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Research article

Anaerobic fermentation of rice bran with rumen liquor for reducing their fiber components to use as chicken feed

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ABSTRACT

Rice bran is a very cheap and abundant agricultural by-product in rice producing countries. Additionally, many developing countries used these by products as poultry feed. Rice bran (RB) contains high fiber and chicken is not capable to digest those fibrous feed effectively, resulting in low production performance. The main objectives of this study were to decrease fiber components of RB through fermentation by adding rumen liquor to increase their utilization efficacy in chicken. A single-step fermentation of RB was conducted for 12 h (n = 6) under anaerobic conditions, maintaining proper temperature and ideal pH. Rice bran, buffer substances, and rumen liquor were mixed at the ratio of 1:2:3, respectively. The fresh and fermented rice brans were examined for the content of acid detergent fiber (ADF), crude fiber (CF), dry matter (DM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent lignin (ADL). Other fiber components were determined by substracting the value of ADL from ADF (celluloses) and ADF from NDF (hemicelluloses), respectively. One-way analysis of variance was done to compare the mean nutrient components followed by Tukey's multiple comparison tests at $P < 0.05$. The pH of fermented brans was decreased with growing fermentation period but appropriate pH was maintained due to the developed protocol. After fermentation, the fiber components of RB were reduced significantly ($P < 0.05$). However, CP component was not altered significantly after the fermentation of brans. The NDF, ADF, cellulose, hemicellulose contents were reduced by 16.2 ± 0.52, 7.2 ± 0.32, 20.0 ± 0.38 and 23.6 ± 0.54%, respectively compared to the fresh brans. As the fiber content reduced significantly after fermentation that clearly, increases the usability of brans as chicken feed.

1. Introduction

Bran is the most important milling by-product and outer layer of most cereal grains containing high fiber and anti-nutritional substances (Kaur et al., 2011) that are the major limiting factors for the nutritional value and feed quality. One of the most available brans in the developing countries is the rice bran (RB) because it is a very chief by-product of rice production (Rezaei, 2006). More than 50 % of the world population consumes rice (*Oryza sativa*) as a staple food. Rice is the most produced and consumed grain in the world (Christ Ribeiro et al., 2017) and consequently produce large amount of RB as a by-product. On the other hand, many people of developing countries have been suffering from malnutrition due to the insufficient consumption of essential amino acids (FAO, 2018). In this regard, chicken production plays a major role in mitigating amino acid malnutrition in developing countries as chicken meat is the cheapest meat among all the livestock meats (OECD/FAO, 2018). Additionally, chicken production is considered as one of the most economical and efficient animal protein producing systems due to its short production cycle, high feed conversion efficiency and low production costs (Thirumalaisamy et al., 2016). Usually, corn and soybean meal are used as main ingredients in chicken feed formulation. However, high price and unavailability of these ingredients limit their use and result in the use of less expensive high fiber feed stuffs. Among these fibrous feed, RB was used abundantly as feedstuff for chicken in many developing countries (Ravindran, 2013). Although RB is cheap and available, this by-product has a high fiber content (Gallinger et al., 2004) and contains anti-nutritional substance i.e. β-Glucans (Kaur et al., 2011). Rice bran is composed of insoluble cellulose and hemi-cellulose to a great extent (Shaheen et al., 2015) and only about 5% of soluble fiber. The use of this ingredient in chicken feed is advantageous from a cost standpoint,

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but has detrimental effects on poultry production performance (Supriyati et al., 2015). High fiber (33–40 % NDF) (Tahir et al., 2002) present in RB reduces the digestibility of other nutrients and the chicken is not capable to produce enzymes to digest fiber effectively (Kras et al., 2013; Ghimire et al., 2016). The recommended level of inclusion of RB in broiler nutrition is 10–20 %. However, the level of inclusion of RB as 20 % in broiler feeds reduces growth production and the accumulation of 10 % RB reduces feed efficiency of broilers (Gallinger et al., 2004). Thus, the biological value of RB could be increased by decreasing the fiber content and increasing the protein content and quality. This would make RB a valuable ingredient for chicken feed. In literature, fermentation is indicated to progress the nutritional quality of fibrous animal feedstuffs with additional increasing protein as well as decreasing fiber content (Hardini, 2010; Supriyati et al., 2015). Therefore, using rumen liquor during fermentation might be an appropriate technique to upgrade the nutritional value of RB via decreasing fiber components, which was shown in a previous study (Debi et al., 2019). Rumen microbes can degrade all kinds of fiber components of feed stuffs (Adeyemi and Familade, 2003; Wang and McAllister, 2003).

