Received: 27 October 2023 | Revised: 25 January 2024 | Accepted: 5 February 2024

DOI: 10.1002/aro2.55

ARTICLE

AROH ANIMAL RESEARCH AND ONE HEALTH WILEY

Solid-state fermentation pro-enzymes supplementation benefits growth performance, health, and intestinal microbiota of broiler chickens fed wheat-based diet

Jiaheng Li[1,2] | Guosong Bai[1] | Yan Gao[3] | Qingtao Gao[1] | Ruqing Zhong[1] | Liang Chen[1] | Yunlong Wang[3] | Teng Ma[1] | Hongfu Zhang[1]

[1]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China

[2]Precision Livestock and Nutrition Unit, Gembloux Agro-Bio Tech, TERRA Teaching and Research Centre, Liège University, Gembloux, Belgium

[3]Hangzhou Bio-Com Biotechnology Co. LTD, Hangzhou, China

Correspondence

Teng Ma, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, No. 2 Yuan Ming Yuan West Road, Haidian District, Beijing 100193, China.
Email: mateng@caas.cn

Funding information

National Key Laboratory of Animal Nutrition, Grant/Award Number: 2004DA125184G2102; China Agriculture Research System of Ministry of Finance (MOF) and Ministry of Agriculture and Rural Affairs (MARA), Grant/Award Number: CARS-41; Agricultural Science and Technology Innovation Program, Grant/Award Number: ASTIPIAS07

Abstract

Wheat as a kind of diet material can be used for broiler production. However, due to non-starch polysaccharides in wheat, wheat may lead to lower growth performance and worth health. To reverse the negative effect, solid-state fermentation pro-enzymes were added. In this experiment, growth performance, intestinal health-related genes, short chain fatty acids (SCFAs) and intestinal microbiota were detected to find the effects of wheat meal and combined with enzymes on broiler chickens from 15 to 42 days of age. A total of 432 1-day-old Arbor Acres broiler chickens were fed corn-based diet (CD) for 14 days as the preparation stage of the experiment. Then, they were randomly divided into three groups and fed three different kinds of diets which were corn-based diet (CD group), wheat-based diet (WD group), and SFP enzymes supplementation in WD (Enzymes+Wheat-based diet group). The results showed that compared with broilers in CD group, broilers in WD group had lower weight gain and higher Feed conversion ratio ($p < 0.05$) during the whole experimental period especially from day 15 to day 21, but there was no significant effect on feed intake ($p > 0.05$). Moreover, SFP enzymes decreased the spleen index ($p < 0.05$). Wheat also had trends to decrease the expression of *ZO-1* ($p = 0.096$) and increase the concentrations of acetate ($p < 0.05$), butyrate ($p < 0.05$) and total SCFAs ($p < 0.05$), in which SFP enzymes caused the opposite results except for butyrate, and SFP enzymes even increased the expression of *ZO-1* ($p < 0.001$) and OCCLUDIN ($p = 0.075$) and decreased the expression of *TNF-α* ($p < 0.01$). Meanwhile, wheat enhanced the abundances of *Barnesiella* and *Bifidobacterium* ($p < 0.05$) and inhibited the abundances of *Flavonifractor*, *Sellimonas*, *Lachnospiraceae_NK4A136_group*, *Subdoligranulum*, and *Ruminococcus_gauvreauii_group* ($p < 0.05$), and SFP enzymes could reverse the negative effects, and the changes in microbiota could explain the other different parameters. Collectively, wheat results in inflammation and worse growth performance, but SFP enzymes supplementation in WD benefits chickens' growth performance by improving intestinal barrier function, decreasing inflammation, modulating cecal microbiota and SCFAs production.

Jiaheng Li and Guosong Bai are contributed equally to this work.

Keywords
broiler chickens, intestinal barrier, microbes, short chain fatty acids, solid-state fermentation pro-enzymes

INTRODUCTION

Wheat as a poultry diet material has been widely used worldwide, it could apply energy and nutrients for broiler chickens. Meanwhile, the nutrients in wheat diet are higher than that in corn diet such as crude protein (CP), but the digestibility of wheat is fewer [1]. For example, the apparent metabolic energy (AME) and net energy (NE) of wheat for broiler chickens are 3331.66 Kcal/kg and 2655.29 Kcal/kg which are lower than that of corn's 3623.24 Kcal/kg and 2920.58 Kcal/kg [2]. Moreover, the significant disadvantage of wheat is that chickens fed Wheat-based diet (WD) may get lower growth performance and more inflammation which leads to diarrhea affecting the whole production system compared with which fed Corn-based diet (CD) [3]. One of the reasons is that there are more antinutritional factors, which mainly refer to non-starch polysaccharides (NSP), in wheat [4].

NSP can be divided into soluble non-starch polysaccharides (SNSP) and insoluble non-starch polysaccharides (INSP) [5], in which SNSP such as arabinoxylans as the major component show the main anti-nutritional effects which can impair chickens' growth performance and health [4, 6, 7]. In specific, the SNSP in wheat are almost twice than in corn [8], these SNSP can combined with water in gut to increase the intestinal viscosity, which may increase the feed's AME and change the microflora [8, 9]. Therefore, to reduce the anti-nutritional effects, many experiments have been conducted, and there have been several methods such as chemical method, soaking method, and antibiotics method [10–12]. However, these methods had many disadvantages such as pollution problem (chemical method) [13], lower efficiency (soaking method) [10], and policies forbidden (antibiotics method) [14]. Except these methods, there were many other suggestions, in which the efficient way should be adding enzymes.

NSPases could successfully decrease the negative effect of NSP. NSPases such as xylanase and β-glucanase can decompose the NSP in gut, therefore it may reverse wheat's negative effects on broiler chickens [15]. Meanwhile, Munyaka et al. [16] found that xylanase and β-glucanase could benefit chickens' body weight and feed conversion ratio (FCR), and there were many similar results from other experiments [17, 18], and De Keyser et al. [19] found that after NSPases supplementation, the growth performace of chickens were equal to control group. Meanwhile, NSPases had many other benefits on chickens such as and increasing the concentrations of short chain fatty acids (SCFAs) [20]. Yaghobfar and Kalantar [21] also found that NSPases could decrease the abundances of microbes in gut, which was because NSPases improved nutrient utilization and selectively reduced microbial population to maintain chickens' health [22].

However, NSP in wheat are various such as arabinoxylan, beta-glucan, cellulose, and pectin [23], therefore single NSPase could not eliminate the effects of NSP. Furthermore, antinutritional factors in wheat are not only NSP but also other factors such as allergic protein, trypsin inhibitors, and phytic acid [24–26]. Although their content is few, they also be harmful for host's health or they could cause the lower nutrients digestibility [25], and some enzymes such as phytase and protease could help host to digest these antinutritional factors to benefit host [26, 27]. Therefore, to benefit broiler chickens' health and growth, the supplied enzymes in diet should be made of various enzymes. solid-state fermentation pro-enzymes (SFP enzymes) were produced commercially by *Asperjillus Niger* after solid fermentation technology, it contains many enzymes such as xylanase, β-Glucoamylase, pectinase, β-mannanases, cellulose enzyme, α-galactosidase, and protease. It could help host to digest many antinutritional factors to eliminate the negative effects. However, the mechanisms of SFP enzymes on broilers' health should be further studied.

Therefore, this study investigated the effects of SFP enzymes on growth performance, slaughter performance, intestinal barrier and inflammation genes expression, intestinal microorganism, and SCFAs in broilers chickens fed WD from 15 days of age to 42 days of age.

MATERIALS AND METHODS

Experimental broilers and husbandry practices

There were 432 broiler chicks (Arbor Acres) from same hatchery which were randomly allocated into cages with 12 broilers per cage, and all chicks were fed from 1 day to 42 days of age in which pre-feeding period were began from day 1 to day 14. All broilers were fed in 2-level cages (150 × 70 × 60 cm). To eliminate 2-level's effects, the treatments of 2 cages in same column were same. Feed and water were supplied ad

libitum, and the light was scheduled for 1-h darkness and 23-h light per day during the whole experiment. The temperature, humidity, and air flow rate were automatically controlled by fans and cooling pad. The temperature was gradually decreased from 32°C on day 1–24°C on day 28, then maintained 24°C until day 42.

Experiment design and dietary treatment

During the first 14 days, chicks were fed CD. On day 15, the chicks were weighed individually and allocated into 36 cages (3 treatments × 6 columns × 2 cages) by random stratification based on their weight. There were three treatments which were (1) CD as control group, (2). WD, (3). Enzymes + Wheat-based diet (EWD), and each treatment had 12 replicate cages. All ingredients and composition of grower and finisher diets are presented in Table S1 according to Feeding standard of Chicken (NY/T 33–2004). All diets were made to pellet form without antibiotics.

SFP enzymes supplied

SFP enzymes were added as 0.16 kg/t in EWD group, which were provided by Hangzhou Bio-Com Biotechnology Co. LTD. The main types and content of SFP enzymes are shown in Table S2.

