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Fermentation Time and Environmental Conditions Changes Nutritional Composition of Fermented Rice and Wheat Bran

Momota Rani Debi[1], Khan Md. Shaiful Islam[1] and Biplob Kumar Saha[2,*]
[1]Department of Animal Nutrition, Bangladesh Agricultural University, Mymensingh-2202
[2]Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh-2202
[*]Correspondance: bksaha@bau.edu.bd

ABSTRACT

The utilization of rice bran (RB) and wheat bran (WB) in broiler is limited due to low protein and high fiber content. Therefore, this study was conducted to increase the nutritive value of these by-products through fermentation with rumen liquor. Rumen microbes are very sensitive to pH for their proper functioning. To achieve these goals, precise fermentation of RB and WB using rumen liquor was conducted in a different environmental condition. Environment 1: fermentation in a hot plate with magnetic stirrer at 39°C; Environment 2: fermentation in an incubator at 39°C; Environment 3: fermentation in a room temperature. The fermentation was performed for different periods from 3 to 72 hours with 3 replications (n=3). The fermentation was initiated with pH 7 by adding some buffer substances. Results showed that pH changed significantly ($p<0.05$) in a different environment as well as with duration of fermentation. After some hours, pH reduced to a level that is harmful for fiber degrading microbes. For nutritional composition, better results were obtained in the case of environment 1 compared to others explaining that this environment was more appropriate for rumen microbes for their proper function. It can be concluded from the results that pH is very sensitive with time of fermentation as well as with different environmental conditions. However, further research is required to verify that the addition of supplementary buffer during fermentation might improve or stable the pH of the fermentation which might produce better fermented product.

KEYWORDS: Fermentation, Poultry nutrition, Rice bran, Rumen microbes, Wheat bran

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INTRODUCTION

Poultry industry plays a dynamic role to fulfil the protein requirements for human consumption and the proficiency of poultry to alter feed into products which is crucial for the profit of poultry industry (Mandal and Khan, 2017; Mottet and Tempio, 2017; Sell-Kubiak et al., 2017). But lack of quality poultry feed and their high prices are the main reasons for low poultry production specially in developing countries like Bangladesh (Korver, 2023). Soybean and maize are the main feed ingredients for protein and energy sources for poultry nutrition in our country. However, in developing countries, humans also compete with poultry for some grains for their daily nutritional requirements e.g., maize, wheat, and soybean (Mottet and Tempio, 2017). Consequently, to minimize feed cost and to overwhelm the scarcity of quality feed, cereal brans are being used as poultry feed in developing countries (Korver, 2023). Among these existing sources, rice and wheat bran are the plentiful agricultural by-products and very cheap in rice and wheat producing countries (Hemery et al., 2007; Supriyati et al., 2015) and can be used as poultry feed (Korver, 2023). Even though these are low-priced and easily available, but high fiber content confines their use as poultry feed. These ingredients have low protein as well as have some anti-nutritive factors e.g.phytic acid, ß-glucans which interfere with other nutrients (Babiker et al., 2009; Arte et al., 2015; Guo et al., 2015). In rice and wheat bran, crude fiber content varies from 13.6 to 15.5% (Babiker et al., 2009) and phytic acid content is 42.8 and 41.7 mg g^{-1}, respectively (Kaur and Sharma, 2011). Among the common animal feed stuffs, rice

bran has the highest level of phytic acid content (Singh, 2008; Kaur and Sharma, 2011). Phytic acid form phytate mineral complexes, changing their solubility and amount of absorption (e.g., calcium and phosphorous) during digestion. Phytic acid also binds with protein and forms strong insoluble phytate protein complexes (Arte et al., 2015; Guo et al., 2015). The poultry is not capable to produce enzymes for the hydrolysis of non-starch polysaccharides and anti-nutritional substances present in brans (Krás et al., 2013; Kang and Kim, 2016) and therefore depress performance by reducing the availability of nutrients to monogastric animals (Selle and Ravindran, 2007; Cowieson and Bedford, 2009; Supriyati et al., 2015; Cowieson et al., 2017). Hesselman and Åman (1986) reported that ß-glucans produce very sticky situations in the small intestine of the poultry that badly distresses the utilization of all other nutrients, particularly starch and protein utilization. So, it is vital to decrease fiber components and improve other nutritional value of rice and wheat brans by any processing methods for enhancing poultry feed quality and sustainable poultry production. In this connection, fermentation with rumen microorganism could be an appropriate technique to reduce fiber content and improve nutritional quality of rice and wheat bran. Rumen microbes are accomplished to produce beta-glucanases, the enzymes essential for the degradation of different fibrolitic and phenolic polymers (Wang and McAllister, 2002). Rumen microorganisms have capability to degrade phytic acid efficiently (Haese et al., 2014; Haese et al., 2017).

