Pak. J. Agri. Sci., Vol. 57(6),1629-1636; 2020
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/20.419.
http://www.pakjas.com.pk

PROBIOTICS-BASED FERMENTATION ENHANCED THE PROTEIN CONTENT OF THE DISTILLER'S GRAIN FOR ITS APPLICATIONS IN ANIMAL HUSBANDRY

Hui Zhu[1,†], Ning Wang[1,†], Muhammad Aamer Mehmood[1,2], Muhammad Kashif Saleemi[3], Yong Tang[4], Muhammad Qamar Bilal[5], Jun Xie[1], Baoxiang Gu[1] and Huibo Luo[1,*]

[1]School of Bioengineering, Sichuan University of Science & Engineering, Zigong, China; [2]Department of Bioinformatics and Biotechnology, Government College University Faisalabad, Faisalabad, Pakistan; [3]Department of Pathology, University of Agriculture, Faisalabad, Pakistan; [4]College of Food and Biological Engineering, Xihua University, Chengdu, China; [5]Institute of Animal and Dairy Sciences, University of Agriculture Faisalabad, Pakistan.
[†] Hui Zhu and Ning Wang contributed equally to this work
***Corresponding author's e-mail: huiboluo@126.com**

Residual biopolymers in the distiller's grains (DGs) can be used as a protein source in animal husbandry. Aimed at this, nine probiotics strains including *Bacillus methylotrophicus, Bacillus subtilis, Bacillus megaterium, Acetobacterium balch, Pediococcus acidilactici, Aspergillus oryzae, Candida utilis, Aspergillus carbonarius*, and *Mucor wutungkiao* Fang were screened for their fermenting DG in this study. The biotransformation effect of DGs was evaluated by the changes of crude protein, soluble protein, content, and composition of 17 amino acids. Besides, 20% fermented DGs were added into the basic mouse feed and the apparent digestibility of crude protein was detected. It was shown that the crude protein contents of DGs products fermented by *A. oryzae*, *B. subtilis* and *B. methylotrophicus* fermented DGs were the highest, while the soluble protein contents of DGs fermented by *B. methylotrophicus*, *A. oryzae* and *A. balch* were the highest. The amino acid contents of DGs were increased by 33% after fermentation by *B. subtilis*. In the fermentation products of *Bacillus* spp. and *A. oryzae*, the proportions of seven essential amino acids except for tryptophan in total amino acids were increased significantly with growth rates of 10-13%. In the feeding experiment of Sprague Dawley rats, the apparent digestibility of crude protein in all groups was increased, while the fermentation group of *A. oryzae*, *B. methylotrophicus* and *B. subtilis* were significantly higher than that in the control group. It was demonstrated that the biotransformation effect of DGs in *A. oryzae* and *Bacillus* spp. groups were the highest among nine probiotics, which could be very promising protein feed material. This study provided basic data to support the idea of developing low-cost, efficient, and novel alternative protein-rich feed for the livestock.
Keywords: Probiotics, distiller's grain, fermentation, protein source, livestock feed**.**

INTRODUCTION

Chinese meat and milk industry especially focus to feed the animals with a well-balanced diet where protein ratio and the amino acid profile of the feed are of special concern (Brand *et al.,* 2004; Khan *et al.,* 2019; Guo *et al.,* 2020). Not only will animals have optimal performance on these well-balanced diets, but the producer will also achieve higher profit margins. The cost of feed is the single largest expenditure in husbandry, which is strongly influenced by the cost of protein used because proteins are the most expensive components of the animal diet (Lim *et al.,* 2011). With global population growth, protein is becoming scarcer, more expensive, and in particular less available to be used in animal feeds (Brand *et al.,* 2014; Sridhar and Bhat, 2007; Mousa and Marwan, 2019). Efforts by nutritionists and feed formulators to reduce feed costs have resulted in the increased use of low-cost and high-quality alternative proteins in diet formulations as replacements, alternative options including use of probiotics gained special attraction because of having positive effects on performance of animals (Kalhoro *et al.,* 2019; Mani, 2019; Marwan *et al.,* 2019; Raheel *et al.,* 2019; Patsios *et al.,* 2020; Muhammad *et al.,* 2020).