Sample collection

Weights of chicks were measured on day 14, 28, 42. Total feed intake was also measured on the same day. Mortality was recorded with cage, and dead broilers were weighed. On day 42, 18 broilers (6 random broilers from each treatment in higher level cage of each column) were selected based on their body weight which was closed to the average. After weighed, they were euthanized by intravenous injection of sodium pentobarbitone. Then, they were bled and sub-scalded (removing feathers) to determine net weight. After giblets removed, the broilers were reweighed to calculate dressing percentage. Other samples were collected from other 24 broilers (8 broilers from each treatment in higher level cage of each column except for the third and fourth columns in which two cages were selected) whose body weight was closed to the average. Slaughter process was similar except sub-scalded. Samples of intestinal contents from caeca and mucosa from jejunum were collected on day 42, and snap frozen in liquid nitrogen and transferred to −80°C. The organ indexes of 18 broilers were calculated by the ratio of giblets weight to body weight (%). Dressing percentage with giblets was calculated by the ratio of body weight after slaughter to body weight (%), and dressing percentage without giblets was calculated by the ratio of body weight without giblets to body weight after slaughter (%).

RNA extraction and real-time quantitative PCR detecting system (qPCR)

Tissue/Cell Total RNA Mini Kit (Gene-Better) was used to extract the mRNA of jejunal mucosa. Thereafter, 2 μg total mRNA was reverse transcribed using Prime Script RT reagent Kit (Takara). Primers were produced commercially (Sangon Biotech) (Table S3). Then, 1 μL cDNA was mixed by 5 μL SYBR Premix Ex Taq II, 0.4 μL each of forward and reverse primers (final concentration of 0.4 μM for each primer), and 3.2 μL double distilled water to react for qPCR. QuantStudio 7 Flex (Thermofisher) was used for amplification and detection under the following conditions: (1). Pre-denaturation stage: at 95°C for 30 s. (2). PCR stage (40 cycles): denaturation at 95°C for 5 s, followed by annealing and extension at 60°C for 30 s. (3). Melt curve: at 95°C for 15 s, 60°C for 1 min, and 95°C for 15 s *β-Actin* was used as reference gene to normalize the gene's Ct values. The relative gene expression was calculated using $2^{-\Delta\Delta Ct}$ method.

SCFAs concentration

Methods were according to previous study [28], ultrapure water was used to extract digesta samples from broilers' cecum (around 0.5 g). Then, they were centrifugated at 10,000 × g. After that, they were mixed with Metaphosphoric acid (25%, w/v), in which the amounts of extracts were nine times more than acid. Then, mixture was centrifugated at 12,000 × g, and supernatant was filtered through the 0.45-μm Milled-LG filter (Millipore). Finally, Agilent 7890 N gas chromatograph (Agilent) was used to analyze SCFAs.

Intestinal microbiota

DNA of cecal digesta samples were extracted by DNA isolation kit (Qiagen), primers were 338F (5′-GTGCCAGCMGCCGCGG-3') and 806R (5′-CCGT CAATTCMTTTRAG TTT-3') to amplify V3–V4 region of bacterial 16S rRNA genes. After that, samples were sequenced on the Illumina HiSeq sequencing platform (Illumina). QIIME (version 1.70) was used to analyze raw data. UPARSE (version 7.1) was used to cluster operational taxonomic units (OTUs), and UCHIME (version 7.1) was used to remove chimeric sequences. The data was deposited into NCBI Sequence Read Archive database (Accession number: PRJNA898836).

Statistical analysis

The normality of data was calculated by Shapiro-Wilk test, and based on the normality, statistical significance was determined by Student's *T* test or Mann-Whitney test using IBM SPSS Statistics. The data were adjusted based on mortalities. GraphPad Prism 8 was used to make figures of SCFAs result and mRNA gene expression result. Majorbio I-Sanger Cloud Platform (www.i-sanger.com) was used to analyze microbes. Wilcoxon rank-sum test was used to analyze alpha diversity using four indexes (Sobs, Shannon, Chao and Ace) and the significantly different microbes. Beta diversity was determined by unweighted unifrac and analysis of similarities test. Linear discriminant analysis Effect Size (LDEfSe) was used to further analyze differences.

RESULTS

Growth performance

The growth performance of all broilers ($n = 12$) in three groups is shown in Table 1. There was no significant difference during the whole experiment for the feed intake ($p > 0.05$). However, for other parameters, broilers in WD group showed lower weight gain and higher FCR from day 15 to day 28 ($p < 0.05$) compared with which in CD group. Meanwhile, broilers in EWD group showed significantly higher weight gain and lower FCR from day 15 to day 28 ($p < 0.05$) compared with which in WD group. What is more, from day 28 to 42 period, SFP enzymes showed trend to increase daily weight gain compared with wheat ($p = 0.078$), and SFP enzymes even significantly increased daily weight gain and decreased FCR ($p < 0.05$) compared with wheat during the whole experimental period (from day 15 to 42).

Slaughter performance and organ indexes

The slaughter performance of broilers ($n = 6$) in three groups is shown in Table 2. Except for the body weight before slaughter and spleen index, there was no significant difference for other data ($p > 0.05$). Compared with broilers in WD group, broilers in EWD group had significantly heavier body weight ($p < 0.05$) and lower spleen index (0.12%/0.08%) ($p < 0.05$).

Intestinal barrier and inflammation genes expression

The intestinal barrier and inflammation genes expression of jejunum of broilers ($n = 8$) in three groups are shown in Figure 1. The trends showed that compared with broilers in WD group, broilers in EWD group had higher expression levels of *OCCLUDIN* ($p = 0.075$). Meanwhile,

TABLE 1 Effects of wheat diet supplemented with SFP enzymes or not on growth performance in broiler chickens.

Parameters	1. CD group	2. WD group	3. EWD group	*p* value (WD VS CD)	*p* value (EWD VS WD)
Body weight (g/bird)					
Day 14	407.52 ± 8.51	404.82 ± 12.00	407.58 ± 10.82	0.531	0.559
Day 28	1260.55 ± 57.48	1186.05 ± 67.85	1232.66 ± 34.33	0.008	0.045
Day 42	2180.30 ± 158.51	2089.46 ± 122.30	2225.16 ± 158.65	0.130	0.028
Daily weight gain (g/bird/day)					
Day 15–28	65.62 ± 4.56	60.13 ± 4.85	63.47 ± 2.42	0.009	0.044
Day 28–42	65.70 ± 10.44	64.53 ± 6.56	70.89 ± 9.95	0.746	0.078
Day 15–42	65.66 ± 5.76	62.39 ± 4.26	67.32 ± 5.77	0.129	0.026
Daily feed intake (g/bird/day)					
Day 15–28	95.74 ± 3.26	93.77 ± 5.04	93.81 ± 4.01	0.269	0.985
Day 28–42	127.35 ± 11.63	122.24 ± 18.03	128.02 ± 14.51	0.418	0.397
Day 15–42	112.13 ± 6.91	108.53 ± 9.39	111.55 ± 8.37	0.297	0.416
Feed conversion ratio (g feed/g gain)					
Day 15–28	1.46 ± 0.06	1.56 ± 0.09	1.48 ± 0.07	0.004	0.019
Day 28–42	1.97 ± 0.23	1.90 ± 0.24	1.81 ± 0.10	0.481	0.283
Day 15–42	1.71 ± 0.10	1.74 ± 0.12	1.66 ± 0.06	0.546	0.039

Note: Data are presented as mean ± SD. CD Group: corn-based diet group, WD Group: wheat-based diet group, EWD Group: solid-state fermentation pro-enzymes (SFP enzymes) supplementation in wheat-based diet group. $n = 12$.

TABLE 2 Effects of wheat diet supplemented with SFP enzymes or not on slaughter performance and organ indexes in broiler chickens.

Parameters	1. CD group	2. WD group	3. EWD group	*p* value (WD VS CD)	*p* value (EWD VS WD)
Body weight before slaughter (g)	2179.56 ± 205.45	2213.88 ± 203.99	2432.83 ± 124.31	0.735	0.039
Body weight after slaughter (g)	2026.78 ± 205.08	2050.63 ± 172.09	2255.40 ± 89.94	0.800	0.033
Body weight without giblets (g)	1202.39 ± 126.08	1169.05 ± 95.27	1302.63 ± 71.76	0.552	0.014
Dressing percentage with giblets (%)	92.95 ± 1.89	92.71 ± 2.41	94.19 ± 0.45	0.823	0.130
Dressing percentage without giblets (%)	59.32 ± 1.57	57.19 ± 4.46	58.73 ± 1.49	0.198	0.475
Abdominal fat (%)	3.34 ± 1.32	3.74 ± 0.74	3.73 ± 0.63	0.460	0.982
Heart (%)	0.40 ± 0.07	0.44 ± 0.11	0.38 ± 0.06	0.335	0.261
Liver (%)	2.05 ± 0.24	2.34 ± 0.43	2.10 ± 0.43	0.105	0.332
Spleen (%)	0.10 ± 0.02	0.12 ± 0.02	0.08 ± 0.02	0.193	0.002
Lungs (%)	0.26 ± 0.07	0.31 ± 0.06	0.26 ± 0.06	0.161	0.167
Kidneys (%)	0.25 ± 0.06	0.22 ± 0.09	0.22 ± 0.09	0.391	0.968
Proventriculus (%)	0.34 ± 0.07	0.34 ± 0.06	0.33 ± 0.05	0.850	0.934
Gizzard (%)	1.31 ± 0.16	1.25 ± 0.09	1.18 ± 0.16	0.349	0.339

Note: Data are presented as mean ± SD. CD Group: corn-based diet group, WD Group: wheat-based diet group, EWD Group: solid-state fermentation pro-enzymes (SFP enzymes) supplementation in wheat-based diet group. $n = 6$.

