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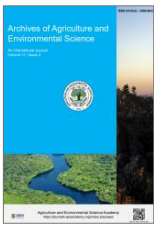

Original Research Article

Enhancing phosphorus bioavailability in rice bran and wheat bran through fermentation with rumen liquor

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ABSTRACT

Rice bran (RB) and wheat bran (WB) are two agro-industrial by-products, generated during the processing of rice and wheat grain and are widely used as a poultry feed in many developing countries. However, their use in poultry diets is limited by their high fiber and presence of anti-nutritional factors, particularly phytate phosphorus, which reduces nutrient availability specially phosphorus. Therefore, this study aimed to reduce phytate phosphorus and increase available phosphorus content of RB and WB through fermentation with rumen liquor (RL). Fermentation was carried out by mixing bran with buffer solution, and diluted RL at ratios of 1:1.5:0.5 and 1:1.5:1 corresponding to 50% and 100% RL inclusion levels, respectively for 12, 24, 48 and 72 h with maintaining an appropriate environment for fermentation. The best result was obtained after 48 h and 72 h fermentation for both 50% and 100% RL. Total phosphorus increased 41% for RB and 27% for WB after 72 h with 100% RL ($p<0.05$). However, after 12 h of fermentation, phytate phosphorus started to reduce with increasing fermentation duration, and it was decreased 57% for RB and 44% for WB after 72 h with 100% RL ($p<0.05$). The available phosphorus level was also increased from 0.372% to 1.202% for RB and from 0.285% to 1.077% for WB after 72 h with 100% RL. Phytate phosphorus decreased, while available phosphorus increased with increasing fermentation time ($p<0.05$). These improvements enhance the nutritional value of RB and WB, making them more suitable and potentially cost-effective ingredients for poultry feed.

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INTRODUCTION

The poultry industry is one of the fastest-growing agricultural sectors worldwide; however, the availability of high-quality, protein-rich feed remains limited and costly in many developing countries. Feed cost is a major constraint to poultry production, necessitating greater attention to nutrient density, ingredient availability, and cost-effective feed resources. Consequently, low-quality agro-industrial by-products such as rice bran (RB) and wheat bran (WB) are commonly used in poultry diets despite their relatively low protein value (Debi *et al.*, 2022b). Global paddy rice (*Oryza sativa*) production exceeds 600 million metric tons annually, generating approximately 48 million metric tons of RB as a major by-product (Bodie *et al.*, 2019; Chen *et al.*, 2012). Similarly, wheat bran (WB) is widely available in cereal-producing regions. Both RB and WB are inexpensive and abundant sources of nutrients (Kang *et al.*, 2015; Xia *et al.*, 2012); however, their utilization in poultry diets is limited by their high fiber content and the presence of some antinutritional factors (Krás *et al.*, 2013; Satinder *et al.*, 2011). Broiler diets are primarily plant-based and contain high levels of phytic acid (Chowdhury *et al.*, 2022), the principal storage form of phosphorus (P) in plants, is present as phytate (myo-inositol 1,2,3,4,5,6-hexakisphosphate). Phytate accounts for approximately 50-90% of total phosphorus in cereals and legumes (Ravindran, 1995), with about 60-80% of total phosphorus existing as phytate phosphorus (Gupta *et al.*, 2015). Average phytate phosphorus accounted for about 0.67 of total phosphorus in cereals and cereal by-products,

indicating that roughly two-thirds of the phosphorus is bound as phytate and thus poorly available to non-ruminant animals (Steiner *et al*., 2007). Because poultry lacks endogenous phytase, phytate-bound phosphorus is poorly digested and largely excreted, reducing mineral and nutrient utilization and increasing fecal phosphorus output (Abbasi *et al*., 2019). Excess phosphorus excretion contributes to environmental pollution and eutrophication (Bohn *et al*., 2008; Chowdhury *et al*., 2022).

