents showed similar trends, with the greatest reduction recorded in $T_6$ representing 51% and 44% reduction, respectively compared to the control and other treatments. These results indicate that supplementation with urea and molasses enhanced the efficiency of rumen liquor fermentation in degrading structural carbohydrates (fiber components). In contrast, acid detergent lignin (ADL) was not significantly affected (p>0.05) by fermentation, although a numerical increase was observed among the treatments. Since lignin is highly resistant to microbial degradation, little changes in ADL content were expected during the fermentation process.

**Table 2.** Effects of additive supplementation on fiber components of rice bran fermented with rumen liquor for use as poultry feed.

| Variables (%) | $T_0$ | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ | P-value |
|---|---|---|---|---|---|---|---|---|
| CF | $15.65^{d}$ ±0.01 | $13.98^{c}$ ±0.01 | $13.10^{b}$ ±0.10 | $12.15^{ab}$±0.01 | $13.15^{b}$ ±0.02 | $11.21^{ab}$ ±0.01 | $10.17^{a}$ ±0.01 | <0.001 |
| NDF | $38.24^{f}$ ±0.01 | $34.57^{e}$ ±0.02 | $32.65^{d}$ ±0.01 | $30.43^{bc}$ ±0.33 | $33.80^{c}$ ±0.33 | $28.37^{b}$ ±0.12 | $25.44^{a}$ ±0.33 | <0.001 |
| ADF | $18.46^{c}$ ±0.02 | $17.77^{bc}$ ±0.01 | $17.07^{b}$ ±0.03 | $16.16^{ab}$ ±0.01 | $17.15^{b}$ ±0.01 | $15.59^{ab}$ ±0.01 | $14.77^{a}$ ±0.00 | <0.001 |
| ADL | $6.53^{a}$ ±0.03 | $6.87^{a}$ ±0.01 | $7.65^{b}$ ±0.01 | $7.96^{b}$ ±0.00 | $7.46^{b}$ ±0.01 | $8.19^{c}$ ±0.00 | $8.97^{d}$ ±0.01 | <0.01 |
| Cellulose | $11.93^{e}$ ±0.03 | $10.90^{d}$ ±0.00 | $9.42^{c}$ ±0.06 | $8.20^{bc}$ ±0.01 | $9.69^{c}$ ±0.01 | $7.40^{b}$ ±0.01 | $5.80^{a}$ ±0.01 | <0.01 |
| Hemicell-ulose | $19.78^{f}$ ±0.03 | $16.80^{e}$ ±0.03 | $15.58^{d}$ ±0.06 | $14.27^{c}$ ±0.58 | $16.64^{e}$ ±0.57 | $12.78^{b}$ ±0.21 | $11.00^{a}$ ±0.01 | <0.001 |

Values are expressed as mean ± standard error (SE) of mean (n=3). Means within the same row bearing different superscripts differ significantly (p<0.05) as determined by Tukey's HSD test. CF= Crude fiber; NDF= Neutral detergent fiber; ADF= Acid detergent fiber; ADL= Acid detergent lignin. $T_0$ = Fresh rice bran; $T_1$ = Fermented rice bran with rumen liquor; $T_2$= Fermented rice bran with rumen liquor+0.5% urea; $T_3$= Fermented rice bran with rumen liquor+1% urea; $T_4$ = Fermented rice bran with rumen liquor+2% molasses; $T_5$ = Fermented rice bran with rumen liquor+0.5% urea+2% molasses; $T_6$ = Fermented rice bran with rumen liquor+1% urea+2% molasses.

Crude fiber (CF) is another nutritional component which inclusion level is very crucial factor in poultry diet. In the present study, CF content of RB significantly decreased (p<0.05) after fermentation. Debi *et al.* (2019; 2022a; 2024) reported that CF content significantly reduced after fermentation of RB with RL. Shi *et al.* (2020) illustrates that CF decreased from 21.5% to 14.0% in the solid-state fermentation of okra with probiotics. Islam *et al.* (2026) also reported that CF content of RB significantly (p<0.05) decreased when RB was fermented with yeast. A significant reduction of other fiber components (NDF, ADF, Cellulose and Hemicellulose) were also occurred after fermentation. Other previous study also revealed that a considerable reduction in total fiber content was detected, accompanied by reductions in both NDF and ADF after fermentation of RB with RL or probiotics (Ullah *et al.*, 2021; Islam *et al.*, 2022; Shuvo *et al.*, 2022; Wang *et al.*, 2024). In the present study, all kinds of fiber components decreased significantly more with increasing levels of additive supplementation. This can be expected that the supplementation of additional nutrients is a critical factor for influencing the fermentation condition that leads to more fiber degradation. The NDF content of RB decreased significantly (p<0.05) that supports by Aro & Aletor (2012), where NDF and ADF decreased after fermentation. The reduction in fiber fractions can be attributed to the activity of fibrolytic microorganisms and enzymes present in rumen liquor including cellulase, hemicellulase, xynalase, $\beta$-Glucosidase, amylase, pectinase etc., which degrade complex structural carbohydrates into simpler compounds. In addition, molasses supplied readily fermentable carbohydrates that stimulated microbial growth and enhanced enzyme production, resulting in greater fiber degradation (Debi *et al.*, 2019; Lukkananukool *et al.*, 2019; Debi *et al.*, 2022a). The present findings are consistent with previous reports showing that rumen liquor fermentation effectively reduces CF, NDF, ADF, cellulose and hemicellulose content of RB, thereby improving its nutritional quality for feeding poultry (Aro & Aletor, 2012).