With all these in mind, our previous study (Debi et al., 2019) investigated the effect of fermentation (two-step) of different brans with addition of rumen microbes on fiber content. It has been shown that the fiber content reduced significantly after 6 h fermentation and found that period of fermentation is a very important factor for the degradation of fiber components. However, fermentation time could not be increased after 6 h in both steps because after 6 h, the pH was too low for the degradation of fiber components in case of wheat bran (WB). Consequently, the study aimed to upsurge the fermentation time only in case of first fermentation step to examine the effect of time on fiber degradation. The hypothesis of this study is that the fiber content will be reduced more with increasing fermentation time.

2. Materials and methods

The fermentation trials and all nutritional components were examined at the Institute of Animal Nutrition laboratory of the Vet Suisse faculty under University of Zurich, Switzerland. All the experiments were performed with following the animal welfare law exixt in Switzerland (Permission no. ZH061/18).

2.1. Procedure of fermentation

McDougall buffer solution was prepared according to the procedure of McDougall (1948). The chemical contents of prepared buffer solution are presented in Table 1. The fermentation process was conducted for 12 h with six replicate samples (n = 6) that were conducted at six different days. Variability of rumen liquor composition was taken into the consideration. That's the reason we have collected rumen liquor at six different days for six replicate samples. The experimental procedure was similar as our previous study (Debi et al., 2019) up to the first fermentation step in a two step fermentation process. Two fermentation step means, 1st fermented dried brans were fermented 2nd time due to desirable fiber reduction was not occurred up to the first step. But single step fermentation is more convenient than two step. That's the reason, in the present study we increased the fermentation time in a single step by improving the environmental condition of the method and found more fiber reduction occurred than the two step fermentation for 6 h.

Table 1. The constituents of prepared McDougall (1948) buffer solution (1 L).

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

First RB (fine particle size nearly 0.16–0.43 mm) and buffer solution (1:2 ratios) was mixed in a fermentation container connected with a plastic bag for collecting gas after fermentation. The fermentation container was kept in an incubator to rise the mixer temperature to 39 °C. After that, rumen liquor was collected before morning feeding from a Brown Swiss cow (cannulated), from the Department for Farm Animals, Vetsuisse Faculty, University of Zurich, Switzerland (Permission no. ZH061/18). The cow was maintained on a hay and concentrate diet during the whole collection period. Rumen liquor was collected in to warmed (39 °C) insulated flask in an anaerobic condition (Weiss et al., 2017). After collection of rumen liquor, the pH, temperature and other physical characteristics (i.e. color, odor, and consistency of the collected rumen liquor) were noted and after that Methylene Blue Reduction test (MBRT) were performed with following the technique of DePeters and George (2015). When all parameters showed the collected liquor was physiological containing with optimum amount of rumen microbes, then the fermentation procedure was started. Proper amount (Debi et al., 2019) of rumen liquor was added to the previously warmed mixture with a continuous supply of CO_2 gas for maintain the anaerobic condition for the fermentation process (Weiss et al., 2017). The mixture was kept for 12 h of fermentation in an appropriate environment for rumen microbes. The general procedure of fermentation of RB with rumen liquor is presented in Figure 1.

2.2. The pH measurement

The pH measurement was done before and after each fermentation through a pH meter (827 pH lab, The Metrohm AG, Herisau, Switzerland). After 3 h of fermentation, an additional $NaHCO_3$ (1% level of the total mixture), were mixed for maintaing an optimum pH during fermentation. It was observed in our previous study (Debi et al., 2019) that the pH value was declined lower than 6.0 after first 3 h. After the addition of $NaHCO_3$, the mixture was kept for another 9 h fermentation. The whole fermentation process was done under anaerobic conditions. After 12 h, the pH of all fermented samples was noated again and a MBRT was also performed.