Distiller's grains (DGs), the solid residues of alcohol extracted from sorghum, corn, wheat, and barley by fermentation and distillation are the main byproducts of the brewing industry (Cookman and Glatz, 2009; Abdou *et al.,* 2019; Goda *et al.,* 2020). Nowadays, the annual output of DGs in China has exceeded 100 million tons (Zuo *et al.,* 2016). As a subsidiary product with a large amount, DGs rot if they are not treated timely. It will not only be a waste of resources but may also cause serious environmental pollution. The DGs are currently readily available and are less expensive than other conventional protein sources on a per unit protein basis (Lim *et al.,* 2011). However, adding DGs to feed directly reduce the palatability of feed and nutrient absorption of animals. Ethanol producers are seeking ways to improve the quality of DGs to increase market penetration and help to stabilize

prices (Tucker *et al.*, 2004). One possible improvement is to increase the protein content of DGs by probiotics fermentation. Probiotics can be used to improve the quality of animal feed, because it has a role to degrade the protein content in animal feed to the simple molecules, such as being short oligopeptides or amino acids, so it can be absorbed easily by the animal cells. Therefore, using DGs fermented by probiotics as a new protein source can realize "ecological to the enterprise" and have a vital significance for China's resources recycling and environmental protection. However, due to the different metabolic characteristics of different probiotics, the nutritional components of fermentation products may be significantly different, especially the amino acid composition.

Keeping in view the scenario, the present study was focused to screen probiotics for the fermentation of DGs. Biotransformation effect of DGs was evaluated by the changes of crude protein, soluble protein, free amino acid composition, and amino acid content. Besides that, fermented DGs were also added into the basic mouse feed and the apparent digestibility of crude protein in rats was determined.

MATERIALS AND METHODS

***Sample preparation*:** Nine strains of probiotics (*B. methylotrophicus, B. subtilis, B. megaterium, A. balch, P. acidilactici, A. oryzae, C. utilis, A.s carbonarius, M. wutungkiao* Fang) were collected from Sichuan Key Laboratory of Brewing Biotechnology and Application, Sichuan University of Science and Engineering, Zigong, China. Distillery's Grains (DGs) used in our study were supplied by a liquor-making enterprise in Yibin, China. Fresh DGs were oven-dried and crushed to a powder containing particles less than 0.4 mm for further use.

***Ethical statement*:** All experimental procedures in the present study were reviewed and approved by the National Institute of Animal Health Animal Care and Use Committee of Sichuan University of Science and Engineering (Zigong, China). All animal experiments were performed by Dashuo Biological Technology Co., Ltd. (Chengdu, China) and animals were raised strictly as per the Regulations for the Administration of Affairs Concerning Experimental Animals (approved by the State Council of the People's Republic of China).

***Activation and expansion of probiotics cultures*:** Under sterile conditions, bacteria were inoculated on nutrient agar medium at 37°C for 24 hr, while yeast and mold were inoculated on Yeast Malt Dextrose Agar and Potato Dextrose Agar medium at 28°C for 24 hr. Single colonies were selected and cultured in a liquid medium and then stored at 4°C for further study.

***Fermentation of DGs*:** DGs powder (5g) was added in 20 mL sterile ddH_2O with 5% (v/v) probiotics in every group with six repeats. In the control group, probiotics were replaced by sterile water. DGs were fermented by probiotic bacteria at 37°C for 48 hr and fungi at 28°C for 72 hr. After fermentation, the samples were extracted using an ultrasonic cell crusher for 30 min. Three samples in each group were centrifuged at 10000 rpm (RCF of 11870 g) for 5 min, then the supernatant was taken for further use, and the remaining three samples were dried and crushed for further use.

***Determination of crude and soluble protein content*:** The Kjeldahl method of nitrogen determination (GB/T 6432-2018) was used to determine crude protein content in fermentation products after drying. Soluble protein content in fermentation products was determined by coomassie brilliant blue method as described previously (Giuffrida *et al.*, 2019). A standard curve was prepared by using a range of Bovine Serum Albumin (BSA) solutions with known concentrations; each dilution was mixed with Coomassie Brilliant Blue (1 × G-250 dye) solution for the reaction. Later, 100 µL of the mixture from each dilution was pipetted into the wells of a 96-well plate and incubated for five min at room temperature, and the absorbance was measured at λ595 nm to make the calibration curve. The fermented samples were treated in the same way and absorbance values were used to calculate the soluble protein content by using the equation derived from the standard curve ($y=3.6997x+0.0168$, $R^2=0.9926$).

***Determination of the content and composition of free amino acids*:** According to instructions for amino acid (AA) content test kit (Solarbio, Beijing, China), free amino acids in all samples were determined. The supernatant of each sample was collected after ultrasonic crushing and mixed with the reagents supplied by the kit. The mixture was kept in boiling water for 15 min. After centrifugation, the supernatant was collected and the absorbance was measured at λ570nm. The absorbance values were used to calculate the amino acid content of the sample following the instructions manual of the kit. While the amino acid composition of the fermentation product was determined by using the acid hydrolysis method as described in GB/T 18246-2000.