Improving the nutritional quality of RB and WB by releasing phytate-bound phosphorus is therefore essential. Fermentation using rumen liquor, which contains phytate-degrading microorganisms, may be an effective approach. Rumen microbes possess strong phytase activity and efficiently degrade phytate phosphorus, making it readily absorbable in ruminants (Haese *et al*., 2014 and 2017). Similarly, soaking and fermentation activate microbial and endogenous plant phytases, leading to the liberation of phytate-bound phosphorus (Carlson & Poulsen, 2003). Previous studies have demonstrated that fermentation of cereal brans with rumen liquor improves nutritional quality by reducing fiber content (Debi *et al*., 2019; Debi *et al*., 2022a) and enhancing protein content and quality (Debi *et al*., 2024; Debi & Ivy, 2024; Debi *et al*., 2022b). However, limited information is available regarding the use of rumen liquor fermentation specifically to enhance phosphorus availability in RB and WB. Therefore, the objective of the present study was to reduce phytate phosphorus and increase its availability in RB and WB through fermentation with rumen liquor.

MATERIALS AND METHODS

Study area and ethical approval

The experiment was carried out in the Animal Nutrition Laboratory, Department of Animal Nutrition, Bangladesh Agricultural University (BAU), Mymensingh with ensuring proper safety and considering hazardous factors that were permitted by the Animal Welfare and Experimentation Ethics Committee, Bangladesh Agricultural University, Mymensingh-2202 {AWEEC/BAU/2026(2)/04(a)}.

Preparation of buffer solution

According to the McDougall protocol, a McDougall buffer solution was prepared (McDougall, 1948). Solution A and solution B were mixed to prepare the final buffer solution. The chemical components of the buffer solution are shown in Table 1. The buffer solution pH ranged from 8.1 to 8.5 and pH was measured using a digital pH meter (NINGBO PHS-25 Digital Bench-Top pH Meter, CE ISO, Zhejiang, China).

Table 1. The chemical components of the prepared McDougall buffer solution (1L).

Constituents (Solution A)	Amount (g)	Constituents (Solution B)	Amount (g)
NaCl	0.47	$NaHCO_3$	9.8
KCl	0.57	Na_2HPO_4	3.725
$CaCl_2.2H_2O$	0.054	Distilled Water (mL)	990
$MgCl_2.6H_2O$	0.128		
Distilled Water (mL)	10		

Collection and processing of rumen liquor before fermentation

Rumen liquor was collected from a cannulated cow, of Shahjalal Animal Nutrition field laboratory under the Department of Animal Nutrition, Bangladesh Agricultural University. The cannulated cow was fed an adequate amount of green grass and concentrates mixtures during the rumen liquor collection period. On the day of fermentation, rumen liquor was collected before morning feeding according to the procedure of (Shen *et al*., 2017). The collection flask was flashed with CO_2 gas to make anaerobic condition in the flask and maintained 39 °C temperature inside the flask (Sechler *et al*., 2017; Weiss *et al*., 2017). After being collected, the rumen liquor was filtered to get rid of any remaining solid particles using a stainless-steel round strainer. Evaluation of rumen liquor was performed before mixing with the bran in the fermentation chamber (conical flask). The filtered rumen liquid was then placed into fermentation containers with continuous CO_2 gas flushing to maintain anaerobic conditions (Weiss *et al*., 2017). The rumen liquor's pH and temperature both assessed simultaneously just after it was collected. Throughout the whole experiment, the collected rumen liquor's pH ranged from 6.7 to 7.4 and its temperature ranged from 37 °C to 39 °C. According to the protocol outlined by DePeters & George (2014), physical parameters including color, odor, and consistency were noted, and a methylene blue reduction test (MBRT) was performed to measure the proportion of functional anaerobic bacteria present in the rumen fluid. The fermentation was only started if the measurements showed that the rumen fluid sample was normal and contained an adequate number of microorganisms.

