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Research article

Effect of a two-step fermentation method with rumen liquor on protein quality of wheat bran and rice bran to use as poultry feed

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ABSTRACT

The availability of high quality protein rich feed in many developing countries is limited as well as expensive. Low-quality agro-industrial by-products, i.e., rice bran (RB) and wheat bran (WB), are therefore used as poultry feed irrespective of their low protein content. The main objective of the present study was to improve the protein content and the amino acid profiles of these by-products through rumen liquor mixed fermentation process. A two-step fermentation of some agricultural by-products (e.g., WB and RB) was performed in a controlled environment for 3 h and 6 h. In the 1st and 2nd steps, feedstuff (brans), McDougall buffer as well as collected rumen liquor were mixed with following the proportion of 1:2:3, respectively. After fermentation, brans were dried at 100 °C in an oven. Dried sample were used to analyze the crude protein (CP) as well as amino acid (AA) content. In 1st and 2nd fermentation of the WB, CP content increased 3.3 ± 0.2% (3 h), 4.3 ± 0.2% (6 h) and 7.7 ± 0.1% (3 h), 8.5 ± 0.1% (6 h), respectively compared to control. On the other hand, RB protein content increased by 3.3 ± 0.1% (3 h), 0.8 ± 0.1% (6 h) and 7.3 ± 0.3% (3 h), 4.0 ± 0.1% (6 h) in the 1st and 2nd fermentation step, respectively compared to control. Majority of the AA increased compared to control during the 1st fermentation step for RB and WB. However, In WB, some of the AA did not show significant difference. A number of AA were decreased after the 2nd step for both RB and WB except Methionine, which increased in both steps. In 1st and 2nd steps, Methionine increased by 24.9 ± 5.1% (3 h), 25.9 ± 5.8% (6 h) for WB and 12.2 ± 3.2% (3 h), 13.0 ± 4.5% (6 h) for RB, respectively compared to control. In conclusion brans protein and amino acid quality optimization might be possible through methodical rumen liquor mixed fermentation process for better utilization as poultry diet.

1. Introduction

Proteinous food products like meat, egg and milk is necessary in order to fulfill the protein requirements of overly growing global population. Poultry plays a significant role to produce two of the major food products (e.g., meat and eggs) that are acceptable globally irrespective of cultural or religious variation. In addition, high feed conversion efficiency of poultry compared to ruminants (Mottet et al., 2017) make them suitable components for rapid protein production. In developing countries, the main constraints of poultry production are the unavailability of quality poultry or expensive feed that farmers cannot afford (Ravindran, 2013). Therefore, it is highly challenging to the farmer to maximize production with the provision of low quality agricultural by products like rice bran (RB) or wheat bran (WB). The WB and RB are low in protein. Further, the essential amino acids like Methionine and Lysine is also limited in WB and RB. It is important to note that poultry's corn and soybean meal-based diets required further addition of methionine and lysine supplementation (Cemin et al., 2017; Vieira et al., 2004). The commonly used protein rich ingredients in poultry diets are soybean meal, other oilseed meal, cereal by-products (i.e., brans), some re-cycled animal by-products (i.e., fishmeal, feather meal) and synthetic amino acids. Previous research suggested that, if soybean products is added to poultry diet high as much as 81% poultry will be considered as direct competitor to human for soybean globally (Mottet and Tempio, 2017). To reduce feed costs, other agricultural by-products like different brans are used as components of poultry feed. However, the presence of low quality protein and high content of fiber in the agricultural by-products like WB and RB may not

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fulfill the requirement to become a suitable alternatives of high quality protein feed substance like as soybean meal (Kras et al., 2013). Some agro-industrial by-products feedstuff are good in protein, however, their lacking in essential AA also create hindrance for their sufficient addition in poultry diets.

The methodological fermentation process with using rumen liquor could be a suitable technique for improving the quality of by-products in respect to protein contents. The rumen microbes are capable of degrading fiber easily and produce single cell protein (Hackmann and Firkins, 2015; Boguhn et al., 2006a) that may increase the protein quality of brans. The microbial population in the rumen of live animal can transform non-protein N (NPN) components into high quality protein in presence of adequate soluble energy (Hackmann and Firkins 2015). Previous study of Debi et al. (2019) and (2022) demonstrates that the fermentation of brans with rumen liquor reduced the fiber content significantly. This research hypothesized that using rumen liquor during fermentation will improve the protein content of RB and WB due to addition of microbial protein. Further, the AA profiles of the fermented brans including limiting AA, which are essential for poultry, will be improved. It is also assumed that microbial protein present in fermented RB and WB will be a good source of quality protein for poultry nutrition. Considering these facts, our research aims to observe the effect of rumen liquor used fermentation process on low-quality fiber containing RB and WB protein value's.