2.3. Crude nutrient analysis

Crude nutrients were analyzed from different samples that were obtained from different phases of this fermentation procedure: samples are RB1: fresh rice bran; RB2: bran before the fermentation, after the inclusion of rumen microbes and buffer; RB3: fermented bran (dried) after 12 h of fermentation. The fresh and fermented brans were examined for proximate analysis and the van Soest fibers (Van Soest et al., 1991) with the procedure of VDLUFA method book III (Naumann and Bassler, 1997). Dry matter was investigated from the fresh and dry fermented brans in a compartment dryer (Binder FED 53-UL Laboratory compartment dryer) at 105 °C for 3 h until to obtain a constant weight. Each bran samples were examined by two times and mean values were considered. Other fiber components were determined by substracting the value of ADL from ADF (celluloses) and ADF from NDF (hemicelluloses), respectively (Lopez et al., 2016) to know how much of these nutrients were actually reduced during the fermentation process. Percent changes were calculated from original data with the following formula [(Initial value – Final value) ×100]/Initial value (Supriyati et al., 2015).

2.4. Statistical analysis

Data were statistically analyzed by the statistical software package IBM SPSS, version 23 (IBM SPSS Statistics for Windows, 2015; IBM Corp, New York, USA). One-way analysis of variance (ANOVA) was performed to analyze the mean values of all the nutrients followed by the Tukey's

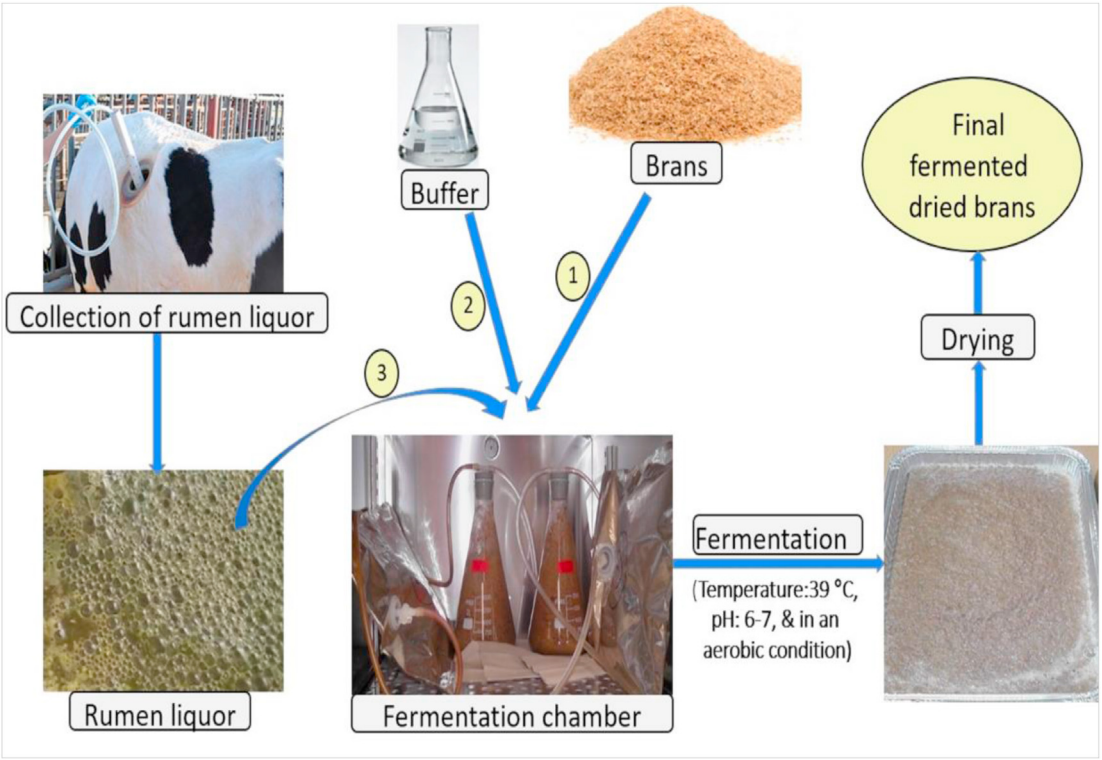

Figure 1. General procedure of fermentation of brans with rumen liquor.

multiple comparison tests ($P < 0.05$). Nutrients and various fermentation stages were considered as factors in the analyses. The pH measurements data were also analyzed similarly. All outcomes are presented in Table and figure as mean ± standard Deviation.