Fermentation of RB and WB with rumen liquor

The fermentation of RB and WB with rumen liquor was conducted by two different rumen liquor ratios (50% & 100% diluted rumen liquor) on four different time durations (12 h, 24 h, 48 h, and 72 h). In the initial step of this method, RB and buffer solution were mixed in an Erlenmeyer flask and preheated to reach the temperature to 39 °C. After that rumen liquor was diluted with warm water (39 °C) that had been collected earlier and mixed in the previous mixture in an Erlenmeyer flask. Finally, the ratio of RB, Buffer solution and rumen liquor were mixed at the ratio of 1:1.5:0.5 and 1:1.5:1 for the quantities of 50% and 100% diluted RL, respectively. After that the bran mixtures were kept for fermentation for 12 h, 24 h, 48 h and 72 h in an appropriate environment like temperature (39 °C), pH (initial pH was nearly 7) and anaerobic condition. A plastic bag was connected in an Erlenmeyer flask for collection of gas after fermentation. The pH was measured before and after fermentation. After fermentation for specific ours, the bran mixture was removed from the fermentation chamber and dried in an oven at 70 °C. The same procedure was followed in case of fermentation of WB.

Sample collection

Throughout the whole fermentation process, samples were taken from fresh bran (RB1/WB1), bran after mixing with rumen liquor and buffer before fermentation (RB2/WB2) and bran after fermentation (RB3/WB3) for phosphorus content measurement.

Determination of total phosphorus

Total phosphorus was determined using an acid digestion followed by a colorimetric method. A 100 mg oven-dried sample was taken in a test tube, to which 2 mL of concentrated H_2SO_4 and 1 mL of H_2O_2 were added. The mixture was heated at 200 °C (or lower) for 1 h. After cooling to room temperature, 2-3 drops of H_2O_2 were added and the sample was reheated at 200 °C (or lower) for 30 minutes. Again, 2-3 drops of H_2O_2 were added, and the sample was allowed to cool at room temperature. The digested sample was then

transferred to a volumetric flask and the volume was adjusted to 100 mL with deionized water. For color development, 1 mL of the digested solution was taken into a test tube, followed by the addition of 3 mL of distilled water and 4 mL of reaction solution. The reaction solution consisted of distilled water, 6 N H_2SO_4 (prepared by adding 163 mL concentrated H_2SO_4 to make 1 L solution), 2.5% ammonium molybdate, and 10% ascorbic acid mixed in a 2:1:1:1 ratio. The mixture was incubated in a water bath at 37 °C for 1 hour. After incubation, 1 mL of the reacted solution was diluted with 10 mL of deionized water. Absorbance was measured at 660 nm using a spectrophotometer.

Determination of phytate phosphorus

Phytate phosphorus was determined by acid extraction followed by iron precipitation. A 100 mg sample was extracted with 10 mL of extraction solution (0.4 N HCl and 10% Na_2SO_4) and kept overnight (16 h) at room temperature with shaking. The extract was centrifuged at 10,000 rpm for 20 min at 4 °C and filtered. From the filtrate, 5 mL supernatant was mixed with 5 mL distilled water and 2.5 mL reagent solution containing 15 mM $FeCl_3$, 0.2 N HCl, and 5% Na_2SO_4. The mixture was heated at 90–100 °C for 30 min, cooled on ice, and centrifuged at 6,000 rpm for 15 min at 4 °C. The supernatant was discarded, and the residue was washed with 5 mL of 0.2 N HCl, followed by centrifugation under the same conditions. The final residue was dried and used for total P determination, which was considered as phytate phosphorus.

Preparation of standard calibration curve

A primary phosphorus standard solution was prepared by dissolving 0.2195 g KH_2PO_4 in deionized water and making the volume up to 500 mL. Then, 25 mL of 7 N H_2SO_4 was added to the solution, and the final volume was adjusted to 1 L with distilled water. A secondary standard solution containing 2 mg P/L was prepared by taking 40 mL from the primary standard solution. From the secondary solution, 5, 10, 15, 20, 25, and 30-mL aliquots were transferred into separate test tubes. To each test tube, 4 mL sulphomolybdic acid was added, and the volume was made up to approximately two-thirds with distilled water. Subsequently, 3-4 drops of stannous chloride were added, and the final volume was adjusted to 100 mL with distilled water. The resulting phosphorus standard solutions had concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 mg/L, respectively. A calibration curve was prepared using these known concentrations in Microsoft Excel.