2. Materials and methods

A two-step fermentation process was conducted under the research laboratory of the Institute of Animal Nutrition, Vetsuisse Faculty, University of Zurich, Switzerland. The rumen liquor was taken conferring to the animal welfare rules of Switzerland (Approval no. ZH061/18). The AA profiles were measured at the research laboratory of the Institute of Animal Physiology and Animal Nutrition, University of Rostock, Germany.

2.1. Fermentation of wheat bran (WB) and rice bran (RB)

A two-step fermentation was conducted for 3 h and 6 h, according to protocol described by Debi et al. (2019), where a 2nd fermentation process on 1st fermented dried WB and RB was performed. Rumen liquor

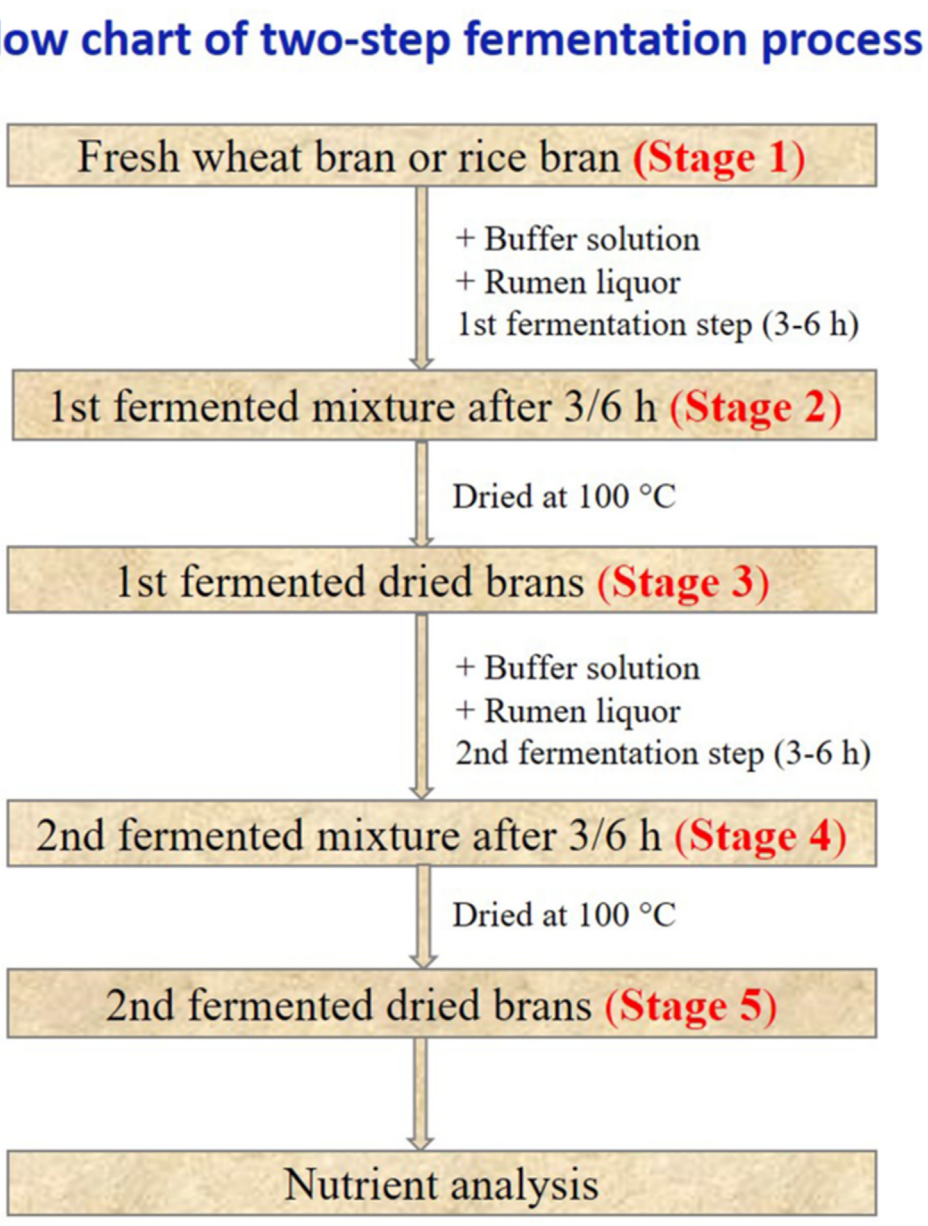

Figure 1. Flow chart of the two-step fermentation process using rumen liquor.