Singh et al. (2018) stated that fermentation improved the nutritive value of fibrous animal feedstuffs by decreasing fiber and increasing microbial protein content. Microbial protein is a good quality protein as amino acid composition of microbial protein is similar to milk and meat. Jazi et al. (2017) described that fermentation of cottonseed meal significantly decreased crude fiber (34.73%, from the initial value 12.58 to 8.21%), increased crude protein (7.92%, from 36.34 to 39.22%) and feeding of fermented feed improved growth performance of broilers (Chiang et al., 2010; Tang et al., 2012). Use of precise fibro lytic enzymes is as usual method, but literature survey shows that no systematic studies have been carried out so far to decrease the fiber component of these kinds of brans by fermentation with rumen microbes through providing suitable environment for microbial activity. Moreover, it would also be a new approach to investigate the effect of different environmental conditions and fermentation time on nutritional changes of rice and wheat bran-based poultry feed. Therefore, this experiment was conducted to find out the optimum fermentation conditions and time for improving the nutritive value of rice and wheat bran by using rumen microbes.

MATERIALS AND METHODS

The fermentation experiment and nutrient analysis was performed at the Department of Animal Nutrition and Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh in accordance with the animal welfare law of Bangladesh.

Preparation of buffer and inoculum for fermentation

McDougall buffer (McDougall, 1948) solution (5000 mL) was prepared before conducting fermentation using standard procedures. First, solution A (50 mL) and solution B (4950 mL) were prepared separately then mixed these two solutions together to make the final buffer. Temperature, pH and anaerobic condition of rumen liquor was maintained before and during fermentation. The pH of the rumen liquor ranged from 6.5 - 7.5 on different days with an average pH of 6.9 ± 0.04 and temperature was 37-40°C. Methylene Blue Reduction test (MBRT) was conducted to assess the number of functional microorganisms present in the rumen liquor (Shen et al., 2012).

Table 1. Constituents of McDougall buffer solution (5000 mL)

Solution-A		Solution-B	
Constituents	Amount	Constituents	Amount
NaCl	2.35g	$NaHCO_3$	49.00 g
KCl	2.85 g	Na_2HPO_4	18.625 g
$CaCl_2.2H_2O$	0.27g	Distilled water	4950 mL
$MgCl_2.6H_2O$	0.64g	-	-
Distilled water	50 mL	-	-

Preparation of bran mixture for fermentation

Rice and wheat bran were collected from the commercial market in Bangladesh. In the present study, fermentation of rice bran (RB) and wheat bran (WB) were carried out in a different environmental condition; environment 1: fermentation in a hot plate with magnetic stirrer at 39°C; environment 2: fermentation in an incubator at 39°C; environment 3: fermentation in a room temperature. Fermentation was conducted for the period of 3 to 72 hours for both RB and WB fermentation. The composition mixture of each fermentation was 100 g bran + 100 mL buffer solution + 100 mL rumen liquor in case of three different environmental conditions. All these experiments were carried out with 3 replications (n=3). First, the required amount of buffer solution was mixed with required amount of RB and WB and kept these mixtures in the incubator for one hour to raise the mixture temperature 39°C (Debi et al., 2019; Debi et al., 2022a; Debi et al., 2022b). Then the required amount of rumen liquor was added with the formerly warmed mixture with constant flow of CO_2 gas to maintain aerobic condition for the entire period. Before starting fermentation, the pH of the all-replication mixtures was measured and allowed for fermentation for different durations (3-72 h). After that, pH of the fermented mixtures was measured in case of all time duration and environmental conditions. The flow chart of the fermentation procedure is presented in Fig. 1.