### Total phosphorus content of RB

Total phosphorus content of RB before and after fermentation among different treatments are presented in Table 3. Total phosphorus increased progressively with increasing levels of additive supplementation (p<0.05). With only rumen liquor, there is no significant difference of total phosphorus content before after fermentation with value change from 1.160 to 1.191%. However, total phosphorus content significantly increased in $T_2$, $T_5$ and $T_6$ with 6%, 17% and 22% compared to fresh RB ($T_0$), respectively (p<0.05). The highest improvement was occurred in case of $T_6$ with highest addition of urea and molasses supplementation together. Phosphorus is a very essential nutrient for the maintenance and development of the skeletal systems, and it is required for the metabolism of carbohydrate and fat. Additionally, phosphorus availability is essential for rumen microbial metabolism and fiber degradation. Alkaline phosphatase catalyzes the release of the final phosphate group from myo-inositol monophosphate (InsP1), a

substrate relatively resistant to phytase hydrolysis (Durand & Komisarczuk, 1988). The phosphate released from phytic acid is commonly quantified using the modified molybdenum blue colorimetric assay and expressed as total phosphorus or phytic acid content (Fiske & Subbarow, 1925). Adequate phosphorus supply is also critical for optimal cellulolytic activity, as phosphorus deficiency markedly reduces cellulose digestion and consequently impairs feed utilization and animal performance (Bravo *et al*., 2000).

Previous studies reported comparable total phosphorus concentrations before and after rumen liquor fermentation, indicating limited phosphorus release under those conditions (Islam *et al*., 2022). Likewise, Pujaningsih (2004) found that rumen liquor alone had little effect on phytate degradation compared with microbial phytase supplementation. In contrast, the present study demonstrated a significant ($p<0.05$) increase in total phosphorus following rumen liquor fermentation supplementation with necessary nutrients energy and protein for increasing microbial activity, suggesting enhanced phosphorus release from phytate and other phosphorus-containing compounds. These findings indicate that the fermentation conditions adopted in this study effectively improved phosphorus availability in RB than the previous study (Debi *et al*., 2026), thereby enhancing their nutritional value as poultry feed ingredients. In RB, increasing additive concentration significantly increased total phosphorus content, while the significant increase total phosphorus indicated that higher energy and protein levels further enhanced the positive effect of total phosphorus concentration. However, the variation in phosphorus release across different levels and combination of additive supplementation, particularly at the highest combination of molasses and urea, suggests that optimizing additional nutrient concentration is important for maximizing phosphorus availability.

**Table 3.** Effects of additive supplementation on phosphorus content of rice bran fermented with rumen liquor for use as poultry feed.

| Variables (%) | $T_0$ | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ | P-value |
|---|---|---|---|---|---|---|---|---|
| Total phosphorous | $1.160^{a}$ ±0.0-1 | $1.191^{ab}$ ±0.-06 | $1.232^{b}$ ±0.-01 | $1.290^{bc}$±0.-08 | $1.206^{ab}$ ±0.-02 | $1.354^{c}$ ±0.-01 | $1.410^{d}$ ±0.-01 | <0.00-1 |
| Phytate phosphorous | $0.732^{ef}$ ±0.-03 | $0.672^{e}$ ±0.1-2 | $0.552^{d}$ ±0.-01 | $0.414^{c}$ ±0.-33 | $0.595^{d}$ ±0.-30 | $0.337^{b}$ ±0.-12 | $0.297^{a}$ ±0.-33 | <0.00-1 |
| Available phosphorous | $0.428^{a}$ ±0.0-2 | $0.519^{ab}$ ±0.-01 | $0.680^{c}$ ±0.-03 | $0.876^{d}$ ±0.-02 | $0.611^{c}$ ±0.0-1 | $1.017^{e}$ ±0.-01 | $1.113^{f}$ ±0.-00 | <0.00-1 |