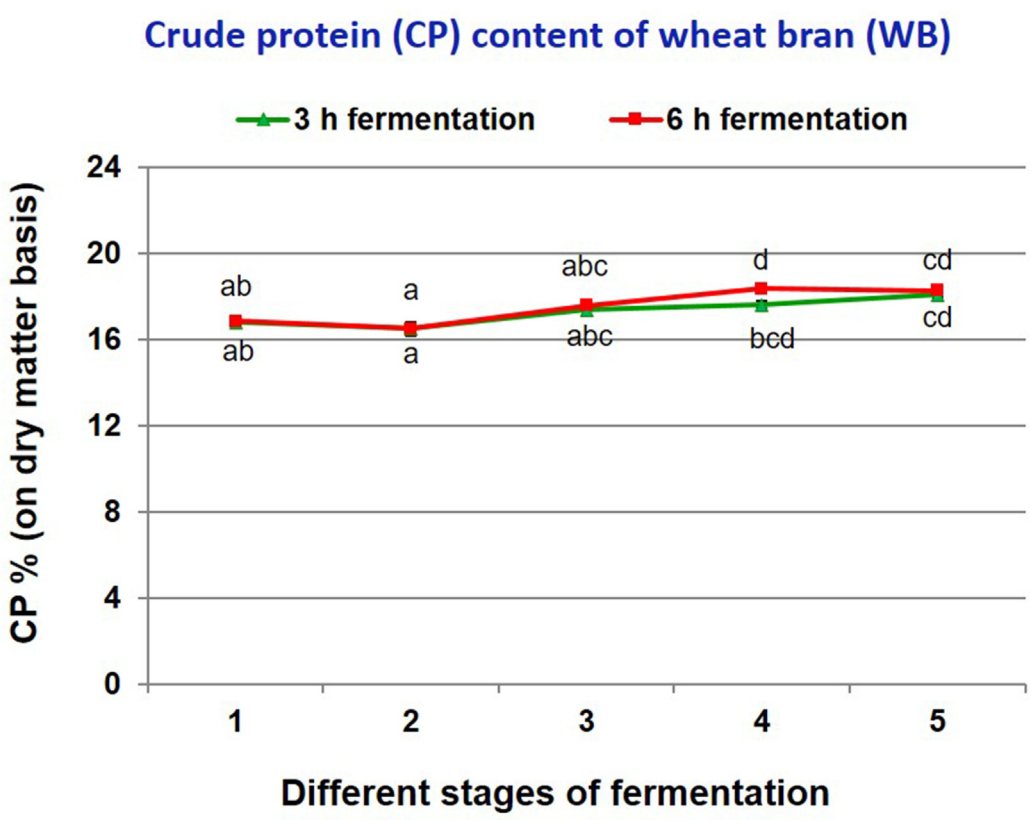

Figure 2. Crude protein (CP) content (%) of fermented and unfermented wheat brans (WB). Stage 1: Fresh WB; Stage 2: WB + rumen liquor + buffer (before 1st fermentation step); Stage 3: 1st fermented dried WB; Stage 4: 1st fermented dried WB + rumen liquor + buffer (before 2nd fermentation step); Stage 5: 2nd fermented dried WB. Values with different letters differ significantly ($p < 0.05$). Values indicated Mean ± SEM; $n = 6$.

was obtained from a cannulated Broun Swiss cow under the Department of Farm Animals, Vetsuisse Faculty, University of Zurich, Switzerland. The nutrition of the cow was maintained with grass (silage and hay) and concentrate according to the requirements during the whole collection period in order to ensure adequate amount of microbes in the rumen liquor for rapid proliferation in the low quality fibrous brans during fermentation. Rumen liquor was taken in the morning time before giving feed in a wormed (39 °C) flask with proper insulation and maintained an anaerobic condition continuously providing CO_2 gas. Simultaneously, pH, temperature and physical characteristics were recorded immediately after collection. After that, a reduction test with methylene blue (MBRT) was performed to evaluate the total live bacterial count of the collected liquor conferring to the method stated by DePeters and George (2015). After satisfactory result of maximum microbial count in the liquor, further fermentation process was activated.

First, a two-step fermentation was conducted for 3 h in a glass container close-fitting with a plastic bag for collection of gas during fermentation and temperature was maintained with 39 °C. After 1st step fermentation, brans were dried, and fermentation was done again with the 1st 3 h fermented dried brans. The process was done in order to resolve the pH fluctuation. After 1st step, pH was reduced to a level that is harmful for rumen microbes for their activity (Debi et al., 2019). Additionally, only in the first step, fermentation time was two shorts for microbial activity. Therefore, 2nd fermentation step was performed for increasing the fermentation time to degrade fiber components as well as to produce microbial protein. In the 1st and 2nd step, feed stuffs (WB and RB), McDougall buffer (McDougall EI, 1948) as well as collected rumen liquor were mixed with the proportion of 1:2:3, respectively. The buffer solution was used to control the pH within a suitable range for the rumen micro-organisms. The duration of fermentation in case of 1st and 2nd step was identical and an adequate temperature e.g., 39 °C, proper pH (6–7) and anaerobic environment was maintained throughout the experimental period. All the fermentation steps were performed six times ($n = 6$) with 6 different collection days. After both fermentation step, the fermented liquid brans were placed in a dryer for drying at 100°. Another two-step fermentation protocol was conducted with WB for 6 h, where a second buffer substance ($NaHCO_3$) was added. Otherwise, every other steps of WB fermentation process for 3 h is similar to RB fermentation process. Figure 1 showed the flow chart of overall fermentation procedure.