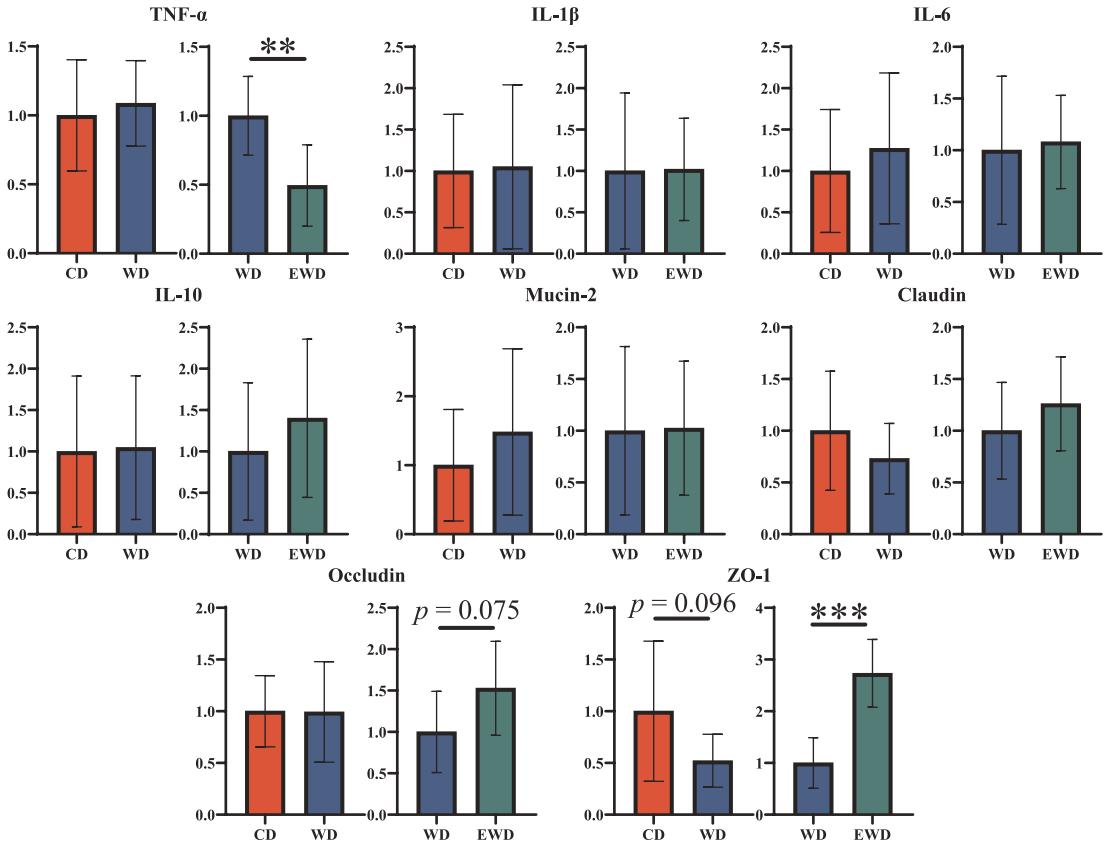

FIGURE 1 Effects of wheat diet supplemented with SFP enzymes or not on intestinal barrier and inflammation gene expression in broiler chickens. CD: corn-based diet group, WD: wheat-based diet group, EWD: solid-state fermentation pro-enzymes (SFP enzymes) supplementation in wheat-based diet group. Data are presented as mean ± SD, and statistical significance was determined by the Student's *T* test; $n = 8$. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

broilers in EWD group had two significantly differentially expressed genes which were higher expression of *ZO-1* ($p < 0.001$) and lower expression of *TNF-α* ($p < 0.01$) compared with broilers in WD group, in which the trend also showed that broilers in WD group had lower expression of *ZO-1* compared with which in CD group ($p = 0.096$). For the expressions of other genes, there were no significant differences ($p > 0.05$).

Concentrations of SCFAs

The concentrations of SCFAs in the cecum of broilers in three groups are shown in Figure 2. Broilers in CD groups had significantly lower concentrations of acetate, butyrate, and total SCFAs compared with which in WD group ($p < 0.05$). The trends showed that for acetate and total SCFAs, broilers in EWD group had lower concentrations compared with which in WD group but not to the control level, and for butyrate, broilers in EWD group had highest concentration. For other parameters, there were no significant differences.

Intestinal microbes in broilers

The microbes in cecum of broilers ($n = 8$) in three groups are shown in Figure 3. Both cecal microbes of chickens in WD group and EWD group were similar, and microbes in both two groups were different from that in CD group (Figure 3A). Meanwhile, even though broilers in three groups had similar OTUs (Figure 3B), broilers in CD group had highest abundance of microbes and broilers in EWD group had lowest data (Figure 3C). The specific different microbes at genus and phylum level are shown in Figure 3D, in which broilers in EWD group showed much similar community with broilers in CD group. For example, *unclassified_f__Lachnospiraceae* showed higher both in broilers in CD group and EWD group and Actinobacteriota showed opposite trends. Moreover, 10 microbes were found significantly differential in TOP 50 abundance of microbes at genus level (WD VS CD), and only two microbes were found significantly differential comparing between EWD group and WD group (Figure 4A). Thereafter, we further analyzed the specific abundance of each significantly differential microbes (Figure 4B), in which only four genera in WD VS CD result which was significantly decreased by wheat can be increased by SFP enzymes, and only two genera were significantly increased by wheat in which only *Bifidobacterium* could be decreased by SFP enzymes. Other genera were all decreased by wheat, but they cannot be increased by SFP enzymes. In EWD VS WD result, *Eubacterium_hallii_group* only significantly increased in broilers in EWD group, and *Ruminococcus_gauvreauii_group* significantly abundant in both CD croup and EWD group. The main microbes of each group are shown in Figure 4C.

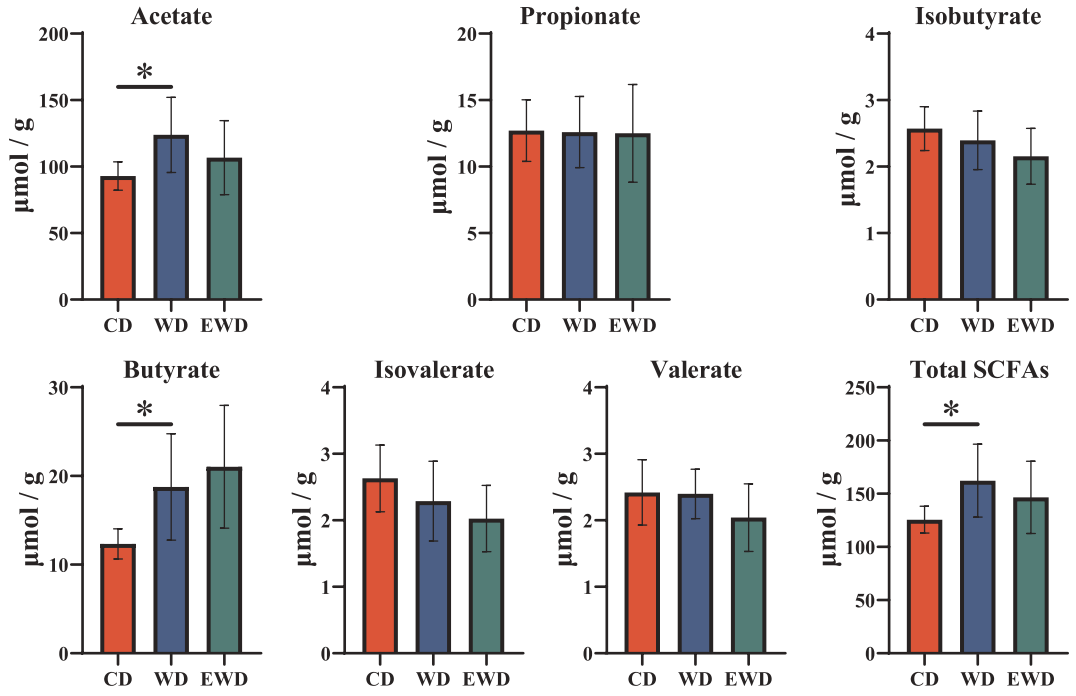

FIGURE 2 Effects of wheat diet supplemented with SFP enzymes or not on the concentration of short chain fatty acids in broiler chickens. CD: corn-based diet group, WD: wheat-based diet group, EWD: solid-state fermentation pro-enzymes (SFP enzymes) supplementation in wheat-based diet group. Unit: μmol/g digesta. Data are presented as mean ± SD, and statistical significance was determined by the Student's *T* test; $n = 8$. *$p < 0.05$.