Determination of available (Inorganic) phosphorus

Total phosphorus in feed consists of both organic and inorganic forms, with a major proportion present as phytate phosphorus (organic form). In this study, available (inorganic) phosphorus was estimated by calculating the difference between total phosphorus and phytate phosphorus, assuming that the non-phytate fraction represents the bioavailable form for poultry

Total phosphorus = Phytate phosphorus + Available phosphorus

Statistical analysis

Statistical analyses were performed using IBM SPSS 20 (IBM SPSS statistics for window, 2012 IBM Crop, New York, USA). All nutrients' data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests ($p<0.05$), rumen liquor and hours were considered as independent variables, and two-way analysis of variance (ANOVA) was performed. The results are shown as mean ± standard deviation of mean.

RESULTS AND DISCUSSION

Total phosphorus content of RB and WB

The results from Table 2 show the changes in total phosphorus content of RB across different treatments. The RB_1 is the fresh bran and there was no change in total phosphorus levels. In RB_2, there were slight increases observed due to the addition of 50% and 100% rumen liquor. But, in RB_3 (after fermentation), a significant increase ($p<0.05$) in total phosphorus percentage was noted after fermentation with both 50% and 100% RL and gradually increased with increasing fermentation time. The highest value was observed in case of 72 h fermentation. The total phosphorus content was consistently higher in case of 100% RL compared to 50% RL across all the time periods. The significant main effects of RL ($p<0.001$), fermentation time (Hour, $p<0.001$), and their interaction (RL*H, $p<0.001$) indicate that both factors play a crucial role in enhancing total phosphorus release. The total phosphorus contents of WB are presented in Table 3. It is found that the total phosphorus contents of WB increased significantly ($p<0.05$) from fresh bran to fermented bran over an increasing time period and the highest value was observed at 72 h of fermentation. The total phosphorus contents of WB increased ($p<0.05$) from 1.260 (fresh bran) to 1.413 and 1.620 for 50% and 100% RL respectively after 72 h of fermentation. RL and hour had a significant impact in total phosphorus content of WB over different time periods. But the interaction between RL and fermentation time (RL*H) was not statistically significant and overall, there was no significant combined effect. Total phosphorus increased progressively with increasing fermentation duration and rumen liquor (RL) concentration. After 12 h of fermentation with 50% RL, total P in rice bran (RB) and wheat bran (WB) increased slightly compared with the unfermented samples. Further extension of fermentation to 24 h, 48 h, and 72 h resulted in a marked improvement in total phosphorus content. Similarly, fermentation with 100% RL produced a gradual and more pronounced increase in total phosphorus at comparable time intervals. In contrast, total phosphorus content of defatted RB oilcake (DORB) remained unchanged at different moisture levels (30%, 40%, and 50%) and fermentation durations (24 h, 48 h, and 72 h), showing comparable values before and after fermentation (Islam *et al*., 2022).

Phosphorus availability plays a crucial role in rumen microbial activity. Enzymatic dephosphorylation by alkaline phosphatase facilitates the release of the final phosphate group from myo-inositol monophosphate (InsP1), which is relatively resistant to phytase action (Durand & Komisarczuk, 1988). The released phosphate from phytic acid is commonly quantified using a modified molybdenum blue colorimetric assay and expressed as total phosphorus or phytic acid content (Fiske & Subbarow, 1925). Previous studies have also demonstrated that phosphorus is essential for maximum cellulose digestion by rumen microorganisms, and phosphorus deficiency rapidly reduces cellulolytic activity, leading to impaired diet utilization and animal performance (Bravo *et al*., 2000). Earlier findings indicated that rumen liquor had no significant effect on phytate degradation compared with microbial phytase supplementation (Pujaningsih, 2004). In agreement with these reports, the present study showed a significant ($p<0.05$) increase in total phosphorus following fermentation. Overall, these results highlight the effectiveness of rumen liquor fermentation in