2.2. Nutrient analysis

The fresh and fermented dried WB and RB were investigated for the crude protein (CP) content ($n = 6$) in duplicates of each sample. The dry matter (DM) content was measured with drying at 105 °C until persistent DM weight was accomplished. The CP was measured according to the VDLUFA method book III by Kjeldahl method (Naumann and Bassler, 1997). The AA profiles ($n = 5$) of fresh and fermented brans were analyzed from 6 h fermentation only. The AA profiles of fresh and fermented WB and RB were measured by high performance liquid chromatography (Modular HPLC, Shimadzu, Kyoto, Japan). Changes in nutrient contents are given as percent.

2.3. Statistical analysis

For statistical analyses of the data, IBM SPSS, version 23 (IBM SPSS Statistics for Windows 2015, IBM Corp, New York, USA) was used. A two-way analysis of variance (ANOVA) test with following Tukey's multiple comparison tests ($p < 0.05$) were used to analyze the CP content where "fermentation time" was considered as factor 1 and "fermentation stages" considered as factor 2. Further a one-way ANOVA was used to analysis the changes in AA profiles, where fermentation time (6 h) and different stages of fermentation was considered as factor. The results are presented as Mean ± Standard error of mean (SEM).

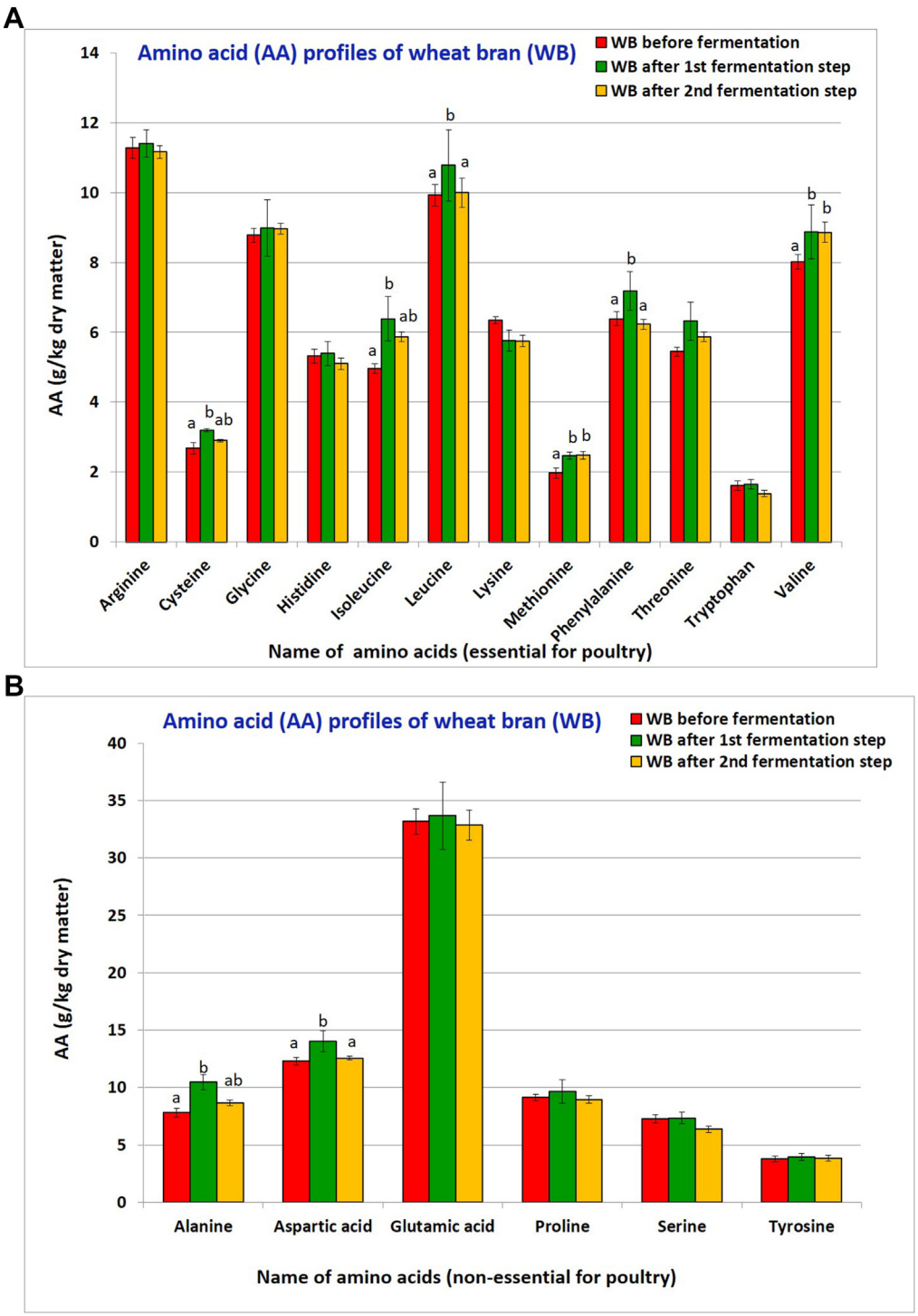

Figure 3. Amino acid profiles of fermented and unfermented wheat brans (WB). Figure 3A and 3B for essential and non-essential amino acid for poultry, respectively. Values with different letters differ significantly ($p < 0.05$). Values indicated Mean ± SEM; n = 6.

3. Results

3.1. CP% of WB (on DM basis)

The detailed results are presented in Figure 2. There it was found that the CP content was improved gradually from unfermented WB to 1st and 2nd fermented WB in both 3 h and 6 h fermentation period. The CP content (%) of WB in 2nd fermentation step was significantly higher than that of fresh WB for both 3 h and 6 h ($p < 0.05$), respectively. Whereas, in the 1st fermented WB, the CP content did not differ significantly from the fresh WB. However, an increasing trend was observed in case of both fermentation periods without any significant ($p > 0.05$) difference between two-time duration (3 h and 6 h).

3.2. AA profiles of WB (g/kg DM)

The AA profiles of the brans are shown in Figure 3A and 3B for essential as well as non-essential AA for poultry. In the 1st fermentation step, the amount of Cysteine, Isoleucine, Leucine, Methionine, Phenylalanine, Threonine, Valine, Alanine and Aspartic acid was increased ($p <$