3. Results

3.1. The pH during fermentation

The pH values of fermentation are presented in Figure 2. The fermentation was started at a pH of 6.9 ± 0.02 and significantly ($P < 0.05$) decreased to pH of 6.3 ± 0.85 during the first 3 h of fermentation. During the next 9 h of fermentation, the pH did not decrease as fast as in the first 3 h but however, the pH decreased significantly ($P < 0.05$) at the termination point of the fermentation process but remained within an appropriate range for proper fermentation.

3.2. Crude nutrient content (%) of fresh and fermented brans

3.2.1. Crude protein (CP) content (%)

The CP content of fresh and fermented brans are presented in Figure 3. The CP values of bran was not significantly changed ($P > 0.05$) in the fermented brans in comparison to the fresh brans.

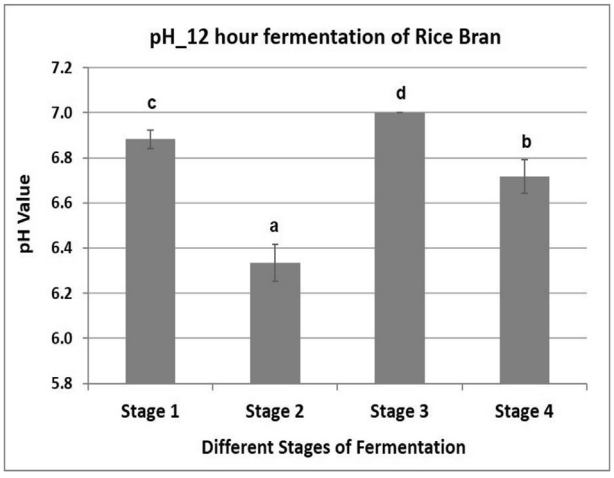

Figure 2. The pH values of brans (RB) fermented adding rumen liquor for 12 h. Stage1: before the fermentation; Stage2: after 3 h fermentation; Stage3: after the addition of $NaHCO_3$; Stage4: after 12 h fermentation. The values with different letters differ significantly at $P < 0.05$ level (Tukey's HSD). Values are Mean ± Standard Deviation; n = 6.

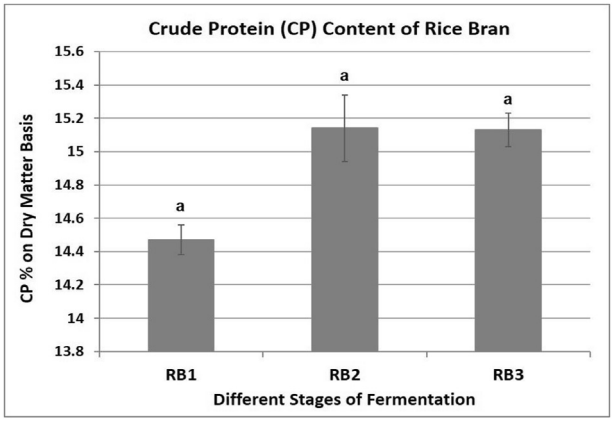

Figure 3. Crude protein (CP) content (%) of brans (RB) fermented adding rumen liquor for 12 h. RB1: fresh RB; RB2: bran before the fermentation after the addition of rumen liquor; RB3: bran after 12 h fermentation. The values with different letters differ significantly at $P < 0.05$ level (Tukey's HSD). Values are Mean ± Standard Deviation; n = 6.

3.2.2. CF, ADF and NDF content (%)

The CF, ADF and NDF content of fresh and fermented brans are presented in Figure 4. The CF and ADF were significantly ($P < 0.05$) reduced after fermentation in comparison to fresh brans. After fermentation, brans contained 12.7 ± 0.41 and 7.2 ± 0.32 % less CF and ADF, respectively than that of fresh brans. Fermentation of RB with rumen liquor played a very significant role in case of NDF content of bran and the NDF content differed ($P < 0.05$) at different stages of this fermentation process. After 12 h of fermentation, the NDF of brans were decreased significantly ($P < 0.05$) and found that the fermented brans contained 16.2 ± 0.52 % less NDF compared to the fresh brans.