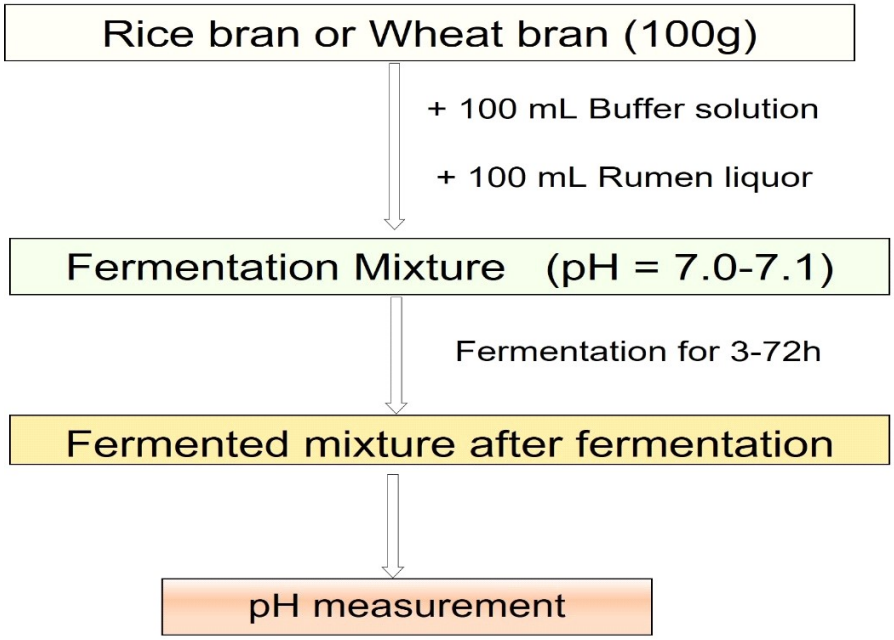

Fig 1. Flow chart of a fermentation process in a different environmental condition pH measurement and nutritional analysis

All samples from 48 h fermentation were analyzed for crude nutrients (proximate analysis) and van Soest fiber fractions (Van Soest et al., 1991). Dry matter (DM) was analyzed from fresh as well as dry fermented samples by drying at 105°C in a compartment dryer for 3 h until weight constancy. Celluloses and hemicelluloses were calculated from the difference of acid detergent fiber (ADF) - acid detergent lignin (ADL) and neutral detergent fiber (NDF) - ADF, respectively (López et al., 2016) to know how much the fiber content decreased during the fermentation process.