0.05) and there was an increasing trend ($p > 0.05$) found for other AA (Arginine, Glycine, Histidine, Tryptophan, Glutamic acid, Proline, Serine, Tyrosine). On the contrary, a decreasing trend ($p > 0.05$) was found in case of Lysine in case of the 1st fermentation step. During the 2nd step fermentation, the Methionine and Valine content increased further ($p = 0.035$) compared to the fresh WB. The remaining AA were not changed ($p > 0.05$) significantly afterwards the 2nd step fermentation compared to the fresh WB. However, the amount of Cysteine, Isoleucine, Leucine, Threonine, Alanine, Aspartic acid, and Tryptophan was slightly higher ($p > 0.05$) in the 2nd fermented WB in contrast to the fresh WB. Methionine, the first limiting AA for poultry increased 24.9 ± 5.1% and 25.9 ± 5.8% in the 1st and 2nd fermented WB, respectively compared to the fresh WB.

3.3. CP% of RB (on DM basis)

The amount of CP in RB was gradually increased in the 1st and 2nd fermented RB than the fresh RB in both 3 h and 6 h fermentation period (Figure 4). No significant difference was found between fresh RB and 1st fermented RB, only a slight increase was found in the case of 3 h fermentation ($p > 0.05$). However, the CP content of the 2nd fermented bran was significantly ($p = 0.000$) higher in comparison to the fresh RB during 3 h. In case of 6 h fermentation, only an increasing trend of CP content was observed after the 2nd fermentation step ($p > 0.05$).

3.4. AA profiles of RB (g/kg DM)

The AA profiles were also changed significantly due to fermentation of RB with rumen liquor (Figure 5A and 5B). During the 1st fermentation step, the content of Isoleucine, Leucine, Methionine and Aspartic acid was significantly ($p < 0.05$) increased. In addition, no AA were decreased ($p > 0.05$) afterwards the 1st step fermentation and only a decreasing trend was found for Arginine, Cysteine, Histidine, Lysine and Alanine. Moreover, an increasing trend was observed for the remaining AA (Glycine, Phenylalanine, Threonine, Tryptophan, Valine, Glutamic acid, Proline, Serine, and Tryptophan) after the 1st step fermentation. After the 2nd step fermentation, some AA (Isoleucine, Leucine, Methionine, Phenylalanine and Aspartic acid) were improved ($p < 0.05$) in comparison to the fresh RB. For the remaining AA, no significant changes were observed between 1st and 2nd fermented RB. However, an increasing trend ($p > 0.05$) of AA was observed for Threonine, Valine, Glutamic acid, Proline, Serine, and Tyrosine but other AA (Cysteine, Glycine, Histidine, Tryptophan and Alanine) tended to decrease after the 2nd fermentation step. The content of Methionine increased 12.2 ± 3.2% and 13.0 ± 4.5% in the 1st and 2nd fermented RB, respectively compared to the fresh RB.