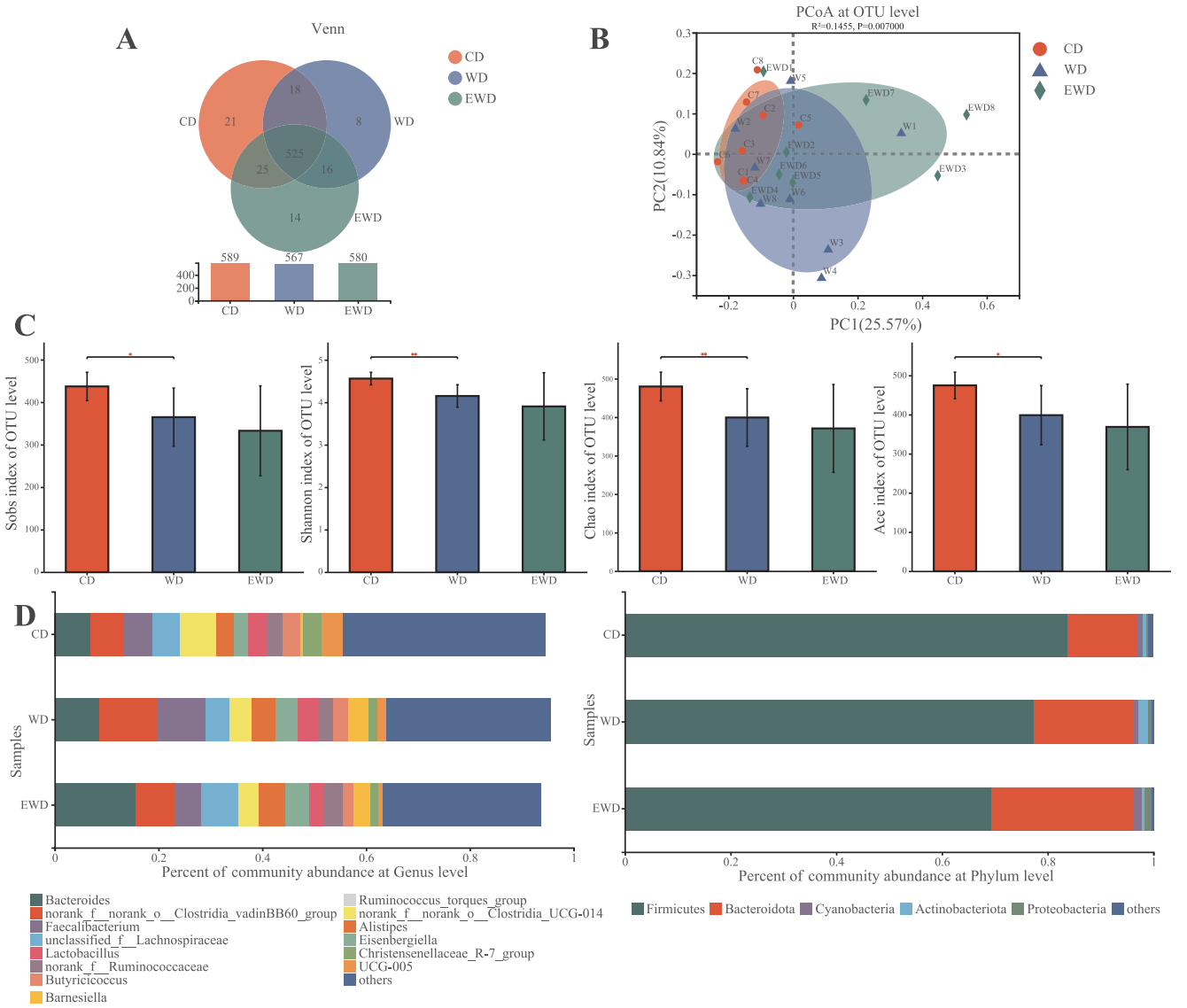

FIGURE 3 Effects of wheat diet supplemented with SFP enzymes or not on intestinal microbiome in broiler chickens. (A) Venn diagrams of each group. (B) PCoA results of each group. (C) α-Diversity of each group. (D) Community analysis of each group. CD: corn-based diet group, WD: wheat-based diet group, EWD: solid-state fermentation pro-enzymes (SFP enzymes) supplementation in wheat-based diet group. Data are presented as mean ± SD, and statistical significance was determined by the Wilcoxon rank-sum test; $n = 8$. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

DISCUSSION

Basically, there were many studies showed the effects of WD with or without enzymes on broiler chickens [16, 29–31]. However, the results were inconformity. Our result showed that wheat could influence chickens' growth performance and health, and SFP enzymes could reverse some of these negative effects.

Firstly, although the differences from day 28 to day 42 were not significant, the trends were same as the significantly differential parameters which were from day 15 to day 28. In specific, our study found that broiler chickens fed WD had lower weight gain and higher FCR but no significant effect on feed intake compared with CD. This result is similar with other studies [30, 32]. The reason is that NSP in wheat can increase the intestinal viscosity to reduce the nutrients digestibility and absorption [33]. Meanwhile, in this experiment, the wheat particle size was coarse which could cause higher feed intake [34]. Besides, some studies found that NSPases can reduce the effect of NSP's negative effects to benefit chickens' growth performance [1, 16] which are similar to our results. In our experiment, SFP enzymes supplementation could improve chickens' weight gain and FCR to the control group's (CD) level or even better especially from day 15 to day 28 which was significantly different. Meanwhile, although from day 28 to day 42, the difference was not significant, SFP enzymes had