***Apparent digestibility of crude protein*:** Sixty-six female Sprague Dawley (SD) laboratory rats were randomly divided into eleven groups: blank, control, and nine fermentation groups. The powdered fermented-DGs were mixed into the basic feed of the rats. The initial crude protein content of the basic feed was 20.3% (w/w). The blank group was fed with the basic feed, the control group was fed with basic diet plus 20% (w/w) unfermented DGs feed, and the fermentation groups were fed with basic diet plus 20% different fermented DGs feed. After fifteen days of adaptive feeding in the early stage, the weight and food intake of every rat was recorded. Feces were collected for 5 consecutive days to record the defecation. The fecal samples were dried at 105°C, milled, and screened for 1 mm. The crude protein contents of the feed, as well as fecal samples, were determined as described above. The apparent digestibility of the protein provided in the feed was calculated by using the following formula;

Apparent digestibility of crude protein = [protein intake – protein excreted] / protein intake

Chemical analysis were conducted according to standard methods as described in recent studies (Hussain *et al.,* 2018; Demir *et al.,* 2019; Raza *et al.,* 2019; Hussain *et al.,* 2020).

***Data analysis*:** All the data in this study were analyzed using one-way ANOVA by SPSS19 and expressed as mean ± standard deviation.

RESULTS

***Increase in the crude and soluble protein content of probiotic-fermented DGs*:** After fermentation of the DGs by the probiotics, a loss in the mass of the DGs was observed. Moreover, the crude protein content of most of the fermentation products increased when compared to the control group (Table 1). While the highest increase (27.290±0.052%) in crude protein content of the *A. oryzae* fermented products of the DGs was observed, which was significantly increased when compared with the control group. Followed by the fermentation products of *Bacillus* genus, the crude protein contents were 16.98-34.05%. Among them, the growth rate of fermented DGs by *B. subtilis* was shown to be the highest. Instead, the crude protein content of the fermentation products of *A. balch* was lower when compared to the control group.

Table 1. Crude protein content of fermentation products.

Group	Weight loss ratio	Crude protein content (%)	Increase ratio (%)
Control	8.50%	19.17±0.017	-
B. methylotrophicus	9.50%	24.546±0.031	28.04*
B. subtilis	7.90%	25.697±0.027	34.05*
B. megaterium	8.70%	22.426±0.146	16.98 *
A. balch	8.10%	18.822±0.09	-1.82
P. acidilactici	8.70%	20.518±0.034	7.03
A. oryzae	8.60%	27.290±0.052	42.36 *
C. utilis	9.30%	22.447±0.029	17.09*
A. carbonarius	8.00%	23.549±0.033	22.84*
M. wutungkiao Fang	9.30%	22.369±0.028	16.69*

Note: Statistically significant differences between the control group and each fermentation groups were determined by Student's t-test ($P < 0.05$)

Interestingly, the soluble protein content of the fermented samples significantly increased in all groups (Fig. 1). Where, the soluble protein content of DGs fermented products by *B. methylotrophicus* was found to be highest (0.164±0.001 mg/mL), which was 28.13% higher as compared to the control group, followed by the DGs fermented products of *A. oryzae*, and where soluble protein content was 24.22%. However, in other groups, a 7.03% -19.53% increase in the soluble protein content was observed.

***Improvement in the content and composition of amino acids after fermentation*:** The free amino acids in all the fermented samples increased when compared with the control group (Fig. 2). Where, *B. subtilis* fermentation group showed the highest increase, reaching 32.84%, followed by *A. oryzae* and *A. carbonarius* fermentation groups, which gave 28.56% and 25.87% increase, respectively. Moreover, the free amino acid contents were shown to increase in the range of 12.33-20.08% in almost all groups except *P. acidilactici* fermentation group.

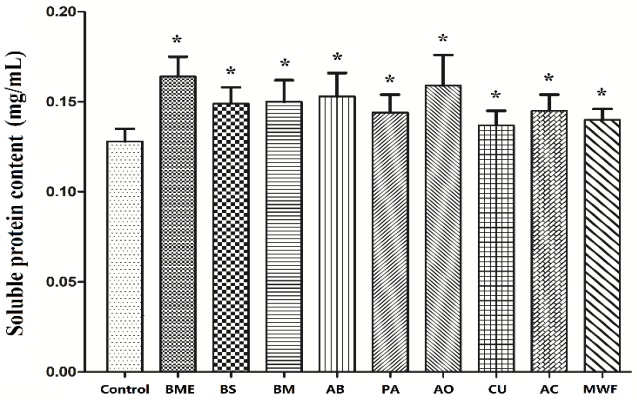

Figure 1. Soluble protein content of DGs fermented by different probiotics strains. BME stands for *B. methylotrophicus*, BS stands for *B. subtilis*, BM stands for *B. megaterium*, AB stands for *A. balch*, PA stands for *P. acidilactici*, AO stands for *A. oryzae*, CU stands for *C. utilis*, AC stands for *A. carbonarius*, MWF stands for *M. wutungkiao* Fang. Statistically significant differences between the control group and each fermentation groups were determined by Student's t-test ($P < 0.05$).