4. Discussion

Developing countries urgently need to enhance livestock production to mitigate protein malnutrition and to expand animal protein supplies for the growing population. Poultry production plays a significant role because poultry products are acceptable foods in many cultures, irrespective of religious issues. Therefore, ensuring a constant supply of good quality protein feedstuffs is likely to be the highest priority in poultry nutrition to maximize production. However, low-quality fibrous feed ingredients are used to reduce feed costs for poultry production in developing countries. This leads to low production performance. In this regard, the new fermentation method using rumen liquor containing microbes is suitable to improve the quality of the low-quality by-products. In the previous findings of Debi et al. (2019) and (2022) was illustrated that this fermentation technique with rumen liquor significantly reduced the fiber content of WB and RB for use in poultry feed. The present study evaluated the protein content and quality of fermented brans. The rumen microorganism can alter the non-protein N compounds into high-quality protein in the presence of sufficient soluble energy (Hackmann and Firkins, 2015). Furthermore, adding microbes is Advantageous in the fact that microbes themselves are a protein component that further improves the protein content and quality of the fermented brans.

In the present study, the CP content was increased in the 1st and 2nd fermented brans in response to the addition of rumen liquor. The CP

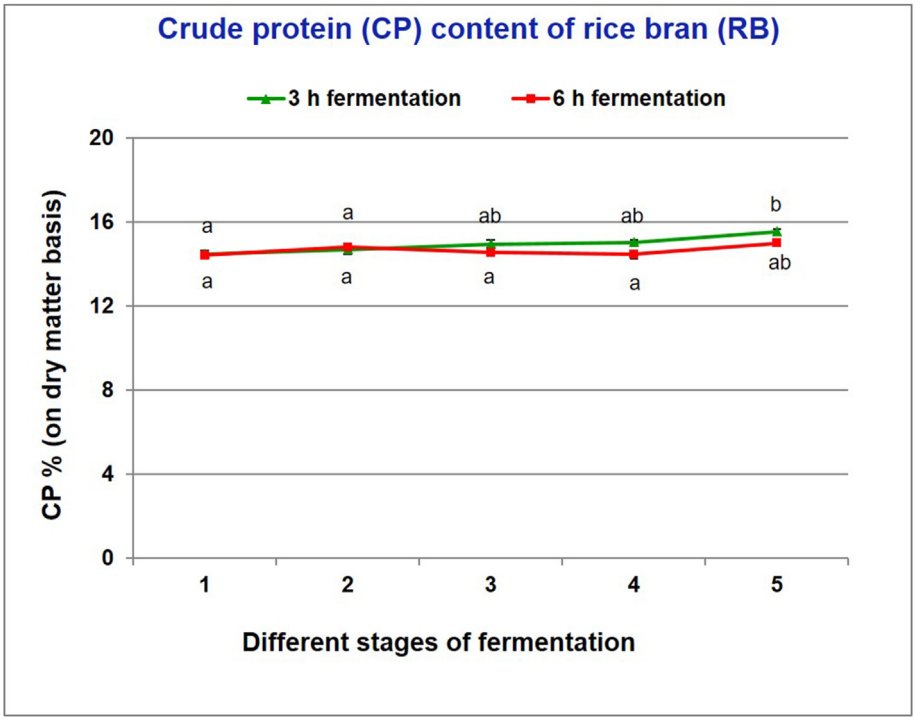

Figure 4. Crude protein (CP) content (%) of fermented and unfermented rice brans (RB). Stage 1: Fresh RB; Stage 2: RB + rumen liquor + buffer (before 1st fermentation step); Stage 3: 1st fermented dried RB; Stage 4: 1st fermented dried RB + rumen liquor + buffer (before 2nd fermentation step) Stage 5: 2nd fermented dried RB. Values with different letters differ significantly ($p < 0.05$). Values indicated Mean ± SEM; $n = 6$.