Values are expressed as mean ± standard error (SE) of mean (n=3). Means within the same row bearing different superscripts differ significantly ($P<0.05$) as determined by Tukey's HSD test. $T_0$ = Fresh rice bran; $T_1$ = Fermented rice bran with rumen liquor; $T_2$= Fermented rice bran with rumen liquor+0.5% urea; $T_3$= Fermented rice bran with rumen liquor+1% urea; $T_4$ = Fermented rice bran with rumen liquor+2% molasses; $T_5$ = Fermented rice bran with rumen liquor+0.5% urea+2% molasses; $T_6$ = Fermented rice bran with rumen liquor+1% urea+2% molasses.

## Phytate phosphorus content of RB

Phytate phosphorus content of RB before and after fermentation among different treatments are presented in Table 3. Phytate phosphorus content of RB decreased significantly with increasing levels of additive supplementation ($p<0.05$). In case of $T_1$ with only rumen liquor and without urea and molasses, there was no significant difference of phytate phosphorus content before after fermentation with value change from 0.732 to 0.672%. However, phytate phosphorus content significantly decreased in $T_2$, $T_3$, $T_4$, $T_5$ and $T_6$ with 25%, 44%, 19%, 54% and 59% compared to fresh RB ($T_0$), respectively ($p<0.05$). The lowest phytate phosphorus content of RB was found in case of $T_6$ with addition of 1% urea and 2% molasses together ($p<0.05$). Phytase plays an important role in animal nutrition by improving phosphorus utilization through the hydrolysis of phytic acid and the release of phosphorus bound within phytate complexes (Dersjant-Li *et al*., 2015). Phytic acid is predominantly present in plant ingredients as phytate (phytin), which has low phosphorus availability and can exert antinutritional effects by binding minerals and other nutrients, thereby reducing their utilization and potentially impairing animal performance (Woyengo & Nyachoti, 2013). This limitation is particularly important in poultry because endogenous intestinal phytase activity is insufficient to hydrolyze a substantial proportion of dietary phytate phosphorus (Navales *et al*., 2025). Rumen microorganisms are recognized as a potential source of phytase, and considerable phytate degradation can occur through microbial activity in the rumen (Ellis & Tillman, 1961). Consequently, rumen liquor fermentation may provide a natural source of phytase-producing microorganisms capable of degrading phytate in plant-based feed ingredients. Previous fermentation studies have reported substantial reductions in phytate phosphorus after 24h of fermentation, with further effects observed at 48h, together with improvements in other nutritional characteristics (Islam *et al*., 2022). These findings support the potential role of microbial fermentation in reducing phytate-associated phosphorus binding.

In the present study, fermentation of RB) with 50% rumen liquor (RL) further supplemented with molasses and urea for 48h resulted in significant reduction in phytate phosphorus. In case of fermentation of RB with only rumen liquor did not significantly vary about the phytate phosphorus content of RB before and after fermentation. Debi *et al.* (2026) reported that phytate phosphorus level decreased with increasing fermentation time. However, in the present study, fermentation time was not extended after 48 h but fermentation was extended with nutrient supplementation and found significant effect of nutrient supplementation for phytate phosphorus reduction. Extending the fermentation period to 24, 48, and 72h produced a progressive decline in phytate phosphorus, indicating that prolonged fermentation enhanced phytate degradation (Debi *et al*., 2026). The greater reduction was observed at the higher level of combined urea and molasses supplementation may be associated with increased abundance or activity of phytase-producing rumen microorganisms, although direct phytase activity was not measured in the present study. The significant reduction in phytate phosphorus with increasing level of additive supplementation indicates that the factors contributed

to more phytate degradation. The significant phytate phosphorus reduction further suggests that the additional nutrient supplementation is very important for rumen microorganisms for degrading phytate phosphorus concentration of RB. This pattern is consistent with the recognized role of microbial phytase in hydrolyzing phytate and releasing phosphorus from phytate-bound forms (Dersjant-Li *et al*., 2015). Overall, rumen liquor fermentation effectively reduced phytate phosphorus in RB with the greatest reduction observed at higher levels of additive supplementation. These findings suggest that rumen liquor fermentation with some nutrient supplementation may be a simple biological approach for reducing the phytate fraction of bran and potentially improving the proportion of phosphorus available for utilization in poultry diets. However, because phytase activity and phosphorus digestibility were not directly measured, the observed reduction in phytate phosphorus should be interpreted as evidence of phytate degradation rather than a direct measure of phosphorus bioavailability.