3.2.3. The other fiber components cellulose and hemicellulose content (%)

Figure 5 represents the cellulose as well as hemicellulose content of fresh and fermented brans. Both components were reduced significantly ($P < 0.05$) after 12 h of fermentation. Fermented bran contained 20.0 ± 0.38 and 23.6 ± 0.54 % less cellulose and hemicellulose in comparison to fresh bran. Hemicellulose content of RB decreased more than the cellulose content after fermentation of 12 h.

4. Discussion

Nutritional improvement of low quality fibrous by-products (i.e. brans) is very essential to increase the usability of these by products for chicken feed to enable maximum production at minimum price. Fermentation technique is one of the most promising strategies to decreased the fiber components of brans. In this context, fermentation using rumen liquor is an easy and cheap method for nutritional improvement of fibrous feed. In this investigation, a single-step fermentation of RB was conducted taking into account the results of our earlier study (Debi et al., 2019). In this study, fermentation time was increased from 3 h or 6 h, respectively to 12 h in one fermentation step only to investigate the effect of increased fermentation period on fiber components of RB. The outcomes of this study indicated that a longer fermentation time is more effective in reducing the fiber content, what was assumed due to the result of Debi et al. (2019). The fermentation time was not increased further after 12 h because the CP content started to decrease after this time.

The rumen contains a variety of bacterial population, which constitutes the majority of the microorganisms that live in an anaerobic environment with a pH range of 6.0–7.0 (Pitta et al., 2010). To improve the efficiency of rumen microbial activity, it is very crucial to maintain the proper environment during fermentation period. In this aspect, pH is one of the very important factors (Santra et al., 2003). The pH of rumen constantly changes due to fermentation of feed (Russell and Strobel, 1989). In the present experiment, it was found that pH of fermented mixture was constantly changing ($P < 0.05$) with increasing fermentation time. The pH decreased because of the production of different kinds of volatile fatty acids (VFA) and also lactic acids in the process of fermentation (Dijkstra et al., 2012). The findings of this study can be supported by our previous findings (Debi et al., 2019) and the study of Mourino et al. (2001). The pH was decreased rapidly during the first 3 h of fermentation. This can be explained by the microbial fermentation of different carbohydrates producing different organic acids (Aschenbach et al., 2011) that further dissociate and with this reduce the pH and change the microbial ecosystem. It is clear that this also determines the growth of cellulose degrading bacteria and the fermentation rate (Russell and Rychlik, 2001). Mourino et al. (2001) found that, cellulose degradation was at its optimum if the initial pH was 6.8 and cellulose degradation declined with decreasing ruminal pH. Cellulolytic bacteria are not capable to grow at a pH below 6.0 (Santra et al., 2003). A low pH with large period of fermentation time can cause the reduction of fiber degradation (Krause and Oetzel, 2006). To enable an optimal pH for a longer time in the present investigation, additional buffer substance $NaHCO_3$ was added after 3 h of fermentation to increase the pH. Sodium bicarbonate ($NaHCO_3$) has strong buffering capacity to prevent acidic conditions in the fermentation system (Kang and Wanapat, 2013; Santra et al., 2003). After 12 h of fermentation with the addition of $NaHCO_3$, it was observed that, the pH did not decrease as much as during the first 3 h without $NaHCO_3$.