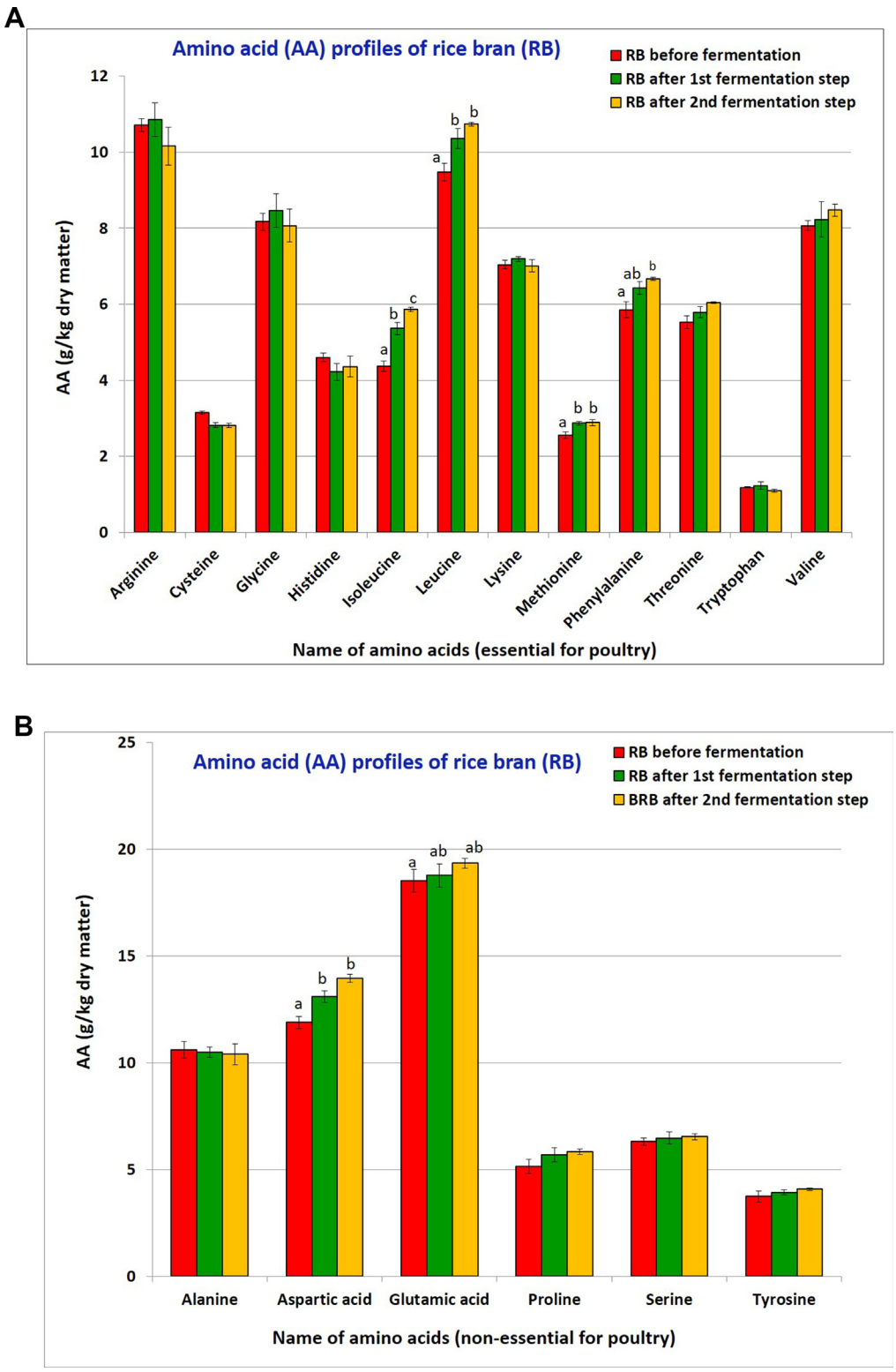

Figure 5. A and B) Amino acid profiles of fermented and unfermented rice brans (RB). Figure 5A and 5B for essential and non-essential amino acid for poultry, respectively. Values with different letters differ significantly ($p < 0.05$). Values indicated Mean ± SEM; n = 6.

content was increased 7.7% (3 h), 8.5% (6 h) in WB and 7.3% (3 h), 4.0% (6 h) in RB in the 2nd fermentation step. The present results are similar with the findings of Supriyati et al. (2015). In their study, the CP content (%) increased from 12.1% to 13.4% when RB was fermented with humic substances and cellulolytic bacteria. Similarly, other studies observed that fermentation of cotton seed meal by adding a proper amount of *Bacillus subtilis*, *Aspergillus niger* and *Aspergillus oryzae* for the period of 7 days increased the CP content (7.9%, from 36.34 to 39.22%) significantly ($p < 0.05$) (Jazi et al., 2017). Another study found that the CP content was increased by approximately 3% in the fermented feed for poultry (Engberg et al., 2009). In this study, the content of CP of the 2nd fermented bran was significantly higher for both 3 h and 6 h of fermentation

in WB and only for 3 h in the case of RB fermentation than fresh bran. This slight variation of reaction of the CP content of fermented WB and RB is might be the nutritional variation of WB and RB and collected rumen liquor used. The CP content is increased due to the addition of some nitrogen-containing substances (rumen liquor-containing microbes) in the present investigation. The CP content of the diet significantly affects broilers' body weight gain (Law et al., 2018). The total AA (or true protein) fractions in bacteria represented 82.4% of CP (Sok et al., 2017). As microbial proteins are high-quality proteins for ruminants, fermented brans with microbial protein are also assumed to be a good source of quality protein for poultry nutrition. Therefore, incorporating fermented brans might improve the production performance of poultry.