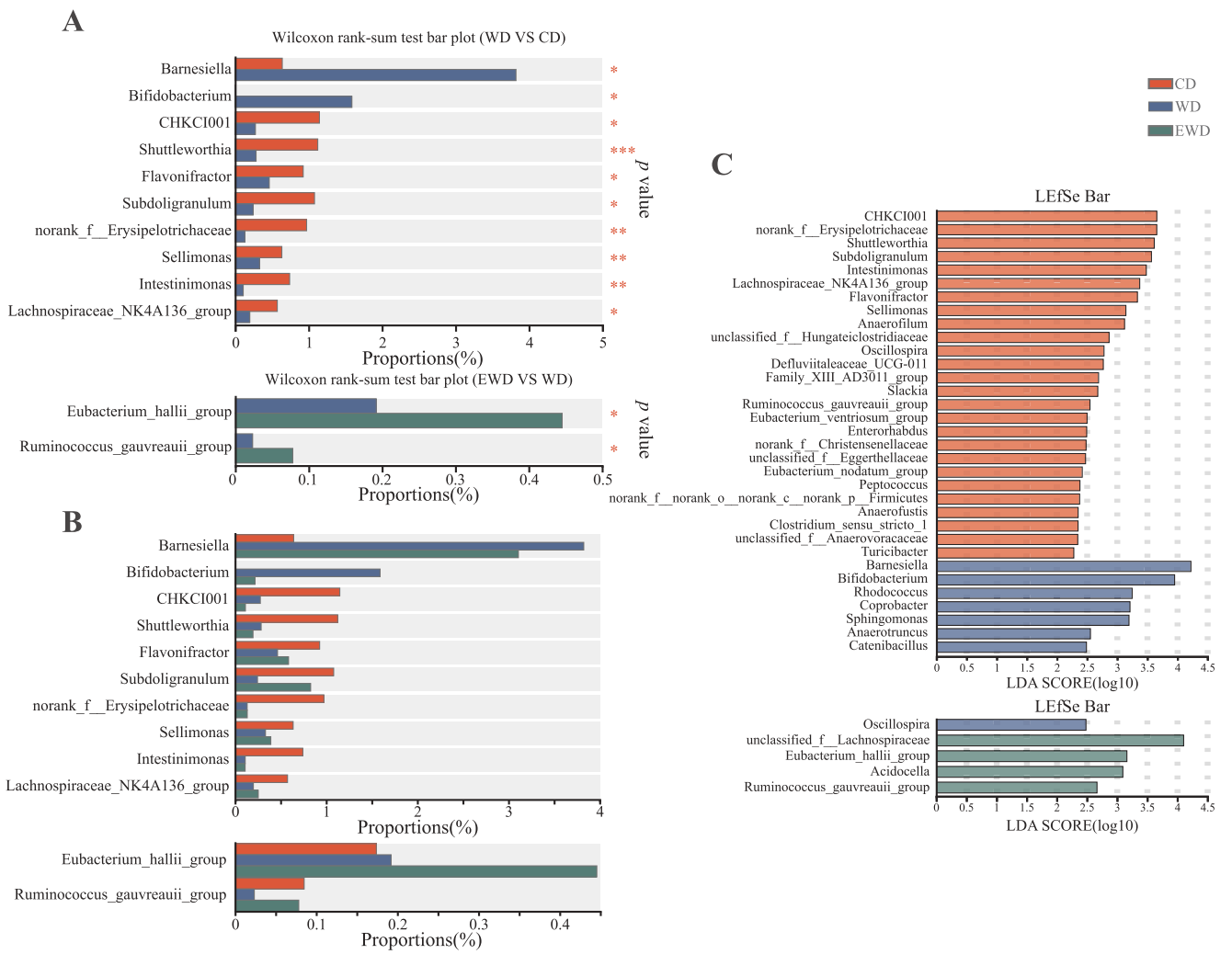

FIGURE 4 The specific significantly differential microbes. (A) Significantly differential microbes in different groups (WD VS CD, EWD VS WD). (B) The abundances of significantly differential microbes in three groups. (C) LDfSe results of each group. CD: corn-based diet group, WD: wheat-based diet group, EWD: solid-state fermentation pro-enzymes (SFP enzymes) supplementation in WD group, LDfSe: Linear discriminant analysis Effect Size. Data are presented as proportions' mean and statistical significance was determined by the Wilcoxon rank-sum test; $n = 8$. *$p < 0.05$ and **$p < 0.01$.

trends to increase weight gain during this period, and in the whole period, the SFP enzymes showed significantly effect on weight gain and FCR improving. The better growth performance for the chickens in EWD group is because SFP enzymes help chickens to digest NSP. Specifically, firstly, the digested NSP are nutrients to benefit chickens [16, 35]. Then, there is NSP in corn to affect chickens, SFP enzymes make the content of NSP in wheat even lower than in corn to benefit chickens [33]. However, some studies found that NSPases had no effect on chickens' growth performance [19, 21], whose reasons could be enzymes' concentration or activity or other factors. Therefore, SFP enzymes supplementation improved broiler chickens' growth performance especially in early stage (15–24 days of age).

Slaughter performance and organ indexes of broiler chickens were significant for their commercial value and health. Previous studies showed that WD with or without enzymes do not change chickens' slaughter performance [30, 36]. Similarly, in our result, there was no significant difference for chickens' slaughter performance except body weight. The reason for different body weight is linked to broilers' growth performance. What is more, previous studies found that wheat with or without enzymes did not change the organ indexes compared with corn [30, 37]. In contrast, the present result showed that wheat could increase the spleen index which is caused by the higher inflammation [38], and SFP enzymes could reverse this negative effect.

To determine the intestinal health, we detected the related genes expressions. We found that wheat might promote inflammation by enhancing the expression of *TNF-α* which is a kind of pro-inflammatory cytokine as a major regulator of inflammatory responses main factors leading to inflammation [39]. Meanwhile, wheat had

trend to reduce the expression of *ZO-1* which is a main factor of tight junction to benefit intestinal barrier [40]. Contrarily, SFP enzymes showed ability to significantly increase the expression of *ZO-1* and decrease the expression of *TNF-α*. Furthermore, it can enhance the expression of *OCCLUDIN* which is another representative tight junction to maintain gut barrier function [41]. Therefore, it was showed that SFP enzymes can adjust these genes expressions to maintain intestinal health. Similarly, Chuang et al. [42] found that NSPases can promote the expreesions of *OCCLUDIN* and *CLAUDIN*, and decrease the expreesions of *IL-1β* and *IL-6*, and Gao et al [43] found that NSPases can inhibit the expression of *TNF-α*. To sum up, wheat negatively affected the intestinal health of broilers by affecting the intestinal barrier function and inflammation, but SFP enzymes can eliminate this effect.

The SCFAs are affected by many reasons such as microbes, environment, and broiler's age, and it can affect animals gut barrier function, energy metabolism, and immunity [44]. In this study, WD resulted in higher concentrations of acetic, butyrate, and total SCFAs but lower or similar content of other SCFAs, which is similar with other research [1, 45]. SFP enzymes could reverse the increasing of acetate and total SCFAs caused by wheat but not to the level of control group, and SFP enzymes did not decrease the concentration of butyrate which is significant to animals' immune system such as inhibiting pro-inflammatory immune cells of whole intestine [46, 47]. Many researchers found that enzymes increase the content of acetic, butyrate, and total SCFAs [20, 48, 49] which are similar with this experiment's result. Differently, based on the discovery of Józefiak et al. [50] which showed that SCFAs in chickens fed barley or oats could not be affected, our result may rely on the different types of enzymes and wheat.

The microbes in the intestines of animals are considered essential for gut health and nutrient absorption. The changes of microbes in cecum can affect the health of not only whole intestine but also whole body [51]. Therefore, to find the bacterial evidence leading to the differences above, we analyzed the microbes in broilers' cecum. The result showed that broilers in CD group had significantly highest α-diversity and chickens in EWD group's α-diversity were lowest. Therefore, wheat could decrease the diversity of cecal microbes. SFP enzymes seem focused on affecting particular microbes, which is because although enzymes lower the intestinal viscosity to benefit microbes, they mainly promote the growth of some competitive probiotics communities and limit the nutrients absorbed by other bacteria [16]. Therefore, fewer cecal bacteria existed in the chickens of EWD group. The community abundance at Phylum level results showed that wheat would change the composition of microbes such as decreasing Firmicutes and increasing Bacteroidota. SFP enzymes further decreased the microbial diversity but benefited the growth of some probiotics. The ratio of Firmicutes to Bacteroidota is linked to fat produced, and obesity individuals have higher ratio [52]. Therefore, broilers fed WD may have more adipose tissue. Besides, based on the community abundance results, wheat promoted the growth of Actinobacteriota which is main pathogen of animals [53], and SFP enzymes could decrease it to normal level to benefit animals. What is more, SFP enzymes increased the abundance of *unclassified_f__Lachnospiraceae* to the high abundance even higher than chickens in CD group. *Unclassified_f__Lachnospiraceae* is negatively correlated with the expression of *TNF-α* [54], which is the reason of the enhancing of its expression in the broilers of WD and CD group and SFP enzymes reduced its expression.

In specific, in 10 differentially abundant microbes (WD VS CD), there were four kinds of bacteria decreased by wheat but increased by SFP enzymes which were *Flavonifractor*, *Sellimonas*, *Lachnospiraceae_NK4A136_group*, and *Subdoligranulum* in which *Flavonifractor* is related to carbohydrate metabolism to promote growth [55], and *Sellimonas* is a potential biomarker to adjust intestinal recovery [56]. *Sellimonas* can be decreased when the host's gut is damaged by arsenic exposure [57], which shows that the abundance of *Sellimonas* is necessary for intestinal health. Similarly, Zhang et al. [58] also found that CD can increase the abundance of *Sellimonas*. Moreover, *Lachnospiraceae_NK4A136_group* as a kind of probiotic can maintain intestinal health and improve broilers' growth [59, 60]. Finally, *Subdoligranulum* is found to colonize in healthy host to maintain animals' metabolism especially SCFAs [61], and it is linked to metabolic health improving [62]. Therefore, WD reduced the proportion of these probiotics which affected the intestinal health and growth performance, and SFP enzymes promoted the proliferation of these intestinal probiotics which related to carbohydrate metabolism and gut health in WD. These may be important reasons why SFP enzymes increase production performance by increasing these taxa of microorganisms. However, the detailed microbial mechanism needs further analysis. What is more, the abundances of other probiotics were decreased by wheat, but SFP enzymes could not make them colonize again. What is more, many studies showed that inulin and fructooligosaccharide, which are two kinds of NSPs in wheat, can stimulate *Bifidobacterium* to absorbed Fe [63, 64]. Tako et al. [31] also found that wheat promotes *Bifidobacterium* in broiler chickens. In our result, broilers in WD group had significantly higher *Bifidobacterium*, and SFP enzymes could decrease them. The reason might be the digestion of NSP of broilers in EWD group. Moreover, *Bifidobacterium* is a kind of SCFAs producer which can explain the higher

concentrations of acetate and total SCFAs in WD group [63]. Meanwhile, *Barnesiella*, which was increased by wheat and did not be affected by SFP enzymes, has ability to produce butyrate [65]. Therefore, the chickens in WD and EWD group had higher butyrate. Moreover, CD also enhanced the abundances of some SCFAs producer which were *norank_f__Erysipelotrichaceae* [66], *Shuttleworthia* [67], and *Intestinimonas* [68] to benefit chickens' growth, but SFP enzymes could not affect them after which were decreased by wheat. Therefore, SFP enzymes seems to affect the proliferations of specific probiotics. Finally, in 2 differentially abundant microbes (EWD VS WD), the abundance of *Ruminococcus_gauvreauii_group* only significantly decreased in WD group compared with other two groups, and this genus is well known as a SCFAs producer [69], which may explain why broilers in CD group had similar concentrations of other SCFAs compared with which in WD group. This genus is also showed as the probiotic to maintain hosts' health such as the strong associate between the deficiency of it and coronary artery disease [70], which proves the benefits of SFP enzymes again. Moreover, *Eubacterium_hallii_group* was only enriched by SFP enzymes in our result which is a butyrate producer [71], which can explain the highest butyrate of broilers in EWD group, meanwhile, *Eubacterium_hallii_group* was found that it is positively associated with *ZO-1* and *OCCLUDIN* [72] which can explain the highest expressions of these two genes in broilers in EWD group, and it is necessary to maintain gut metabolic balance [71], which again showed that SFP enzymes seem focused on affecting particular probiotics. Therefore, compared with broilers in WD group, chickens in CD group had more probiotics to benefit health and growth, and SFP enzymes could increase some of these probiotics. Meanwhile, proliferation of all probiotics explains the results of mRNA expression, SCFAs concentrations and intestinal health or growth performance improving.