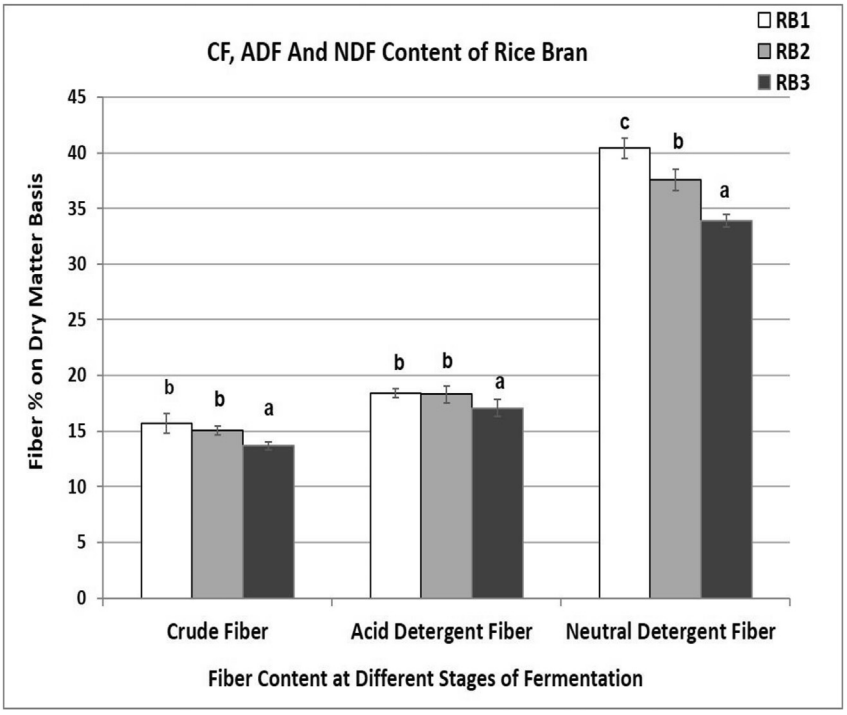

Figure 4. Crude fiber (CF), Acid detergent fiber and Neutral detergent fiber content (%) of brans (RB) fermented adding rumen liquor for 12 h. RB1: fresh RB; RB2: bran before the fermentation after the addition of rumen liquor; RB3: bran after 12 h fermentation. The values with different letters differ significantly at $P < 0.05$ level (Tukey's HSD). Values are Mean ± Standard Deviation; n = 6.

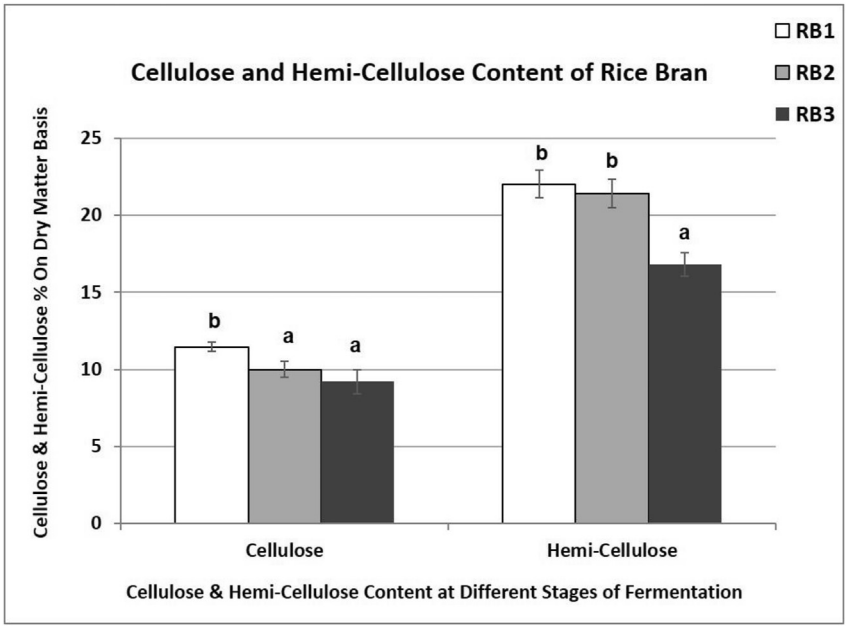

Figure 5. Cellulose and Hemi-Cellulose content (%) of brans (RB) fermented adding rumen liquor for 12 h. RB1: fresh RB; RB2: bran before the fermentation after the addition of rumen fluid; RB3: bran after 12 h fermentation. Cellulose % were calculated from the difference of ADF – ADL. The values with different letters differ significantly at $P < 0.05$ level (Tukey's HSD). Values are Mean ± Standard Deviation; n = 6.