Microbial protein is high in quality, and their AA composition is relatively persistent (Korhonen et al., 2002). In the present investigation, some AA contents were significantly higher in the 1st and 2nd fermented brans than the fresh ones. The rumen microorganisms (bacteria, protozoa, fungi, and archaea) are multiplied in the rumen and move to the lower part abomasum, where these microbes symbolize a substantial quantity of AA for the animals. In the present *in-vitro* fermentation of brans using rumen microbes, microbial protein could be produced like in the rumen, which further could improve the AA profiles of the fermented brans.

In total, only a slight increase of AA was observed in the brans after fermentation. This alone will not be sufficient as the only protein source for poultry feed, as the required protein content and amount of essential AA will not be covered by the protein and AA content of the fermented brans produced in this study. However, the microbial growth and the increase of CP and AA be governed by the accessibility of soluble sugar and nitrogen content of the diet that is obtainable during fermentation (Bach et al., 2005; Boguhn et al., 2006b). In this context, another study stated that adding of urea improved the proficiency of microbial protein production ($p < 0.05$) (Currier et al., 2004; Devant et al., 2001). However, in our fermentation system, additional nutrients were not added. This could be the reason for the slight differences in the AA profile of the fermented brans.

In the same way, also the composition of AA varies in bacterial protein depending on the microorganisms and the diet with its nutritional composition (Ellison et al., 2017). The amount of nitrogen and the structure of the protein present in brans are key factors for rumen microorganisms to produce ammonia nitrogen for the microbial protein synthesis (Bach et al., 2005). Therefore, it seems necessary to improve the method with the addition of missing nutrients to obtain a higher CP content and improved protein quality in the fermented brans.

Some AA were decreased or tented to decrease after fermentation, particularly after the 2nd step. In the case of ruminant, deamination of AA in the rumen leads to the loss of NH_3 across the ruminal wall, negatively affecting the AA profile of microbial protein (Leng and Nolan, 1984). Previous studies described that microbial AA production was affected negatively in the case of missing fermentable carbohydrates (Zhu et al., 2013) or low nitrogen content (Molina-Alcaide et al., 2009). The AA from feed could be assimilated into microbial protein or deaminated to volatile fatty acid, CO_2 and NH_3. In the 2nd fermentation step, the availability of soluble energy could have been reduced. This may have caused a reduction in some of the AAs' amount after the 2nd fermentation step. During the fermentation process, few important nutrients, i.e., free AA, may work as a substrate during the microbial fermentation process and, therefore some AA might not be available in the fermented brans (Canibe et al., 2007). Bach et al. (2005) also reported that many kinds of microorganism utilize feed AA and sugar as energy sources for their activities and multiplication, which could be other reasons for decreasing some AA after fermentation. In the present fermentation system, a decreasing trend was found for Arginine and Lysine during fermentation of both WB and RB. However, there were no significant changes observed between this two AA. Robinsona et al. (2006) described that a substantial proportion of Lysine is quickly converted to ammonia by the rumen microorganism. In this connection, Engberg et al. (2009) noted that the concentration of Lysine (g/kg protein) was reduced by 6% in the fermented feed ($p < 0.01$). However, Lysine is the most required AA for poultry and is used as a reference AA. Therefore, all needed compounds to produce Lysine must be available during the fermentation process.

Another point is that the amount of essential AA was much higher in the rumen digesta than in the microbial fractions in rumen liquor (Wang et al., 2018). Therefore, it could be useful to change the use of rumen liquor to rumen digesta in the fermentation of brans. In the present study, the changes in AA profiles were different between fermentation of WB and RB. The fiber, soluble energy and nitrogen content differ between the two brans. It can be assumed that the degradability of protein by rumen microbes also differs between WB and RB and that this could be the reason for these variations.

5. Conclusions

The percent of CP was increased in the fermented brans compared to the fresh brans. However, this increase was minimal compared to the CP requirement of poultry. Additionally, the content of AA (except a few AA) was increased up to the 1st fermentation step. Moreover, Methionine, the first limiting AA for poultry, was little increased in the 1st and 2nd fermented brans compared to the fresh brans for both WB and RB. Only if a higher increase of these AAs could be generated, this might expand the nutritive quality of the brans to be used in the poultry diet as a replacement for protein source like soybean meal. Therefore, a further improvement of the fermentation method is necessary. Methionine is regarded as the first limiting AA in most commercial diets for practical poultry. So it would be advantageous to incorporate fermented brans with a higher protein and Methionine content into poultry feed. This might increase the profitability of the poultry industry. In the present study, it was not possible to quantity the kinds and number of microbes present before and after fermentation, this is the limitation of this study. However, the developed fermentation method is only a first step in this direction and has to be further improved to have a significant effect for use as poultry feed.

Declarations

Author contribution statement

Momota Rani Debi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

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

Petra Wolf: Analyzed and interpreted the data.

Annette Liesegang: Conceived and designed the experiments; Analyzed and interpreted the data; 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 interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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