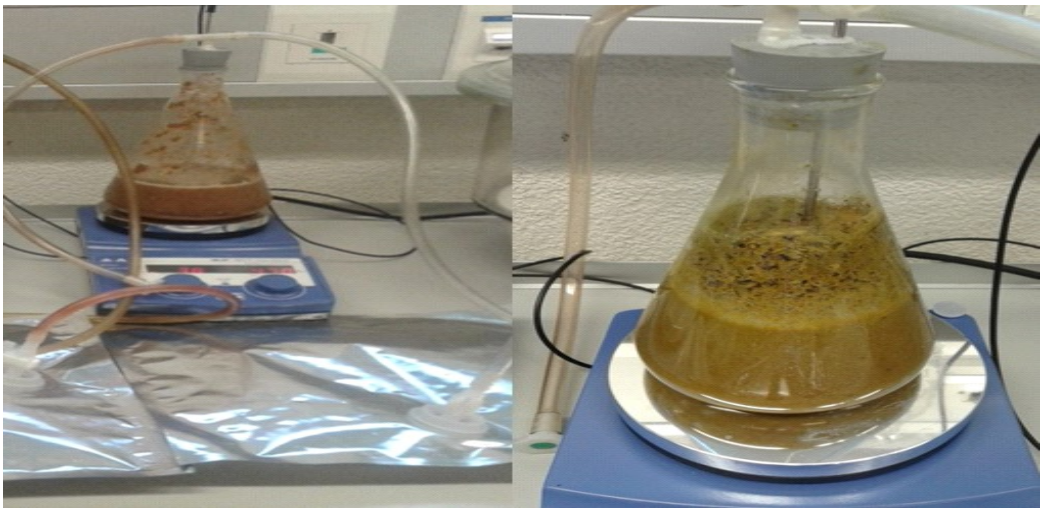

Fig 2. Fermentation of WB and RB in a hot plate with magnetic stirrer at 39°C

Fig 3. Fermentation of WB and RB in an incubator at 39°C

Statistical analysis

Statistical analysis was carried out with IBM SPSS Statistical software package, version 20. All data were analyzed by two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test ($p<0.05$). Factors in the analyses were time duration and different environmental conditions of fermentation. The results were expressed as the mean ± standard error of mean.

RESULTS AND DISCUSSION

pH

All pH measurement data are presented in Table 2 for RB and Table 3 for WB. During fermentation of RB, different pH values were obtained than that of the fermentation of WB. These differences were due to the variation of nutritional composition of WB and RB. Here it was observed that pH significantly ($p< 0.05$) reduced with increasing fermentation time

in case of all the environmental conditions. Optimum pH was maintained up to 24 h and 48 h in case of environment 1 (6.1) and 2 (6.1), respectively. On the other hand, in case of environment 3, optimum pH was maintained for the whole experimental period from starting to 72 h (6.6). In room temperature, here it might be possible to continue fermentation for another some hours as pH was good enough for cellulose degrading bacteria.

In the case of WB, it was observed that pH reduced gradually with increasing the duration of fermentation in case of all the environmental conditions. In these experiments, the starting pH was similar (7.0) in every time duration and decreased with increasing the fermentation time. There was no significant ($p>0.05$) difference was found between environment 1 and 2 during the duration of fermentation. However, pH values from environment 3 are significantly ($p<0.05$) different from environment 1 and 2. In environment 1 and 2, pH reduced significantly after each period of fermentation. In the case of environment 3, pH reduced significantly after 12 h and no significant difference was found from 12 h to 72 h. Optimum pH for fiber degrading rumen microbes (6-7) were maintained up to 6 h in case of environment 1 and 2. However, in case of environment 3, pH was not reduced to as much as that is harmful for these microbes after 72 h of fermentation.

Table 2. The pH values of fermentation of rice bran (RB) in a different environmental condition for different time duration.

pH_ RB	pH before fermentation	pH after 3 h	pH after 6 h	pH after 12 h	pH after 24 h	pH after 48 h	pH after 72 h
Environment 1	7.0±0.00	6.8±0.05^{b}	6.5±0.11^{b}	6.2±0.04^{c}	6.1±0.03^{c}	5.7±0.06^{c}	5.3±0.07^{b}
Environment 2	7.0±0.00	6.8±0.06^{b}	6.7±0.06^{ab}	6.5±0.10^{b}	6.2±0.07^{b}	6.1±0.03^{b}	5.5±0.06^{b}
Environment 3	7.0±0.00	6.9±0.02^{a}	6.8±0.04^{a}	6.7±0.07^{a}	6.7±0.06^{a}	6.6±0.09^{a}	6.6±0.08^{a}

The values in a column with different letters differ significantly at $p< 0.05$ level (Tukey's Honestly Significant Difference).

Table 3. The pH values of fermentation of wheat bran (WB) in a different environmental condition for different time duration.

pH_ WB	pH before fermentation	pH after 3 h	pH after 6 h	pH after 12 h	pH after 24 h	pH after 48 h	pH after 72 h
Environment 1	7.0±0.00	6.6±0.10^{b}	6.1±0.05^{c}	5.6±0.10^{c}	5.4±0.09^{c}	4.8±0.05^{c}	4.1±0.05^{b}
Environment 2	7.0±0.00	6.7±0.08^{ab}	6.3±0.07^{b}	5.8±0.09^{b}	5.6±0.09^{b}	5.1±0.03^{b}	4.2±0.04^{b}
Environment 3	7.0±0.00	6.8±0.02^{a}	6.7±0.06^{a}	6.5±0.20^{a}	6.4±0.10^{a}	6.1±0.04^{a}	6.1±0.06^{a}

The values in a column with different letters differ significantly at $p< 0.05$ level (Tukey's Honestly Significant Difference).