enhancing phosphorus availability in RB and WB, thereby improving their nutritional value. For RB, both increasing rumen liquor concentration and extending fermentation time significantly enhanced total phosphorus content. The significant RL × H interaction suggests a synergistic effect; whereby higher rumen liquor levels amplified the benefits of prolonged fermentation. A similar trend was observed in WB, with 100% RL producing greater improvements than 50% RL. However, variability across fermentation durations, particularly at lower rumen liquor levels, indicates the need for further optimization of fermentation time to maximize phosphorus release

Table 2. Influence of different hours of fermentation with different doses of rumen liquor on total phosphorous content of rice bran (Total phosphorous; %).

RL Doses	RB_1	RB_2	RB_3 (After fermentation)				Source of Variation			
			12 h	24 h	48 h	72 h	Overall	RL	Hour	RL*H
50% RL	$1.065^{i} \pm 0.001$	$1.096^{h} \pm 0.002$	$1.202^{g} \pm 0.010$	$1.286^{e} \pm 0.006$	$1.364^{d} \pm 0.008$	$1.394^{c} \pm 0.002$	<0.001	<0.001	<0.001	<0.001
100% RL	$1.065^{i} \pm 0.001$	$1.097^{h} \pm 0.001$	$1.188^{g} \pm 0.029$	$1.259^{f} \pm 0.012$	$1.435^{b} \pm 0.018$	$1.502^{a} \pm 0.014$	<0.001	<0.001	<0.001	<0.001

RB_1 = Fresh rice bran; RB_2 = Rice bran with rumen liquor and buffer before fermentation; RB_3 = Rice bran after fermentation at different time interval; RL = Rumen liquor; RL*H = Interaction between rumen liquor and hour; % = percent; $^{a-i}$ = at similar row and columns are statistically significant.

Table 3. Influence of different hours of fermentation with different doses of rumen liquor on total phosphorous content of wheat bran (Total phosphorous; %).

RL Doses	WB_1	WB_2	WB_3 (After fermentation)				Source of Variation			
			12 h	24 h	48 h	72 h	Overall	RL	Hour	RL*H
50% RL	$1.260^{ef} \pm 0.006$	$1.271^{ef} \pm 0.010$	$1.279^{ef} \pm 0.031$	$1.323^{e} \pm 0.015$	$1.387^{e} \pm 0.059$	$1.413^{d} \pm 0.081$	<0.001	0.003	0.516	0.999
100% RL	$1.260^{ef} \pm 0.006$	$1.325^{e} \pm 0.010$	$1.510^{cd} \pm 0.165$	$1.543^{c} \pm 0.193$	$1.607^{ab} \pm 0.240$	$1.620^{a} \pm 0.251$	<0.001	0.003	0.516	0.999

RB_1 = Fresh rice bran; RB_2 = Rice bran with rumen liquor and buffer before fermentation; RB_3 = Rice bran after fermentation at different time interval; RL = Rumen liquor; RL*H = Interaction between rumen liquor and hour; % = percent; $^{a-f}$ = at similar row and columns are statistically significant.