CONCLUSION

In this study, wheat could decrease weight gain and increase FCR of broiler chickens, and it also led to inflammation, whereas SFP enzymes could reverse these negative effects. Meanwhile, SFP enzymes supplementation in WD could benefit tight junction genes expression and inhibit *TNF-α* expression to benefit chickens. Finally, SFP enzymes could promote proliferation of probiotics which improved SCFAs production and intestinal health in wheat-base diet chickens.

To sum up, WD results in lower growth performance than CD, and SFP enzymes supplementation in WD benefits chickens' growth performance and health by affecting the growth of particular microbes.

AUTHOR CONTRIBUTIONS

Jiaheng Li and Guosong Bai: Conceptualization; data curation; formal analysis; writing & hyphen; original draft. **Gao Yan**: Software; writing & hyphen; review & editing. **Qingtao Gao**: Formal analysis; methodology. **Ruqing Zhong**: Resources; supervision; writing & hyphen; review & editing. **Liang Chen and Yunlong Wang**: Resources. **Teng Ma**: Funding acquisition; Project administration; resources; supervision; writing & hyphen; review & editing. **Hongfu Zhang**: Funding acquisition; methodology; project administration; resources.

ACKNOWLEDGMENTS

This work was supported by China Agriculture Research System of Ministry of Finance (MOF) and Ministry of Agriculture and Rural Affairs (MARA) (CARS-41), National Key Laboratory of Animal Nutrition (2004DA125184G2102), and Agricultural Science and Technology Innovation Program (ASTIPIAS07).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

ETHICS STATEMENT

The Animal Ethics Committee of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences approved the experimental protocol (Ethics Approval Code: IAS2021-233).

ORCID

Jiaheng Li https://orcid.org/0009-0004-8903-6663
Teng Ma https://orcid.org/0000-0003-0014-1105

REFERENCES

1. Kiarie, E., Romero, L., & Ravindran, V. (2014). Growth performance, nutrient utilization, and digesta characteristics in broiler chickens fed corn or wheat diets without or with supplemental xylanase. *Poultry Science*, *93*(5), 1186–1196. https://doi.org/10.3382/ps.2013-03715
2. Wu, S-B., Swick, R. A., Noblet, J., Rodgers, N., Cadogan, D., & Choct, M. (2019). Net energy prediction and energy efficiency of feed for broiler chickens. *Poultry Science*, *98*(3), 1222–1234. https://doi.org/10.3382/ps/pey442
3. Steenfeldt, S. (2001). The dietary effect of different wheat cultivars for broiler chickens. *British Poultry Science*, *42*(5), 595–609. https://doi.org/10.1080/00071660120088416
4. Annison, G. (1993). The role of wheat non-starch polysaccharides in broiler nutrition. *Australian Journal of Agricultural Research*, *44*(3), 405–422. https://doi.org/10.1071/ar9930405
5. Molist, F., De Segura, A. G., Gasa, J., Hermes, R., Manzanilla, E., Anguita, M., & Pérez, J. (2009). Effects of the insoluble and soluble dietary fibre on the physicochemical properties of digesta and the microbial activity in early weaned piglets. *Animal Feed Science and Technology*, *149*(3–4), 346–353. https://doi.org/10.1016/j.anifeedsci.2008.06.015

6. Choct, M., Kocher, A., Waters, D., Pettersson, D., & Ross, G. (2004). A comparison of three xylanases on the nutritive value of two wheats for broiler chickens. *The British Journal of Nutrition*, *92*(1), 53–61. https://doi.org/10.1079/bjn20041166
7. Nguyen, H. T., Bedford, M. R., Wu, S-B., & Morgan, N. K. (2022). Dietary soluble non-starch polysaccharide level influences performance, nutrient utilisation and disappearance of non-starch polysaccharides in broiler chickens. *Animals*, *12*(5), 547. https://doi.org/10.3390/ani12050547
8. Marcotuli, I., Colasuonno, P., Hsieh, Y. S., Fincher, G. B., & Gadaleta, A. (2020). Non-starch polysaccharides in durum wheat: A review. *International Journal of Molecular Sciences*, *21*(8), 2933. https://doi.org/10.3390/ijms21082933
9. Kermanshahi, H., Shakouri, M. D., & Daneshmand, A. (2018). Effects of non-starch polysaccharides in semi-purified diets on performance, serum metabolites, gastrointestinal morphology, and microbial population of male broiler chickens. *Livestock Science*, *214*, 93–97. https://doi.org/10.1016/j.livsci.2018.04.012
10. Ward, A. T., & Marquardt, R. R. (1989). Effect of various treatments on the nutritional value of rye or rye fractions. *British Poultry Science*, *29*(4), 709–720. https://doi.org/10.1080/00071668808417099
11. Esteve-Garcia, E., Brufau, J., Perez-Vendrell, A., Miquel, A., & Duven, K. (1997). Bioefficacy of enzyme preparations containing beta-glucanase and xylanase activities in broiler diets based on barley or wheat, in combination with flavomycin. *Poultry Science*, *76*(12), 1728–1737. https://doi.org/10.1093/ps/76.12.1728
12. Miafo, A-P. T., Koubala, B. B., Kansci, G., & Muralikrishna, G. (2019). Free sugars and non-starch polysaccharides–phenolic acid complexes from bran, spent grain and sorghum seeds. *Journal of Cereal Science*, *87*, 124–131. https://doi.org/10.1016/j.jcs.2019.02.002
13. Tapiwa, K. A. (2019). Polyphenols in sorghum, their effects on broilers and methods of reducing their effects: A review. *Biomedical Journal of Scientific & Technical Research*, *19*(1), 14058–14061. https://doi.org/10.26717/bjstr.2019.19.003243
14. Annison, G., & Choct, M. (1991). Anti-nutritive activities of cereal non-starch polysaccharides in broiler diets and strategies minimizing their effects. *World's Poultry Science Journal*, *47*(3), 232–242. https://doi.org/10.1079/wps19910019
15. Choct, M. (2006). Enzymes for the feed industry: Past, present and future. *World's Poultry Science Journal*, *62*(1), 5–16. https://doi.org/10.1079/wps200480
16. Munyaka, P., Nandha, N., Kiarie, E., Nyachoti, C., & Khafipour, E. (2016). Impact of combined β-glucanase and xylanase enzymes on growth performance, nutrients utilization and gut microbiota in broiler chickens fed corn or wheat-based diets. *Poultry Science*, *95*(3), 528–540. https://doi.org/10.3382/ps/pev333
17. Iji, P., Hughes, R. J., Choct, M., & Tivey, D. (2001). Intestinal structure and function of broiler chickens on wheat-based diets supplemented with a microbial enzyme. *Asian-Australasian Journal of Animal Sciences*, *14*(1), 54–60. https://doi.org/10.5713/ajas.2001.54
18. Wu, Y., Ravindran, V., Thomas, D., Birtles, M., & Hendriks, W. (2004). Influence of phytase and xylanase, individually or in combination, on performance, apparent metabolisable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate level of phosphorus. *British Poultry Science*, *45*(1), 76–84. https://doi.org/10.1080/00071660410001668897
19. De Keyser, K., Kuterna, L., Kaczmarek, S., Rutkowski, A., & Vanderbeke, E. (2016). High dosing nsp enzymes for total protein and digestible amino acid reformulation in a wheat/corn/soybean meal diet in broilers. *The Journal of Applied Poultry Research*, *25*(2), 239–246. https://doi.org/10.3382/japr/pfw006
20. Wang, Z., Qiao, S., Lu, W., & Li, D. (2005). Effects of enzyme supplementation on performance, nutrient digestibility, gastrointestinal morphology, and volatile fatty acid profiles in the hindgut of broilers fed wheat-based diets. *Poultry Science*, *84*(6), 875–881. https://doi.org/10.1093/ps/84.6.875
21. Yaghobfar, A., & Kalantar, M. (2017). Effect of non-starch polysaccharide (nsp) of wheat and barley supplemented with exogenous enzyme blend on growth performance, gut microbial, pancreatic enzyme activities, expression of glucose transporter (sglt1) and mucin producer (muc2) genes of broiler chickens. *Brazilian Journal of Poultry Science*, *19*(4), 629–638. https://doi.org/10.1590/1806-9061-2016-0441
22. Huyghebaert, G., Ducatelle, R., & Van Immerseel, F. (2011). An update on alternatives to antimicrobial growth promoters for broilers. *The Veterinary Journal*, *187*(2), 182–188. https://doi.org/10.1016/j.tvjl.2010.03.003
23. Rosenfelder, P., Eklund, M., & Mosenthin, R. (2013). Nutritive value of wheat and wheat by-products in pig nutrition: A review. *Animal Feed Science and Technology*, *185*(3–4), 107–125. https://doi.org/10.1016/j.anifeedsci.2013.07.011
24. Jenab, M., & Thompson, L. U. (1998). The influence of phytic acid in wheat bran on early biomarkers of colon carcinogenesis. *Carcinogenesis*, *19*(6), 1087–1092. https://doi.org/10.1093/carcin/19.6.1087
25. Nadeem, M., Anjum, F. M., Amir, R. M., Khan, M. R., Hussain, S., & Javed, M. S. (2010). An overview of anti-nutritional factors in cereal grains with special reference to wheat-a review. *Pakistan Journal of Food Sciences*, *20*, 54–61.
26. Stefańska, I., Piasecka-Jóźwiak, K., Kotyrba, D., Kolenda, M., & Stecka, K. M. (2016). Selection of lactic acid bacteria strains for the hydrolysis of allergenic proteins of wheat flour. *Journal of the Science of Food and Agriculture*, *96*(11), 3897–3905. https://doi.org/10.1002/jsfa.7588
27. Moss, A. F., Chrystal, P. V., Truong, H. H., Liu, S. Y., & Selle, P. H. (2017). Effects of phytase inclusions in diets containing ground wheat or 12.5% whole wheat (pre-and post-pellet) and phytase and protease additions, individually and in combination, to diets containing 12.5% pre-pellet whole wheat on the performance of broiler chickens. *Animal Feed Science and Technology*, *234*, 139–150. https://doi.org/10.1016/j.anifeedsci.2017.09.007
28. Tang, S., Zhong, R., Yin, C., Su, D., Xie, J., Chen, L., Liu, L., & Zhang, H. (2021). Exposure to high aerial ammonia causes hindgut dysbiotic microbiota and alterations of microbiota-derived metabolites in growing pigs. *Frontiers in Nutrition*, *8*, 8. https://doi.org/10.3389/fnut.2021.689818
29. Cowieson, A., Ptak, A., Maćkowiak, P., Sassek, M., Pruszyńska-Oszmałek, E., Żyła, K., Świątkiewicz, S., Kaczmarek, S., & Józefiak, D. (2013). The effect of microbial phytase and myo-inositol on performance and blood biochemistry of broiler chickens fed wheat/corn-based diets. *Poultry Science*, *92*(8), 2124–2134. https://doi.org/10.3382/ps.2013-03140
30. El-Katcha, M. I., Soltan, M. A., El-Kaney, H. F., & Karwarie, E. (2014). Growth performance, blood parameters, immune response and carcass traits of broiler chicks fed on graded levels of wheat instead of corn without or with enzyme supplementation. *Alexandria Journal of Veterinary Sciences*, *40*(1), 95–111. https://doi.org/10.5455/ajvs.48232
31. Tako, E., Glahn, R. P., Knez, M., & Stangoulis, J. C. (2014). The effect of wheat prebiotics on the gut bacterial population and iron status of iron deficient broiler chickens. *Nutrition Journal*, *13*, 1–10. https://doi.org/10.1186/1475-2891-13-58
32. Liu, S., Cadogan, D., Péron, A., Truong, H., & Selle, P. (2014). Effects of phytase supplementation on growth performance, nutrient utilization and digestive dynamics of starch and protein in broiler chickens offered maize-sorghum-and wheat-based diets. *Animal Feed Science and Technology*, *197*, 164–175. https://doi.org/10.1016/j.anifeedsci.2014.08.005
33. Polovinski-Horvatović, M. (2021). A mini review of the effects of nsp and exogenous enzymes in broiler diets on digestibility and