### Available phosphorus contents of RB

The available phosphorus content of RB before and after fermentation among different treatments are presented in Table 3. Available phosphorus increased gradually with increasing levels of additive supplementation. In case of $T_1$ with only rumen liquor and without addition of additional additive, there is no significant difference of available phosphorus content before after fermentation with value change from 0.428 to 0.519%. However, available phosphorus content of RB significantly increased in $T_2$, $T_3$, $T_4$, $T_5$ and $T_6$ with 59%, 105% 43%, 138% and 160% compared to fresh RB ($T_0$), respectively ($p<0.05$). The highest improvement was occurred in case of $T_6$ with highest level of urea (1%) and molasses (2%) supplementation together ($p<0.05$). In the present study, increasing the level of additive supplementation or combined effect of additive supplementation resulted in a gradual increase in total phosphorus and a corresponding reduction in phytate phosphorus. Available phosphorus was estimated as the difference between total phosphorus and phytate phosphorus. Previous research has shown that reducing dietary non-phytate phosphorus can depress serum calcium and phosphorus concentrations in broilers, whereas phytase supplementation can alleviate the adverse effects of phosphorus deficiency (Islam *et al*., 2022). Similarly, fermentation has been reported to reduce phytate phosphorus and increase available phosphorus with increasing fermentation duration and moisture level (Islam *et al*., 2022). Phytase-mediated hydrolysis of phytate is considered an important mechanism for increasing the proportion of phosphorus in a potentially available form (DePeters & George, 2014).

In the present study, available phosphorus increased significantly ($p<0.05$) with extended level of additive supplementation, particularly at the combination of urea and molasses supplementation, while phytate phosphorus decreased gradually. The reduction in phytate phosphorus, relatively changes in total phosphorus, resulted in an increased the level of calculated available phosphorus fraction of RB after fermentation. This pattern may indicate enhanced phytate degradation during fermentation of RB for 48h with additive supplementation, potentially due to more microbial phytase activity. However, because phytase activity was not directly measured, this mechanism should be considered a possible explanation rather than a confirmed mechanism. Across the different treatments, available phosphorus generally increased with increasing level of additive supplementation. However, no significant difference was observed between $T_0$ and $T_1$ in case of most of the parameters. The fermentation period 48h was selected because Debi *et al*. (2026) reported that, extending fermentation period beyond 48h provided limited additional improvement in the measured phosphorus profile under the conditions tested. Overall, rumen liquor fermentation with different levels and combination of urea and molasses supplementation improved the phosphorus profile of RB by reducing phytate phosphorus and increasing the calculated available phosphorus fraction. These findings suggest that rumen liquor fermentation with little number of additives (energy and protein) may be a promising approach for improving the nutritional quality and feeding value of RB that might potentially enhance the phosphorus utilization by poultry.

## Conclusion

The present study demonstrated that rumen liquor fermentation supplemented with urea and molasses significantly improved the nutritional quality of rice bran. Fermentation increased crude protein content and phosphorus availability while reducing crude fiber, neutral detergent fiber, acid detergent fiber, cellulose and hemicellulose, thereby enhancing the feeding value of rice bran for poultry. Among the treatments, $T_6$ (50% rumen liquor + 1% urea + 2% molasses) produced the most promising nutritional composition for poultry. These findings suggest that rumen liquor fermentation supplemented with appropriate additives is a simple, effective and economical approach for upgrading rice bran as an alternative feed ingredient for poultry.

## DECLARATION

**Author contribution statement:** Conceptualization: M.R.D. and M.N.H.; Methodology: M.R.D. and M.N.H.; Software and validation: M.R.D., M.N.H., M.S.R.S. and T.D.; Formal analysis and investigation: M.R.D., M.N.H. and T.D.; Resources: M.R.D.; Data curation: M.R.D. and M.N.H.; Writing—original draft preparation: M.R.D. and M.N.H.; Writing—review and editing: M.R.D., M.N.H., M.S.R.S. and T.D.; Visualization: M.R.D., M.N.H., M.S.R.S. and T.D.; Supervision: M.R.D.; Project administration: M.R.D.; Funding acquisition: M.R.D. All authors have read and agreed to the published version of the manuscript.

**Conflicts of interest:** The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

**Ethics approval:** This study was conducted in view of the institutional ethical guidelines and does not harm the human participants that were permitted by the Animal Welfare and Experimentation Ethics Committee, Bangladesh Agricultural University, Mymensingh-2202 {Ethical approval number: AWEEC/BAU/2026(2)/04(a)}.

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