Phytate phosphorus content of RB and WB

Phytate phosphorus content of RB data are presented in Table 4 and found that a significant decrease ($p<0.05$) in phytate phosphorus content was found following fermentation, with a more pronounced reduction observed at 48 h and 72 h. For the addition of 50% and 100% RL, the levels decreased to 0.493% and 0.430% at 48 h, and to 0.330% and 0.300% at 72 h fermentation, respectively, compared to RB_1, which had a phytate phosphorus percentage of 0.693. The decrease was particularly significant ($p<0.05$) at 72 h, with reductions of (0.330) 37.95% and (0.300) 56.71% for the 50% and 100% RL treatments, respectively. RL and fermentation time (H) and the interaction between both RL*H was significant that the rate of phytate phosphorus declined between the two RL levels over time. The overall statistical significance ($p<0.001$) confirmed that all the effects played a role in declining phytate phosphorus. Phytate phosphorus content of WB before and after fermentation is presented in Table 5. It is revealed that the phytate P content of WB decreased significantly ($p<0.05$) after fermentation with increasing fermentation time. Maximum reduction was observed at 72 h of fermentation. Value decreased ($p<0.05$) from 0.975 in the fresh bran to 0.560 and 0.543 in the fermented bran for 50% and 100% RL, respectively, after 72 h of fermentation. RL, fermentation time (H), interaction between both RL*H and the overall statistical model had no significant effect on the rate of phytate P declined between the two RL levels over time. Phytase is widely used in animal nutrition to improve nutrient utilization, particularly phosphorus availability. Phytase hydrolyzes phytic acid in plant-based feeds, releasing bound phosphorus for absorption (Dersjant-Li *et al.*, 2015). Phytic acid occurs mainly as its salt, phytate (phytin), which is poorly available to animals and exerts antinutritional effects by reducing nutrient digestibility and animal performance (Woyengo & Nyachoti, 2013). In poultry, phytate phosphorus is poorly utilized due to the absence of sufficient endogenous intestinal phytase (Navales *et al.*, 2025).

Rumen microorganisms are known to produce phytase, and rapid phytate hydrolysis has been reported in the rumen (Ellis & Tillman, 1961). Phytase activity derived from rumen microbes or microbial phytase supplementation has been shown to be sufficient for releasing phosphorus from phytate-bound forms. Fermentation studies have demonstrated that phytate P decreases markedly after 24 h, with significantly lower phytate phosphorus observed at 50% moisture across fermentation durations (24 h, 48 h, and 72 h), accompanied by reductions in fiber content (Islam *et al.*, 2022). In the present study, fermentation for 12 h with 50% rumen liquor (RL) slightly reduced phytate phosphorus in RB and WB, while extending fermentation to 24 h, 48 h, and 72 h resulted in a progressive decrease. Similarly, fermentation with 100% RL led to a more pronounced reduction in phytate phosphorus at comparable time points. Dietary phytase supplementation is also recognized as an effective strategy to enhance phytate phosphorus availability (Dersjant-Li *et al.*, 2015). Overall, phytate phosphorus in RB and WB decreased significantly with increasing fermentation time and higher rumen liquor levels. The reduction was more

pronounced at 100% RL, indicating that higher rumen liquor concentrations accelerated phytate degradation. The significant RL × H interaction suggests a synergistic effect; whereby increased rumen liquor levels amplified the impact of prolonged fermentation on phytate P reduction.

Available phosphorus contents of RB and WB

Data on available phosphorus content of RB is presented in Table 6. It is revealed that the available phosphorus percentage increased significantly ($p<0.05$) as the fermentation time progressed for both 50% and 100% RL addition. For RB_1, the initial available phosphorus percentage was 0.372 with 50% RL. After 48h of fermentation, the levels rose significantly to 0.871%, and at 72 h, they further increased to 0.964%. When using 100% RL, the available phosphorus percentage reached 1.105 at 48 h and 1.202 at 72 h. The RL, fermentation time (H), interaction between both RL*H and the overall statistical model had a significant effect that the rate of available phosphorus increased between the two RL levels over the time period.

Table 4. Influence of different hours of fermentation with different doses of rumen liquor on phytate phosphorous content of rice bran (Phytate phosphorous; %).