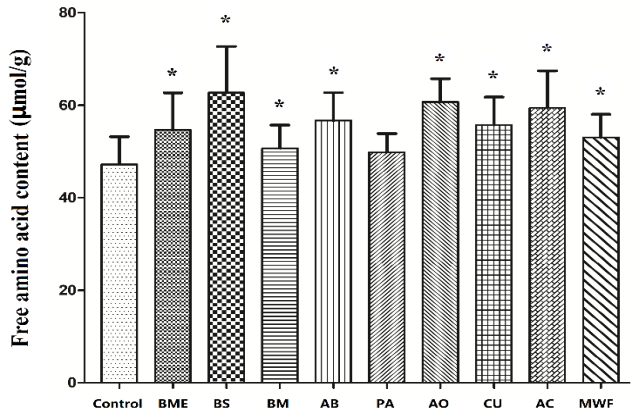

Figure 2. Free amino acid content of DGs fermented by different probiotics strains. BME stands for *B. methylotrophicus*, BS stands for *B. subtilis*, BM stands for *B. megaterium*, AB stands for *A. balch*, PA stands for *P. acidilactici*, AO stands for *A. oryzae*, CU stands for *C. utilis*, AC stands for *A. carbonarius*, MWF stands for *M. wutungkiao* Fang. Statistically significant differences between the control group and each fermentation groups were determined by Student's t-test (* $P < 0.05$).

***Amino acid composition of fermentation products*:** The composition of seventeen amino acids in fermented DGs by probiotics was determined by the acidolysis method. The results showed that the total content of amino acids in fermentation products of each group increased, especially in *Bacillus* genus fermented groups (Table 2). The total amino acids of fermentation products of *B. methylotrophicus*, *B. subtilis*, *B. megaterium* were 9.30±1.03 mg/mL, 9.12±0.89 mg/mL and 8.76±0.90 mg/mL, respectively. Besides, the total amino acids in *M. wutungkiao* Fang, *A. oryzae* and *C. utilis* fermentation groups were also considerably high, which were shown to be 8.73±0.87 mg/mL, 8.30±0.97 mg/mL and 8.00±0.81 mg/mL, respectively. While, there was no significant increase in the amino acid content of other groups. Moreover, in the fermentation products of *Bacillus* genus and *A. oryzae*, the proportion of essential amino acids except tryptophan increased significantly, with an increase of 9.74-13.08%, where the *A. oryzae* fermentation group showed the highest increase (Table 2).

After fermentation by probiotics, seventeen determined amino acids in DGs varied considerably, especially the essential amino acids except for tryptophan (Table 3). Among these, glutamate, methionine, and lysine are the most important essential amino acids for the growth of animals such as pigs, cattle, and sheep (Lee *et al.*, 2012). The content of glutamic acid in the fermentation products of *B. megaterium*, *A. oryzae*, and *P. acidilactici* was significantly higher when compared to the control group. The content of methionine in the fermentation products of *A. oryzae*, *B. methylotrophicus* and *C. utilis* showed the highest increase when compared to the control group, while the content of lysine showed the highest increase in the fermentation products of *B. subtilis*, *A. oryzae* and *B. methylotrophicus*.

***Improved apparent digestibility of crude proteins in fermented DGs*:** The apparent digestibility in the control group decreased when compared with the blank, however, it was improved in all other groups when compared to the control where a 2.63%-10.07% increase in the digestibility

Table 2. Amino acid composition of fermentation products.

	Control	BME	BS	BM	AB	PA	AO	CU	AC	MWF
Total amino acids content (mg/mL)	7.61±0.82	9.30±1.03	9.12±0.89	8.76±0.90	7.98±0.85	7.68±0.95	8.30±0.97	8.00±0.81	7.83±0.79	8.73±0.87
Content of essential amino acids (%)	51.13	56.92	57.32	56.11	52.83	55.88	57.81	54.11	52.81	53.91
Growth rate of essential amino acids content (%)	-	11.33	12.11	9.74	3.33	9.29	13.08	5.83	3.28	5.43

Note: BME stands for *B. methylotrophicus*, BS stands for *B. subtilis*, BM stands for *B. megaterium*, AB stands for *A. balch*, PA stands for *P. acidilactici*, AO stands for *A. oryzae*, CU stands for *C. utilis*, AC stands for *A. carbonarius*, MWF stands for *M. wutungkiao* Fang.