Proximate components of RB and WB after fermentation (48 h)

Nutritional composition of RB and WB used (before fermentation) in this study are presented in Table 4. Nutritional changes after fermentation (48 h) are shown in Table 5 and Table 6 for RB and WB, respectively. In case of crude protein (CP), no significant ($p>0.05$) difference was found among the three environmental conditions for RB and WB. However, CP was little more in case of environment 1 and that was higher compared to unfermented bran (Table 4, 5 and 6). At the same time, there is similar trend results found in case of crude fiber (CF) and no significant ($p>0.05$) difference found among the different environmental conditions. But CF reduced from fresh bran to fermented bran. For the other fiber components, neutral detergent fiber, acid detergent fiber, cellulose, hemi-cellulose also reduced significantly from unfermented bran to fermented bran. There were no significant changes that occurred among the three environmental conditions. However, fiber components reduced more in case of environment 1 compared to environment 2 and 3 for both RB and WB. Other proximate components also changed positively after fermentation that could be beneficial for poultry and other non-ruminant animals.

In the present study, when fermentation time increased, pH gradually reduced, and the optimum pH was maintained up to 12 h of fermentation during all the environmental conditions. Debi et al. (2022b) also reported that pH reduced gradually with increasing duration of fermentation. When fermentation time increased more than 12 h in environment 1 and 2, pH reduced to a level that cellulose degrading bacteria were not capable to survive as well as degradation of fiber components. However, in the case of environment 3, the optimum pH was maintained throughout the entire period of fermentation. At the same time, when fermentation was conducted at room temperature (environment 3), the pH reduced slowly than other two systems that were carried out at 39°C in hot plate and in an incubator. Rumen microbes functioned well at

temperature 37-41°C and unable to survive at temperatures lower or higher than that and which might be the reason for slower fermentation rate in environment 3.

For the fermentation of fibrous components, pH is one of the most important factors (Santra et al., 2003). The normal range of rumen pH is 6-7 and a pH lower than that reduces the degradation of fiber (Mouriño et al., 2001; Russell et al., 2009; Dijkstra et al., 2012) and cellulolytic bacteria are very sensitive to low pH (Russell et al., 2009). In case of live animals, the rumen pH constantly changes and that rely on some aspects e.g., the saliva production, the production and absorption of volatile fatty acids from the rumen wall, extents of feed intake, and the exchange of bicarbonates and phosphates through the ruminal epithelial tissue (Aschenbach et al., 2011). The pH reduced due to rapid fermentation and formation of volatile fatty acids (Dijkstra et al., 2012; Sato, 2016) and sometime lactic acids (Beauchemin and Yang, 2005) through fermentation. Gathering of these acidic components causes detrimental decrease of pH (Chibisa et al., 2016). As volatile fatty acids (VFA) are weak acids, quickly dissociate (Russell and Rychlik, 2001) and release a proton thus reduce pH under most situations. When production of VFA exceeds the ability to neutralize the protons, pH continues to decrease. In case of live animal about 70% of VFA absorbed by rumen wall, some neutralize by salivary buffer, some passage through the liquid to the lower digestive tract (Aschenbach et al., 2009) and these are the major process that control ruminal pH (Dijkstra et al., 2012).

Table 4. Nutritional composition of rice bran (RB) and wheat bran (WB) used in this study (before fermentation for all environment).

Components (%)	Rice Bran	Wheat bran
Dry matter	93.0±0.00	92.0±0.00
Crude protein	14.4±0.07	17.0±0.06
Crude fiber	15.5±0.13	12.8±0.21
Ether extract	12.8±0.09	3.45±0.15
Nitrogen free extract	42.1±0.13	51.02±0.17
Ash	8.11±0.16	7.71±0.09
Neutral detergent fiber	40.3±0.01	48.7±0.16
Acid detergent fiber	18.2±0.08	13.9±0.12
Acid detergent lignin	6.92±0.16	3.99±0.15
Cellulose	11.2±0.18	9.89±0.16
Hemicellulose	22.1±0.26	34.9±0.20

Table 5. Changes of nutritional composition of rice bran (RB) in a different environmental condition (after 48 h fermentation).