RL Doses	RB_1	RB_2	RB_3 (After fermentation)				Source of Variation			
			12 h	24 h	48 h	72 h	Overall	RL	Hour	RL*H
50% RL	$0.693^{a} \pm 0.009$	$0.623^{ab} \pm 0.017$	$0.550^{c} \pm 0.020$	$0.523^{cd} \pm 0.015$	$0.493^{d} \pm 0.015$	$0.430^{e} \pm 0.020$	<0.001	<0.001	<0.001	0.007
100% RL	$0.693^{a} \pm 0.009$	$0.641^{ab} \pm 0.012$	$0.453^{e} \pm 0.015$	$0.360^{f} \pm 0.010$	$0.330^{fg} \pm 0.02$	$0.300^{g} \pm 0.010$	<0.001	<0.001	<0.001	0.007

RB_1 = Fresh rice bran; RB_2 = Rice bran with rumen liquor and buffer before fermentation; RB_3 = Rice bran after fermentation at different time interval; RL = Rumen liquor; RL*H = Interaction between rumen liquor and hour; % = percent; $^{a-g}$ = at similar row and columns are statistically significant.

Table 5. Influence of different hours of fermentation with different doses of rumen liquor on phytate phosphorous content of wheat bran (Phytate phosphorous; %).

RL Doses	WB_1	WB_2	WB_3 (After fermentation)				Source of Variation			
			12 h	24 h	48 h	72 h	Overall	RL	Hour	RL*H
50% RL	$0.975^{a} \pm 0.007$	$0.875^{ab} \pm 0.014$	$0.643^{c} \pm 0.015$	$0.623^{cd} \pm 0.015$	$0.580^{e} \pm 0.010$	$0.560^{ef} \pm 0.010$	<0.001	0.487	0.433	0.664
100% RL	$0.975^{a} \pm 0.007$	$0.873^{ab} \pm 0.012$	$0.600^{c} \pm 0.010$	$0.580^{e} \pm 0.010$	$0.570^{e} \pm 0.010$	$0.543^{ef} \pm 0.006$	<0.001	0.487	0.433	0.664

RB_1 = Fresh rice bran; RB_2 = Rice bran with rumen liquor and buffer before fermentation; RB_3 = Rice bran after fermentation at different time interval; RL = Rumen liquor; RL*H = Interaction between rumen liquor and hour; % = percent; $^{a-f}$ = at similar row and columns are statistically significant.

Table 6. Influence of different hours of fermentation with different doses of rumen liquor on available phosphorous content of rice bran (Available phosphorous; %).

RL Doses	RB_1	RB_2	RB_3 (After fermentation)				Source of Variation			
			12 h	24h	48 h	72 h	Overall	RL	Hour	RL*H
50% RL	$0.372^{h} \pm 0.009$	$0.473^{g} \pm 0.018$	$0.652^{f} \pm 0.030$	$0.763^{e} \pm 0.013$	$0.871^{d} \pm 0.021$	$0.964^{c} \pm 0.021$	<0.001	<0.001	<0.001	<0.001
100% RL	$0.372^{h} \pm 0.009$	$0.456^{g} \pm 0.012$	$0.735^{e} \pm 0.044$	$0.899^{d} \pm 0.022$	$1.105^{b} \pm 0.038$	$1.202^{a} \pm 0.024$	<0.001	<0.001	<0.001	<0.001

RB_1 = Fresh rice bran; RB_2 = Rice bran with rumen liquor and buffer before fermentation; RB_3 = Rice bran after fermentation at different time interval; RL = Rumen liquor; RL*H = Interaction between rumen liquor and hour; % = percent; $^{a-h}$ = at similar row and columns are statistically significant.

The available phosphorus content of WB is presented in Table 7. The results showed that the percentage of available P increased significantly ($p < 0.05$) with increasing fermentation time. Specifically, the value increased ($p < 0.05$) from 0.285 in fresh bran to 0.853 and 1.077 in fermented bran with 50% and 100% RL, respectively, after 72 h of fermentation. However, RL level, fermentation time, and their interaction had no significant effect on the available phosphorus content of WB. In the present study, increasing fermentation duration resulted in a gradual increase in total phosphorus (organic phosphorus) and a corresponding decrease in phytate phosphorus. Available phosphorus was calculated as the difference between total phosphorus and phytate phosphorus. Previous research has shown that reducing dietary non-phytate phosphorus depresses serum calcium and phosphorus concentrations in broilers, whereas phytase supplementation effectively alleviates the negative effects of phosphorus deficiency (Chowdhury *et al.*, 2022). Consistent with these findings, available P increased

significantly ($p<0.05$) with extended fermentation durations (24 h, 48 h, and 72 h), while phytate phosphorus decreased significantly with increasing fermentation time and moisture level (Islam *et al*., 2022). Phytase enzyme increases the availability of available phosphorus content (DePeters & George, 2014).