Table 3. Changes of amino acids in fermentation products (mg/mL)

Types of amino acids	Control	BME	BS	BM	AB	PA	AO	CU	AC	MWF
Aspartic acid	0.722	0.558	0.640	0.661	0.711	0.773	0.690	0.709	0.726	0.715
Threonine	0.345	0.369	0.340	0.317	0.341	0.299	0.334	0.344	0.371	0.346
Serine	0.456	0.494	0.510	0.412	0.450	0.553	0.434	0.407	0.425	0.458
Glutamate	**2.561**	**2.696**	**2.714**	**3.132**	**2.709**	**2.828**	**2.918**	**2.699**	**2.697**	**2.584**
Glycine	0.639	0.483	0.468	0.582	0.644	0.573	0.492	0.633	0.642	0.633
Alanine	0.774	0.720	0.737	0.719	0.778	0.681	0.698	0.7.60	0.776	0.733
Cysteine	0.014	0.017	0.011	0.008	0.012	0.010	0.012	0.007	0.011	0.014
Valine	0.457	0.488	0.507	0.412	0.449	0.582	0.440	0.464	0.452	0.462
Methionine	**0.198**	**0.317**	**0.302**	**0.149**	**0.182**	**0.298**	**0.368**	**0.314**	**0.170**	**0.302**
Isoleucine	0.318	0.358	0.351	0.294	0.319	0.284	0.364	0.320	0.316	0.334
Leucine	0.555	0.613	0.603	0.520	0.560	0.492	0.536	0.542	0.552	0.568
Tyrosine	0.435	0.386	0.346	0.336	0.363	0.329	0.362	0.356	0.367	0.379
Phenylalanine	0.377	0.421	0.410	0.345	0.367	0.359	0.364	0.364	0.369	0.389
Lysine	**0.302**	**0.431**	**0.505**	**0.442**	**0.356**	**0.373**	**0.457**	**0.365**	**0.355**	**0.405**
Histidine	0.302	0.297	0.273	0.290	0.300	0.270	0.271	0.287	0.289	0.306
Arginine	0.479	0.398	0.385	0.371	0.384	0.352	0.353	0.387	0.400	0.397
Proline	1.067	0.955	0.898	1.009	1.073	0.945	0.907	1.044	1.083	0.974

Note: BME stands for *B. methylotrophicus*, BS stands for *B. subtilis*, BM stands for *B. megaterium*, AB stands for *A. balch*, PA stands for *P. acidilactici*, AO stands for *A. oryzae*, CU stands for *C. utilis*, AC stands for *A. carbonarius*, MWF stands for *M. wutungkiao* Fang.

was observed (Fig. 3). In particular, the apparent digestibility of crude protein in *A. oryzae*, *B. methylotrophicus* and *B. subtilis* group was significantly increased.

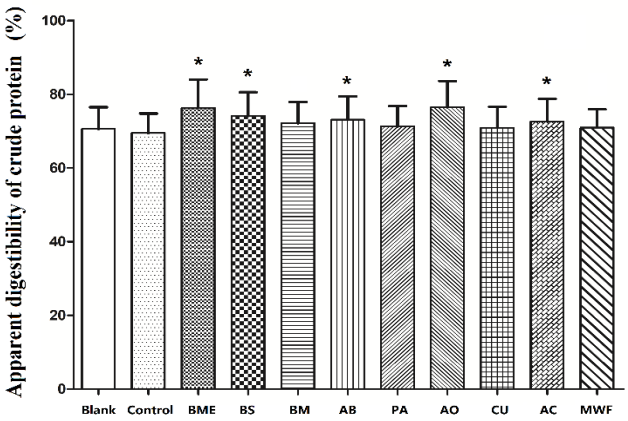

Figure 3. Apparent digestibility of crude protein in rats fed with different feed. The blank group was fed with the basic feed, the control group was fed with basic diet plus 20% (w/w) unfermented DGs, and the fermentation groups were fed with basic diet plus 20% DGs fermented by different probiotics strains. Statistically significant differences between the control group and each fermentation groups were determined by Student's t-test ($P < 0.05$).

DISCUSSION

Proteins are the basic requirement of life which are involved in various metabolic and physiological reactions of the biological system and have a wide range of nutritional functions. Therefore, proteins are a key important component of the diet of animals that we feed to get meat or milk. If the animal diet is lack of proteins, it will slow its growth, reduce its reproductive performance, productivity, and even product quality. The Distillery Grains (DGs) contain high protein, cellulose, fat, vitamins, and various microelements, which may vary with the raw materials and methods of liquor making (Ye *et al.,* 2018). The protein content of dry DGs is around 16-24%, which reflects their huge potential as a new protein source that may be added in animal feed (Li *et al.,* 2018). However, their utilization rate is low because of the high-water content, easy to mildew, and difficult to store. Besides, DGs have high acidity and husk content. The protein denaturation and starch aging are caused by multiple cooking in the process of brewing, which reduces the digestibility and absorption of nutrients by animals.

At present, the use of probiotics to improve the protein quality of feed materials has become an important approach to alleviate the shortage of protein-rich feed (Niemann *et al.,* 2018). Microbial fermentation of raw materials does not only improve the protein content of the animal feed but also ameliorate the composition of amino acids (Tucker *et al.,* 2004). Therefore, the present study was focused on studying the impact of fermentation on the protein content and quality of the Distillery's Grains (DGs) by using probiotics. The total content of crude protein, soluble protein, free amino acid, the apparent digestibility of the crude protein of the fermented DGs was studied and it was observed that some of the studied probiotics greatly improved the protein content and amino acid composition of DGs.