During microbial fermentation, microbes should increase in number as this would improve the protein (microbial protein) quality of feed. Rumen microorganisms provide high quality microbial protein to the small intestine, allowing ruminants to survive and even reproduce under poorer nutritional conditions compared to non-ruminant animals (Fessenden, 2016). In this study, the CP of brans were significantly ($P < 0.05$) increased in RB2 due to the addition of rumen microbes. However, during fermentation, the CP content decreased again. Furthermore, as long as no additional N was added to the fermentation mixture, an increase in CP could not be assumed. In the same way Hardini (2010) reported that the fermentation of RB with *Aspergillus niger* found nearly no significant ($P > 0.05$) consequence on CP content. Conversely, Supriyati et al. (2015) stated that, the CP content (%) of brans increased from 12.1% to 13.4% when RB fermented with cellulolytic bacteria and humic substances. However, this could be explained by the additional humic substances. Although in the present study the amino acid profile was not analyzed, it can be assumed that the fermentation of brans could also have a positive effect on protein quality. To improve the fermentation method to get a valuable component for poultry feed from brans, an addition of nitrogen should be considered.

In the present investigation, the fiber content of RB was reduced more than the both 1st and 2nd fermented RB of our previous study (Debi et al., 2019) (Table 2). This indicates that, microbes in the fermentation process in a longer fermentation time at proper environment led to a higher fiber degradation. Fiber digesting bacteria in the rumen are anaerobic in nature and become less and less active when the pH falls below 6.0 and thereby reduces the extent of fiber degradation (Hu and Murphy, 2005). For this reason, anaerobic conditions, appropriate temperature and an optimal pH have to be maintained during the entire period of fermentation for better fiber degradation. As poultry cannot break down cellulose (Ghimire et al., 2016), such fermented RB with reduced fiber could be a useful ingredient in poultry feed.

However, after fermentation, some amino acids might used by microbes is the limitation of this technique. In future, this technique could be adjusted by improving the nutritional quality specially the changes of amino acid, fatty acid profiles and anti-nutritional substances should be investigated before and after fermentation. Furthermore, this technique could be implemented for a commercial use by feeding broiler and layer to produce meat and eggs with reasonable charge.

5. Conclusions

The overall results suggest that the quality of the RB were improved substantially by reducing the fiber components through fermentation

Table 2. Comparison of different fiber components of rice bran (RB) between two-step fermentation for 3 h, 6 h and single-step fermentation for 12 h.

Nutrients (%)	3 h (two-step fermentation)		6 h (two-step fermentation)		12 h (Single-step fermentation)
	Fresh bran to 1st fermented bran	Fresh bran to 2nd fermented bran	Fresh bran to 1st fermented bran	Fresh bran to 2nd fermented bran	Fresh bran to 1st fermented bran
CF	5.9 ± 0.15 % ↑	0.5 ± 0.26 % ↑	1.3 ± 0.18% ↓	4.1 ± 0.23% ↓	12.7 ± 0.41% ↓
ADF	3.2 ± 0.43 % ↓	8.9 ± 0.70 % ↓	8.8 ± 0.33% ↓	6.9 ± 0.29% ↓	7.2 ± 0.32% ↓
NDF	3.8 ± 0.73 % ↓	7.1 ± 0.91 % ↓	4.6 ± 0.58% ↓	11.8 ± 0.78% ↓	16.2 ± 0.52% ↓
Cellulose	8.6 ± 2.74 % ↓	14.6 ± 3.31 % ↓	15.4 ± 1.76% ↓	15.8 ± 2.20% ↓	20.0 ± 0.38% ↓
Hemicellulose	3.9 ± 3.87 % ↓	5.0 ± 3.90 % ↓	0.5 ± 1.89% ↓	15.9 ± 3.53% ↓	23.64 ± 0.54% ↓

Percentage of nutrient changes are calculated from the original data. Data of 3 h and 6 h fermentation are obtained from our previous study (Debi et al., 2019) except CP. ↑ = Value Increased, ↓ = Value Decreased. Values are Mean ± Standard error of mean; n = 6.

with rumen liquor for 12 h. However, no significant effect of fermentation on CP content in fermented RB was observed. Although, the fermentation method used in this study could improve the nutritional value of brans to use as chicken feed, a further improvement of this method would be necessary to generate an appropriate protein content and quality for this purpose also. This would help to provide better nutrition of chicken which might improve the production performance. This would be a substantial contribution to food security and human nutrition in developing countries.

Declarations

Author contribution statement

Momota Rani Debi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Brigitta A Wichert; Annette Liesegang: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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