some intestinal functions. *Contemporary Agriculture*, *70*(3–4), 116–122. https://doi.org/10.2478/contagri-2021-0017

34. Abdollahi, M. R., Zaefarian, F., Hunt, H., Anwar, M. N., Thomas, D. G., & Ravindran, V. (2019). Wheat particle size, insoluble fibre sources and whole wheat feeding influence gizzard musculature and nutrient utilisation to different extents in broiler chickens. *Journal of Animal Physiology and Animal Nutrition*, *103*(1), 146–161. https://doi.org/10.1111/jpn.13019
35. Aftab, U., & Bedford, M. (2018). The use of nsp enzymes in poultry nutrition: Myths and realities. *World's Poultry Science Journal*, *74*(2), 277–286. https://doi.org/10.1017/s0043933918000272
36. Hussein, E., Suliman, G., Alowaimer, A., Ahmed, S., Abd El-Hack, M., Taha, A., & Swelum, A. (2020). Growth, carcass characteristics, and meat quality of broilers fed a low-energy diet supplemented with a multienzyme preparation. *Poultry Science*, *99*(4), 1988–1994. https://doi.org/10.1016/j.psj.2019.09.007
37. Ghiasvand, A., Khatibjoo, A., Mohammadi, Y., Akbari Gharaei, M., & Shirzadi, H. (2021). Effect of fennel essential oil on performance, serum biochemistry, immunity, ileum morphology and microbial population, and meat quality of broiler chickens fed corn or wheat-based diet. *British Poultry Science*, *62*(4), 562–572. https://doi.org/10.1080/00071668.2021.1883551
38. Yang, L., Liu, G., Zhu, X., Luo, Y., Shang, Y., & Gu, X.-L. (2019). The anti-inflammatory and antioxidant effects of leonurine hydrochloride after lipopolysaccharide challenge in broiler chicks. *Poultry Science*, *98*(4), 1648–1657. https://doi.org/10.3382/ps/pey532
39. Jang, D-I., Lee, A-H., Shin, H-Y., Song, H-R., Park, J-H., Kang, T-B., Lee, S-R., & Yang, S-H. (2021). The role of tumor necrosis factor alpha (tnf-α) in autoimmune disease and current tnf-α inhibitors in therapeutics. *International Journal of Molecular Sciences*, *22*(5), 2719. https://doi.org/10.3390/ijms22052719
40. Li, Q., Gabler, N. K., Loving, C. L., Gould, S. A., & Patience, J. F. (2018). A dietary carbohydrase blend improved intestinal barrier function and growth rate in weaned pigs fed higher fiber diets. *Journal of Animal Science*, *96*, 5233–5243. https://doi.org/10.1093/jas/sky383
41. Feldman, G. J., Mullin, J. M., & Ryan, M. P. (2005). Occludin: Structure, function and regulation. *Advanced Drug Delivery Reviews*, *57*(6), 883–917. https://doi.org/10.1016/j.addr.2005.01.009
42. Chuang, W.-Y., Lin, L.-J., Hsieh, Y.-C., Chang, S.-C., & Lee, T.-T. (2021). Effects of saccharomyces cerevisiae and phytase co-fermentation of wheat bran on growth, antioxidation, immunity and intestinal morphology in broilers. *Animal Bioscience*, *34*(7), 1157–1168. https://doi.org/10.5713/ajas.20.0399
43. Gao, Q., Wang, Y., Li, J., Bai, G., Liu, L., Zhong, R., Ma, T., Pan, H., & Zhang, H. (2022). Supplementation of multi-enzymes alone or combined with inactivated lactobacillus benefits growth performance and gut microbiota in broilers fed wheat diets. *Frontiers in Microbiology*, *13*, 927932. https://doi.org/10.3389/fmicb.2022.927932
44. Den Besten, G., Van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D-J., & Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research*, *54*(9), 2325–2340. https://doi.org/10.1194/jlr.r036012
45. Biggs, P., & Parsons, C. (2009). The effects of whole grains on nutrient digestibilities, growth performance, and cecal short-chain fatty acid concentrations in young chicks fed ground corn-soybean meal diets. *Poultry Science*, *88*(9), 1893–1905. https://doi.org/10.3382/ps.2008-00437
46. Meijer, K., De Vos, P., & Priebe, M. G. (2010). Butyrate and other short-chain fatty acids as modulators of immunity: What relevance for health? *Current Opinion in Clinical Nutrition and Metabolic Care*, *13*(6), 715–721. https://doi.org/10.1097/mco.0b013e32833eebe5
47. Yip, W., Hughes, M. R., Li, Y., Cait, A., Hirst, M., Mohn, W. W., & Mcnagny, K. M. (2021). Butyrate shapes immune cell fate and function in allergic asthma. *Frontiers in Immunology*, *12*, 299. https://doi.org/10.3389/fimmu.2021.628453
48. Yacoubi, N., Van Immerseel, F., Ducatelle, R., Rhayat, L., Bonnin, E., & Saulnier, L. (2016). Water-soluble fractions obtained by enzymatic treatment of wheat grains promote short chain fatty acids production by broiler cecal microbiota. *Animal Feed Science and Technology*, *218*, 110–119. https://doi.org/10.1016/j.anifeedsci.2016.05.016
49. Lee, S., Apajalahti, J., Vienola, K., González-Ortiz, G., Fontes, C., & Bedford, M. (2017). Age and dietary xylanase supplementation affects ileal sugar residues and short chain fatty acid concentration in the ileum and caecum of broiler chickens. *Animal Feed Science and Technology*, *234*, 29–42. https://doi.org/10.1016/j.anifeedsci.2017.07.017
50. Józefiak, D., Rutkowski, A., Jensen, B., & Engberg, R. (2006). The effect of β-glucanase supplementation of barley-and oat-based diets on growth performance and fermentation in broiler chicken gastrointestinal tract. *British Poultry Science*, *47*(1), 57–64. https://doi.org/10.1080/00071660500475145
51. Yuan, S., Wang, K.-S., Meng, H., Hou, X.-T., Xue, J.-C., Liu, B.-H., Cheng, W.-W., Li, J., Zhang, H.-M., Nan, J.-X., & Zhang, Q. G. (2023). The gut microbes in inflammatory bowel disease: Future novel target option for pharmacotherapy. *Biomedicine & Pharmacotherapy*, *165*, 114893. https://doi.org/10.1016/j.biopha.2023.114893
52. Ley, R. E., Turnbaugh, P. J., Klein, S., & Gordon, J. I. (2006). Human gut microbes associated with obesity. *Nature*, *444*(7122), 1022–1023. https://doi.org/10.1038/4441022a
53. Schaechter, M. (2009). *Encyclopedia of microbiology*. Academic Press.