After fermentation, the probiotic would have consumed carbon source, some of which would have discharged in the form of CO_2, and some of which were transformed into microbial protein. Therefore, the crude protein content of the fermentation products was increased. Jiao *et al.* used mixed cultures of *B. subtilis*, *Geotrichum candidum*, and *C. utilis* to ferment DGs, which contained 24.85% true protein and 32.09% crude protein, and the crude fiber content was reduced to 17.66% after fermentation (Jiao *et al.*, 2015). *Li et al*. screened the fine fermentation strains of wheat distiller's grains and optimize its solid-state fermentation technology to improve its feed quality. After fermented by *Lactobacillus* R-02 and *B. Subtilis* KG109, the content of acid-soluble protein in DGs was increased by 4.8 times (Li *et al.,* 2018). In our study, the crude protein content was found to be the highest in the products of DGs fermented by *A. oryzae*, *B. subtilis* and *B. methylotrophicus*, while soluble protein content was shown to be the highest in the fermentation products of DGs by *B. methylotrophicus*, *A. oryzae* and *A. balch*. The soluble proteins present in the feed are easily absorbed and digested by the animals, it is therefore suggested that the latter three probiotic strains could contribute better from the perspective of feed protein transformation and utilization.

The composition and content of amino acids in the feed is an important indicator to measure its quality. For a long time, the husbandry in developing countries overemphasizes the crude protein level in feed and ignored the amino acid balance. Protein is one of the most important nutritional indexes in feed. Animal and microorganism-originated proteins, as well as peptides and amino acids in feed, are more easily absorbed and have higher nutrition value.

The ratio of lysine to crude protein in feed greatly deviates from the ideal value, which reveals the general defect in the formula design of feed enterprises. The imbalance of amino acid will inevitably result in the waste of many feed proteins, namely amino acid resources. Previously, the bacterium 8503 and 8505, which had been selectively bred in the lab, were used to ferment DGs. The crude protein content of DGs was raised to 35.75% from 23.75%, and the amino acid content was increased from 25.35% to 40.68% (Hou *et al.,* 1999). Similarly, using three probiotic strains namely *C. tropicalis*, *T. viride* and *A. oryzae* to ferment DGs increased the amino acids in the fermentation products by 24.94% ($P < 0.05$), and the contents of cysteine and methionine were also significantly improved (Ye *et al.,* 2008). *Ming et al.* utilized *G. candidum*, *Candida tropicalis*, and *Celluomonas* X5 to

fermented DGs. The content of true protein and crude fiber increased by 40.30% and 44.12%, respectively. The total amount of methionine, lysine, and threonine was increased by 28.41% (Ming *et al.*, 2015). This means, that fermentation of the DGs increases the protein content and the balance of the amino acid composition.

The content and composition of seventeen amino acids in DGs fermented by the probiotics in this study were all increased. Moreover, the proportion of 7 essential amino acids except tryptophan in the total amino acids was increased significantly, which was the highest in the DGs fermentation products by *A. oryzae*. The proportion of glutamic acid, methionine, and lysine in animal feed is particularly important, and interestingly the ratios of these three amino acids were increased at most in the DGs products fermented by *B. megaterium*, *A. oryzae* and *B. subtilis*, respectively, which made these strains suitable for the development of protein-rich quality feed.

The apparent digestibility is one of the key indexes to evaluate the nutritional value and bioavailability of feed protein source. A yeast culture (DVAqua from Diamond V. Mills, Cedar Rapids, IA, USA) and *A. oryzae* fermentation extracts were used to feed cows and the apparent digestibility of dry matter, crude protein, and hemicellulose in cows were significantly improved (Wiedmeier *et al.*, 1987). The *in vitro* fermentation of liquor by digestion of pepsin-trypsin improved the digestibility of dry matter, crude protein and crude fiber by 11.4%, 11.3% and 40.7%, respectively (Zhang *et al.*, 2019). However, there are few comparative tests focused on the digestibility of DGs before and after fermentation *in vivo*. In this study, the apparent digestibility of the protein content of DGs fermented by *A. oryzae* showed the most significant increase when compared to the control group in rats, with an increased rate of 10.07%, followed by the fermentation groups of *B. methylotrophicus* and *B. subtilis*. The other groups also showed improved digestibility which indicated that the digestibility and absorption of protein in DGs was improved by probiotic fermentation.