Components (%)	Environment 1	Environment 2	Environment 3
Dry matter	91.0±0.00^{b}	93.0±0.00^{ab}	94.0±0.00^{a}
Crude protein	14.9±0.03	14.5±0.12	14.4±0.04
Crude fiber	14.2±0.17^{a}	14.9±0.10^{a}	12.0±0.11^{b}
Ether extract	14.1±0.19^{ab}	14.4±0.05^{a}	13.9±0.01^{b}
Nitrogen free extract	39.5±0.22^{b}	40.6±0.13^{ab}	42.2±0.23^{a}
Ash	8.32±0.10	8.43±0.14	8.51±0.06
Neutral detergent fiber	33.2±0.04^{b}	34.1±0.21^{ab}	34.2±0.01^{a}
Acid detergent fiber	16.4±0.09^{b}	16.6±0.08^{b}	17.2±0.18^{a}
Acid detergent lignin	6.52±0.17	6.65±0.10	6.00±0.11
Cellulose	9.87±0.09^{b}	10.02±0.19^{ab}	11.2±0.03^{a}
Hemicellulose	16.8±0.05^{b}	17.4±0.08^{a}	17.05±0.10^{a}

The mean values with different superscripts within a row differ significantly at $p < 0.05$ level (Tukey's HSD).

In this study, it was necessary to increase fermentation time anyway because longer time means more chances for the microbes to grow and produce useful enzymes to degrade fiber components (Nigam, 2013). It was also observed that pH reduced more in WB compared to the RB in case of all the environmental conditions. These differences were due to the variation of nutritional composition of RB and WB. It might be the reasons that WB contains more soluble carbohydrate and rapid fermentation occurred during fermentation compared to that of the RB.

Crude nutrients from all time duration were not analyzed from this study as it was a method development part and only pH changes were observed and tried to find out the solution to control pH for longer period of fermentation. Therefore, crude nutrients from 48 h fermentation were only analyzed to see what nutritional changes were occurred due to fermentation.

During microbial fermentation, microbes increase in number that improves the protein (microbial protein) quality of feed because microbes mostly consist of protein. Microbial protein is a good quality protein as amino acid composition of microbial protein is similar to milk and meat (Beauchemin and Yang, 2005). In case of ruminant animal, about 90% of their amino acids reaching the small intestine derived from ruminal microorganisms (Babiker et al., 2009). In the present study, CP increased from fresh bran to fermented bran due to the addition of microbes during fermentation and then proliferation of microbes using bran nutrients. Microbial protein was not analyzed but assumed that it increased as fermentation continued in a proper environment for their growth. The CP content was not influenced ($p > 0.05$) by different environmental conditions. Tang et al. (2012) found that after fermentation crude protein content increased explaining that high microbial population resulted in high crude protein content. Our results are consistent with the results of Babiker et al. (2009) when wheat bran fermented with rumen liquor for 3 days. Previous studies from Elanchezhian and Ally (2016), it has been shown that CP increased when maize was replaced with rice and wheat bran as feed of pig. The present results are also consistent with the previous studies of Jazi et al. (2017)) who reported that, fermentation of cotton seed meal with *Bacillus subtilis*, *Aspergillus niger* and *Aspergillus oryzae* for 7 days significantly ($p<0.05$) increased CP content. Conversely, Hardini (2010) stated that fermentation of RB with *Aspergillus niger* has no significant ($p > 0.05$) effect on CP content. In the present study, CP increased very little in amount. Available energy and nitrogen

sources are the major important factors in maximizing microbial protein synthesis. For improving the efficiency of microbial protein production, it is very important to supply necessary nutrients for microbes that can changes the microbial population and improves the environment for proper fermentation. These factors slowed down the fermentation, whereby less nutrients (e.g., energy) were available for the proliferation of microbes during fermentation.