54. Wang, Y., Tao, H., Huang, H., Xiao, Y., Wu, X., Li, M., Shen, J., Xiao, Z., Zhao, Y., Du, F., Ji, H., Chen, Y., Cho, C. H., & Wang, S. (2021). The dietary supplement rhodiola crenulata extract alleviates dextran sulfate sodium-induced colitis in mice through anti-inflammation, mediating gut barrier integrity and reshaping the gut microbiome. *Food & Function*, *12*(7), 3142–3158. https://doi.org/10.1039/d0fo03061a
55. Meng, Q., Sun, S., Luo, Z., Shi, B., Shan, A., & Cheng, B. (2019). Maternal dietary resveratrol alleviates weaning-associated diarrhea and intestinal inflammation in pig offspring by changing intestinal gene expression and microbiota. *Food & Function*, *10*(9), 5626–5643. https://doi.org/10.1039/c9fo00637k
56. Muñoz, M., Guerrero-Araya, E., Cortés-Tapia, C., Plaza-Garrido, A., Lawley, T. D., & Paredes-Sabja, D. (2020). Comprehensive genome analyses of sellimonas intestinalis, a potential biomarker of homeostasis gut recovery. *Microbial Genomics*, *6*(12). https://doi.org/10.1099/mgen.0.000476
57. Zhong, G., Wan, F., Lan, J., Jiang, X., Wu, S., Pan, J., Tang, Z., & Hu, L. (2021). Arsenic exposure induces intestinal barrier damage and consequent activation of gut-liver axis leading to inflammation and pyroptosis of liver in ducks. *Science of the Total Environment*, *788*, 147780. https://doi.org/10.1016/j.scitotenv.2021.147780
58. Zhang, Y., Liu, Y., Li, J., Xing, T., Jiang, Y., Zhang, L., & Gao, F. (2020). Dietary resistant starch modifies the composition and function of caecal microbiota of broilers. *Journal of the Science of Food and Agriculture*, *100*(3), 1274–1284. https://doi.org/10.1002/jsfa.10139
59. Ma, L., Ni, Y., Wang, Z., Tu, W., Ni, L., Zhuge, F., Zheng, A., Hu, L., Zhao, Y., Zheng, L., & Fu, Z. (2020). Spermidine improves gut barrier integrity and gut microbiota function in diet-induced obese mice. *Gut Microbes*, *12*(1), 1832857. https://doi.org/10.1080/19490976.2020.1832857
60. Wu M.-R., Chou T.-S., Huang C.-Y., Hsiao J.-K. A potential probiotic-lachnospiraceae nk4a136 group: Evidence from the restoration of the dietary pattern from a high-fat diet. 2020.
61. Lin, H., Guo, Q., Ran, Y., Lin, L., Chen, P., He, J., Chen, Y., & Wen, J. (2021). Multiomics study reveals enterococcus and

subdoligranulum are beneficial to necrotizing enterocolitis. *Frontiers in Microbiology*, *12*, 752102. https://doi.org/10.3389/fmicb.2021.752102
62. Van Hul, M., Le Roy, T., Prifti, E., Dao, M. C., Paquot, A., Zucker, J.-D., Delzenne, N. M., Muccioli, G. G., Clément, K., & Cani, P. D. (2020). From correlation to causality: The case of subdoligranulum. *Gut Microbes*, *12*(1), 1849998. https://doi.org/10.1080/19490976.2020.1849998
63. Durieux, A., Fougnies, C., Jacobs, H., & Simon, J.-P. (2001). Metabolism of chicory fructooligosaccharides by bifidobacteria. *Biotechnology Letters*, *23*(18), 1523–1527. https://doi.org/10.1023/a:1011645608848
64. Van De Wiele, T., Boon, N., Possemiers, S., Jacobs, H., & Verstraete, W. (2007). Inulin-type fructans of longer degree of polymerization exert more pronounced in vitro prebiotic effects. *Journal of Applied Microbiology*, *102*(2), 452–460. https://doi.org/10.1111/j.1365-2672.2006.03084.x
65. Lustgarten, M. S. (2019). The role of the gut microbiome on skeletal muscle mass and physical function: 2019 update. *Frontiers in Physiology*, *10*, 1435. https://doi.org/10.3389/fphys.2019.01435
66. Li, J., Hu, Q., Xiao-Yu, D., Zhu, L., Miao, Y-F., Kang, H-X., Zhao, X-L., Yao, J-Q., Long, D., & Tang, W-F. (2020). Effect of shengjiang powder on gut microbiota in high-fat diet-induced nafld. *Evidence-based Complementary and Alternative Medicine*, *2020*.
67. Kelly, A., Mccabe, M., Kenny, D., Guan, L., & Waters, S. (2018). 350 examining the effect of a butyrate-fortified milk replacer on gastrointestinal microbiota and fermentation in dairy calves at weaning. *Journal of Animal Science*, *96*(suppl_3), 174–175. https://doi.org/10.1093/jas/sky404.380
68. Bui, T. P. N., Troise, A. D., Nijsse, B., Roviello, G. N., Fogliano, V., & De Vos, W. M. (2020). Intestinimonas-like bacteria are important butyrate producers that utilize nε-fructosyllysine and lysine in formula-fed infants and adults. *Journal of Functional Foods*, *70*, 103974. https://doi.org/10.1016/j.jff.2020.103974
69. Yin, X., Ji, S., Duan, C., Tian, P., Ju, S., Yan, H., Zhang, Y., & Liu, Y. (2021). Age-related changes in the ruminal microbiota and their relationship with rumen fermentation in lambs. *Frontiers in Microbiology*, *12*, 679135. https://doi.org/10.3389/fmicb.2021.679135
70. Toya, T., Corban, M. T., Marrietta, E., Horwath, I. E., Lerman, L. O., Murray, J. A., & Lerman, A. (2020). Coronary artery disease is associated with an altered gut microbiome composition. *PLoS One*, *15*(1), e0227147. https://doi.org/10.1371/journal.pone.0227147
71. Atallah, E., Mahayri, T., Fliegerová, K., Mrázek, J., Addeo, N., Bovera, F., & Moniello, G. (2023). The effect of different levels of hermetia illucens oil inclusion on caecal microbiota of Japanese quails (coturnix japonica, gould, 1837). *Journal of Insects as Food and Feed*, *1*, 1–19.
72. Xia, B., Zhong, R., Wu, W., Luo, C., Meng, Q., Gao, Q., Zhao, Y., Chen, L., Zhang, S., Zhao, X., & Zhang, H. (2022). Mucin o-glycan-microbiota axis orchestrates gut homeostasis in a diarrheal pig model. *Microbiome*, *10*, 1–21. https://doi.org/10.1186/s40168-022-01326-8

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Li, J., Bai, G., Gao, Y., Gao, Q., Zhong, R., Chen, L., Wang, Y., Ma, T., & Zhang, H. (2024). Solid-state fermentation pro-enzymes supplementation benefits growth performance, health, and intestinal microbiota of broiler chickens fed wheat-based diet. *Animal Research and One Health*, 1–13. https://doi.org/10.1002/aro2.55