Greater phytase activity during fermentation enhanced the conversion of phytate phosphorus to inorganic phosphorus. Although total phosphorus did not increase markedly, the substantial reduction in the phytate fraction resulted in a desirable improvement in phosphorus availability. Comparison across fermentation periods (12 h, 24 h, 48 h, and 72 h) revealed a general improvement in feed quality with increasing fermentation time; however, no significant differences were observed between 48 and 72 h. These results indicate that fermentation for up to 72 h is effective for improving the nutritional quality of RB and WB. The data further demonstrate that fermentation, particularly when combined with rumen liquor, significantly enhances phosphorus availability in RB and WB. Increasing rumen liquor levels and extending fermentation time both contributed to higher available phosphorus, with a significant RL × H interaction indicating a synergistic effect. Overall, these findings highlight fermentation with rumen liquor as a promising strategy to optimize phosphorus utilization in poultry feed, improve nutrient efficiency, and support cost-effective production of meat and eggs.

Table 7. Influence of different hours of fermentation with different doses of rumen liquor available phosphorous content of wheat bran (Available phosphorous; %).

RL Doses	WB_1	WB_2	WB_3 (After fermentation)				Source of Variation			
			12 h	24 h	48 h	72 h	Overall	RL	Hour	RL*H
50% RL	$0.285^{g} \pm 0.009$	$0.396^{f} \pm 0.019$	$0.636^{e} \pm 0.017$	$0.700^{d} \pm 0.020$	$0.807^{c} \pm 0.051$	$0.853^{cd} \pm 0.091$	<0.001	0.487	0.433	0.664
100% RL	$0.285^{g} \pm 0.009$	$0.452^{f} \pm 0.021$	$0.910^{b} \pm 0.173$	$0.963^{b} \pm 0.202$	$1.037^{a} \pm 0.248$	$1.077^{a} \pm 0.248$	<0.001	0.487	0.433	0.664

RB_1 = Fresh rice bran; RB_2 = Rice bran with rumen liquor and buffer before fermentation; RB_3 = Rice bran after fermentation at different time interval; RL = Rumen liquor; RL*H = Interaction between rumen liquor and hour; % = percent; $^{a-g}$ = at similar row and columns are statistically significant.

Conclusion

The primary objective of this study was to enhance the nutritional quality of RB and WB by increasing total and available phosphorus while reducing phytate phosphorus through fermentation with rumen liquor. Fermentation duration significantly affected total phosphorus, phytate phosphorus, and available phosphorus levels of both RB and WB. Extended fermentation time led to a gradual increase in total and available phosphorus, accompanied by a reduction in phytate phosphorus, a major antinutritional factor in poultry nutrition. These results demonstrate that fermentation with rumen liquor is an effective approach for improving the nutritional value of RB and WB by increasing phosphorus availability and decreasing phytate phosphorus content. However, further studies are needed to optimize phosphorus availability for practical application in poultry feeding.

DECLARATIONS

Author contribution statement: Conceptualization: M.R.D. and J.U.K.; Methodology: M.R.D. and J.U.K.; Software and validation: M.R.D., J.U.K., T.D. and B.K.S.; Formal analysis and investigation: M.R.D. and J.U.K.; Resources: M.R.D.; Data curation: M.R.D. and J.U.K.; Writing—original draft preparation: M.R.D. and J.U.K.; Writing—review and editing: M.R.D., J.U.K., B.K.S. and T.D.; Visualization: M.R.D., J.U.K., T.D. and B.K.S.; 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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