Table 6. Changes of nutritional composition of wheat bran (WB) in a different environmental condition (after 48 h fermentation).

Components (%)	Environment 1	Environment 2	Environment 3
Dry matter	93.00±0.00	93.00±0.00	94.00±0.00
Crude protein	17.80±0.05	17.59±0.14	17.35±0.14
Crude fiber	11.10±0.10^{a}	11.40±0.30^{a}	12.00±0.15^{b}
Ether extract	4.10±0.12	4.07±0.06	4.00±0.31
Nitrogen free extract	52.01±0.20	52.15±0.13	53.14±0.20
Ash	7.99±0.30	7.89±0.11	7.51±0.09
Neutral detergent fiber	32.20±0.24^{b}	34.18±0.20^{ab}	35.22±0.21^{a}
Acid detergent fiber	11.19±0.07^{b}	11.97±0.05^{ab}	12.38±0.10^{a}
Acid detergent lignin	3.92±0.14	3.85±0.20	3.98±0.14
Cellulose	7.27±0.19^{b}	8.12±0.12^{ab}	8.40±0.06^{a}
Hemicellulose	21.01±0.15^{b}	22.21±0.09^{ab}	22.84±0.13^{a}

The mean values with different superscripts within a row differ significantly at $p < 0.05$ level (Tukey's HSD).

Rumen inoculums provide suitable microflora that degrades the fiber component of feed over a period of time during fermentation (Mouriño et al., 2001). Fiber is degraded by a combination of ruminal bacteria, fungi and protozoa (Wang and McAllister, 2002), where approximately 80% of this degradation performed by bacteria and fungi, and 20% by protozoa (Dijkstra et al., 2012). In this study, fiber degraded more in environment 1 compared to 2 and 3. Earlier studies reported that fermentation of rice bran with rumen filtrate increased bioavailability of major nutrients by reducing crude fiber (Singh et al., 2019; Reddy et al., 2024). Jazi et al. (2017)) also reported that fermentation of cotton seed meal with *Bacillus subtilis*, *Aspergillus niger* and *Aspergillus oryzae* for 7 days significantly ($p<0.05$) reduced CF (34.73%, from 12.58 to 8.21%). Crude fiber reduced from 10.62 to 8.37 and 8.36% when RB fermented with *Bacillus amyloliquefaciens* for 3 and 5 days, respectively and length of fermentation showed no significant ($p > 0.05$) differences between 3 and 5 days. Conversely, Supriyati et al. (2015) found that CF increased from 6.25 to 6.89% when WB fermented with rumen liquor for 3 days. In the present study, it was observed that fiber reduced more in WB compared to RB. The reasons for these differences were compositional variation of WB and RB. Firstly, RB contains less CP than WB. Guo et al. (2015) reported that, cellulolytic bacteria increased significantly ($p <0.05$) with increasing level of CP content in the diet whereby increased fiber digestibility. Other studies showed that effective degradability of neutral detergent fiber (NDF) increased ($p = 0.045$) with increasing levels of dietary CP (Debi et al., 2022a). Similar results were also observed by Tang et al. (2012) that NDF digestibility increased with increasing level of protein supplementation. However, Chibisa et al. (2016) reported that, NDF digestibility was not affected significantly with increasing dietary CP levels in beef cattle and dairy cattle, respectively but an increasing trend of NDF degradability were observed. Secondly, RB contains more fat compared to other bran including WB which ranges from 4.07-19.31%

depending on the sources of collection (Kaur and Sharma, 2011). It might be another reason for slow degradation of fiber in RB compared to WB.

CONCLUSION

It can be concluded from the present results that the pH is very sensitive with the duration of fermentation time as well as with different environmental conditions. Additionally, the pH also changed differently with kinds of bran. No remarkable variation was observed in crude protein content in the bran due to fermentation. Considering different environmental conditions, fermentation of wheat and rice bran on hotplate with magnetic stirrer resulted in enhancement of nutritional quality of fermented RB and WB than environment 2 and 3.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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