***Conclusion*:** Nine probiotics strains were employed to ferment DGs as protein feed materials, and the changes of crude protein, soluble protein, free amino acid composition, and seventeen amino acid contents were determined, as well as the apparent digestibility of crude protein in rats was also determined. It was found that crude and soluble protein contents, amino acid contents of DGs products fermented by *A. oryzae* and *Bacillus* spp. were significantly increased; while, the ratio of amino acid composition was also improved. Moreover, the fermentation products also had a good protein digestibility and biotransformation effect in rats, which reflected their promising potential as novel protein feed materials. This study provides a preliminary detail to support the idea of developing low-cost, efficient, and novel alternative protein-rich feed for the livestock.

***Acknowledgments*:** This work was supported by funds from the Yibin Science and Technology Plan Project (Grant No.: 2017ZSF008-2), the Talent Introduction Project of Sichuan University of Science & Engineering (Grant No.: 2019RC30), the Academician (Expert) Workstation Project of Sichuan University of Science & Engineering (Grant No.: 2018YSCZZ02), and the Innovation and Entrepreneurship Program for College Students (Grant No.: CX2019055).

REFERENCES

Abdou, A.M., E.A. Fouad, S.S. Alam and A.S. Hakim. 2019. Isolation and identification of probiotic lactobacilli from non-ruminant animals. Inter. J. Vet. Sci. 8:349-354.

Brand, T.S., P.D. Carstens and L.C. Hoffman. 2014. The effect of dietary energy concentrations on production variables of ostrich chicks (*Struthio camelus* var. domesticus). Brit. Poultry Sci. 55:610-618.

Brand, T.S., R.M. Gous, A.C.M. Kruger, B.B. Aucamp, C.J. Nel and J.O. Horbañczuk. 2004. The effect of dietary energy and protein (amino acid) concentrations on the end products of slaughter ostriches. S. Afr. J. Anim. Sci. 34:107-109.

Cookman, D.J. and C.E. Glatz. 2009. Extraction of protein from distiller's grain. Bioresour. Technol. 100:2012-2017.

Demir B, M. Sahin, A.G. Akcacik, S. Aydogan, S. Hamzaoglu and M.K. Demir. 2019.Effect of quinoa (*Chenepodium Quinoa* Wild) flour addition on the nutritional and chemical properties of bread. J. Glob. Inno. Agri. Soci. Sci. 7:171-175.

Giuffrida, F., S. Austin, D. Cuany, B. Sanchez-Bridge, K. Longet, E. Bertschy, J. Sauser, S.K. Thakkar, L.Y. Lee and M. Affolter. 2019. Comparison of macronutrient content in human milk measured by mid-infrared human milk analyzer and reference methods. J. Perinatol. 39:497-503.

Goda, A.M.A., S.R. Ahmed, H.M. Nazmi, M.Z. Baromh, K. Fitzsimmons, W.R. Jr, S. Davies and E. EI-Haroun. 2020. Partial replacement of dietary soybean meal by high-protein distiller's dried grains (HPDDG) supplemented with protease enzyme for European seabass, *Dicentrarchus labrax* fingerlings. Aquacult. Nutr. 26:842-852.

Guo, W., W.J. Li, X.Z. Hu, X.K. Lu, K. Cui and Q.Y. Diao. 2020. Advances in the application of ruminant low protein diets. Food Ind. 41:47-51.

Hou, W.H., Z.Y. Li, L. Yang and D. Zhou. 1999. A study on feed protein producing from distiller's grains by multiple bacteria synergistic fermentation. Environ. Sci. 20:65-67.

Hussain, M., A. Mahmud, J. Hussain, S. Qaisrani, S. Mehmood and A. Rehman. 2018. Subsequent effect of dietary lysine regimens fed in the starter phase on the growth performance, carcass traits and meat chemical

composition of aseel chicken in the grower phase. Braz. J. Poult. Sci. 20:455-462.

Hussain, M., A. Mahmud, J. Hussain, S.N. Qaisrani, S. Mehmood, S. Ahmad and A.U. Rehman. 2020. Effect of dietary amino acid regimens on growth performance and bodyconformation and immune responses in Aseel chicken. Indian J. Anim. Res. 54: 53-58.

Jiao, X.F., J.X. Liu, S.H. Han, X. Li, P.Y. Li, D.L. Luo, W.W. Zhang and B.C. Xu. 2015. Optimization of mixed-culture fermentation conditions for biotransformation of distilled spirit lees. Food Sci. 36:164-168.

Kalhoro, M.S., L.T. Nguyen and A.K. Anal. 2019. Evaluation of probiotic potentials of the lactic acid bacteria (LAB) isolated from raw buffalo (*Bubalus bubalis*) milk. Pak. Vet. J. 39:395-400.

Khan, M., A.A. Anjum, M. Nawaz, A.R. Awan, and M.A. Ali. 2019. Effect of newly characterized probiotic lactobacilli on weight gain, immunomodulation and gut microbiota of *Campylobacter jejuni* challenged broiler chicken. Pak. Vet. J. 39:473-478.

Li, Q., C.X. Pei and Z.S. Wang. 2018. A comparative study on the differences of nutritional components in different types of distiller's grains. Chinese J. Animal Nutr. 30:2369-2376.

Li, S., W.J. Guo, L.M. Li, C. Lu and A.L. Wei. 2018. Screening of strains on fermentation and optimization of fermentation conditions in wheat distiller's grains used for feed. Food Fermentation Ind. 44:105-112.

Lim, C., E. Li and P.H. Klesius. 2011. Distiller's dried grains with solubles as an alternative protein source in diets of tilapia. Rev. Aquacult. 3:172-178.

Mani, A.M.M. 2019. Utilization leaf meal of water hyacinth (*Eichhornia crassipes*) as a replacement protein source for growing Awassi lambs. Inter. J. Vet. Sci. 8:54-60.

Marwan, A.A., S.A. Mousa and A.M. Singer. 2019. Impact of feeding exogenous fibrolytic enzymes (efe) on digestibility, rumen fermentation, haemo-biochemical profile and productive performance in buffalo calves. Inter. J. Vet. Sci. 8:127-133.

Ming, H.M., M.E. Chen, J. Zhou, Z. Guo, J. Liu and X.H. Lan. 2015. Study on protein feedstuff production with distiller grain fermented by complex microbial inoculants. Cereal Food Ind. 12:49-52.

Mousa, S.A. and A.A. Marwan. 2019. Growth performance, rumen fermentation and selected biochemical indices in buffalo calves fed on *Basillis subtilus* supplemented diet. Inter. J. Vet. Sci. 8:151-156.

Muhmmad, A.U.R., T. Zulqurnain, S. Muhammad, Y. Muhammad, M.R. Virk, M. A. Mirza, A. Fawwad, and F. Naeem. 2020. Effect of lysozyme, tributyrin, Bacillus amyloliquefaciens SC06 and enramycin on growth performance, nutrient digestibility and carcass characteristics of broiler during finisher phase. Pak. J. Agr. Sci. 57: 949-956.

Niemann, G.J. 2018. The evaluation of locally produced full-fat canola seed (*Brassica napus*) as an alternative protein source in the diets of slaughter ostriches (*Struthio camelus* var. domesticus). Ph.D. diss., Stellenbosch Univ., Stellenbosch, South Africa.

Patsios, S.I., A. Dedousi, E.N. Sossidou and A. Zdragas. 2020. Sustainable Animal Feed Protein through the Cultivation of YARROWIA Lipolytica on Agro-Industrial Wastes and by-Products. Sustainability 12:1-23.

Raheel, I.A.R., A. Orabi and A. El Masry, 2019. Natural herbs CLEANACTIV®; immune-modulator, health activator and growth promoter in broiler chickens. Inter. J. Vet. Sci. 8:67-270.

Raza H., F. Zaaboul, M. Shoaib, W. Ashraf, A. Hussain and L. Zhang. 2019. Physicochemical and structural characterization of microwave-roasted chickpea. J. Glob. Inno. Agri. Soci. Sci. 7:23-28.

Sridhar, K.R. and R. Bhat. 2007. Lotus-A potential nutraceutical source. J. Agri. Technol. 3:143-155.

Tucker, M.P., N.J. Nagle, E.W. Jennings, K.N. Ibsen, A. Aden, Q.A. Nguyen, K.H. Kim and S. Noll. 2004. Conversion of distiller's grain into fuel alcohol and a higher-value animal feed by dilute-acid pretreatment. Appl. Biochem. Biotech. 115:1139-1159.

Wiedmeier, R.D., M.J. Arambel and J.L. Walters. 1987. Effect of yeast culture and *Aspergillus oryzae* fermentation extract on ruminal characteristics and nutrient digestibility. J. Dairy Sci. 70:2063-2068.

Ye, G., H. Luo, Z. Ren, M.S. Ahmad, C. Liu, A. Tawab, A.B. Alghafari, U.M. Omar, M. Gull and M.A. Mehmood. 2018. Evaluating the bioenergy potential of Chinese Liquor-industry waste through pyrolysis, thermogravimetric, kinetics and evolved gas analyses. Energ. Convers. Manag. 163:13-21.

Ye, J.A., J.J. Wang and G.Z. Xu. 2008. Study on the production of protein feed by solid-state fermentation of rice wine distiller's grains. Animal Prod. 44:58-61

Zhang, H., L. Wei and Z.Y. Liu. 2019. Evaluation of nutritional value of fermented distiller's grains and its effect on growth performance of finishing pigs. China Feed. 3:41-45.

Zuo, S.C., H.Q. Yang and W. Zhou. 2016. Recent progress on the ultilization of Distiller's grains: a review. Food Ind. 1:246-249.

[Received 14 Apr. 2020; Accepted 01 Sept. 2020 Published (Online) 25 Oct. 2020]