



Original Research Article

Co-fermented defatted rice bran alters gut microbiota and improves growth performance, antioxidant capacity, immune status and intestinal permeability of finishing pigs

Weifa Su^{a, b, c, d, e}, Zipeng Jiang^{a, b, c, d, e}, Cheng Wang^{a, b, c, d, e}, Yu Zhang^{a, b, c, d, e},
Tao Gong^{a, b, c, d, e}, Fengqin Wang^{a, b, c, d, e}, Mingliang Jin^{a, b, c, d, e}, Yizhen Wang^{a, b, c, d, e},
Zeqing Lu^{a, b, c, d, e, *}

^a Key Laboratory of Molecular Animal Nutrition, Ministry of Education, Zhejiang University, 866 Yuhang Tang Road, Hangzhou, Zhejiang 310058, China

^b Key Laboratory of Animal Nutrition and Feed, Ministry of Agriculture, Zhejiang University, 866 Yuhang Tang Road, Hangzhou, Zhejiang 310058, China

^c National Engineering Laboratory of Biological Feed Safety and Pollution Prevention and Control, Zhejiang University, 866 Yuhang Tang Road, Hangzhou, Zhejiang 310058, China

^d Key Laboratory of Animal Nutrition and Feed Science of Zhejiang Province, Zhejiang University, 866 Yuhang Tang Road, Hangzhou, Zhejiang 310058, China

^e College of Animal Science, Institute of Feed Science, Zhejiang University, 866 Yuhang Tang Road, Hangzhou, Zhejiang 310058, China

ARTICLE INFO

Article history:

Received 2 September 2021

Received in revised form

15 March 2022

Accepted 25 July 2022

Available online 7 August 2022

Keywords:

Co-fermented defatted rice bran

Finishing pig

Growth performance

Gut microbiota

Gut permeability

Immune status

ABSTRACT

Based on preparation of co-fermented defatted rice bran (DFRB) using *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Lactobacillus plantarum* and phytase, the present study aimed to evaluate the effects of co-fermented DFRB on growth performance, antioxidant capacity, immune status, gut microbiota and permeability in finishing pigs. Ninety finishing pigs (85.30 ± 0.97 kg) were randomly assigned to 3 treatments (3 replicates/treatment) with a basal diet (Ctrl), a basal diet supplemented with 10% unfermented DFRB (UFR), and a basal diet supplemented with 10% fermented DFRB (FR) for 30 d. Results revealed that the diet supplemented with FR notably ($P < 0.05$) improved the average daily gain (ADG), gain to feed ratio (G:F) and the digestibility of crude protein, amino acids and dietary fiber of finishing pigs compared with UFR. Additionally, FR supplementation significantly ($P < 0.05$) increased total antioxidant capacity, the activities of superoxide dismutase and catalase, and decreased the content of malonaldehyde in serum. Furthermore, FR remarkably ($P < 0.05$) increased serum levels of IgG, anti-inflammatory cytokines (IL-22 and IL-23) and reduced pro-inflammatory cytokines (TNF- α , IL-1 β and INF- γ). The decrease of serum diamine oxidase activity and serum D-lactate content in the FR group ($P < 0.05$) suggested an improvement in intestinal permeability. Supplementation of FR also elevated the content of acetate and butyrate in feces ($P < 0.05$). Moreover, FR enhanced gut microbial richness and the abundance of fiber-degrading bacteria such as *Clostridium butyricum* and *Lactobacillus amylovorus*. Correlation analyses indicated dietary fiber in FR was associated with improvements in immune status, intestinal permeability and the level of butyrate-producing microbe *C. butyricum*, which was also verified by the in vitro fermentation analysis. These findings provided an experimental and theoretical basis for the application of fermented DFRB in finishing pigs.

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* Corresponding author.

E-mail address: zqlu2012@zju.edu.cn (Z. Lu).

Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.



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1. Introduction

China is the largest pig-raising country in the world and the grain used annually in feed and husbandry accounts for more than half of China's total grain consumption, which has led to food competition between human beings and livestock. In recent years, to relieve the shortage of feed resources and reduce feed costs, nutrient-poor, less-expensive and locally available agricultural

byproducts are increasingly utilized in livestock diets (Li et al., 2018). Defatted rice bran (DFRB), a result of oil extraction from rice, is a typically inexpensive and easily accessible agricultural byproduct with a global production of 29.3 million tonnes annually (Sohail et al., 2017).

Analysis shows that DFRB contains 32.9% dietary fiber, 14.3% protein and 2.8% lipids (Daou and Zhang, 2014). Moreover, many bioactive compounds in DFRB were reported to play critical roles in combating oxidative stress, reducing inflammation and modulating the gut microbiome and barrier function (Ganda Mall et al., 2018; Kumar et al., 2012; Webber et al., 2014). However, the existence of antinutritional factors (ANF), such as trypsin inhibitors (TI) and phytic acid, plus a high fiber and low protein content have caused DFRB to be currently underutilized in animal husbandry feed (Ranjan et al., 2019). Furthermore, a high oil residue even after the oil extraction process makes DFRB susceptible to oxidation and rancidity during storage (Huang et al., 2018). Hence, comprehensive processing technologies for DFRB are worth researching. Solid-state fermentation (SSF) is an effective, economically viable technique to transform low-value agricultural byproduct efficiently and create high value-added product (Ni et al., 2017). Previous studies have reported that SSF can degrade ANF (phytate and TI), improve dietary fiber composition and protein digestibility, and enhance antioxidant activity of DFRB (Liu et al., 2017; Ranjan et al., 2019). However, the application of fermented DFRB in finishing pigs has not yet been reported.

Based on preparation of co-fermented DFRB using *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Lactobacillus plantarum* and phytase, the present study evaluated the effects of co-fermented DFRB on growth performance, antioxidant activity, immune status, gut microbiota and intestinal permeability in finishing pigs, which provides evidence for the addition of fermented DFRB in the diet of finishing pigs.

2. Materials and methods

2.1. Animal ethics statement

All the procedures were approved by the Institutional Animal Care and Use Committee at Zhejiang University (approval no. ZJU20160400).

2.2. Fermented feed production

In this experiment, *B. subtilis* BSWF (NCBI Accession No. MN696208, *S. cerevisiae* SCWF (NCBI Accession No. MN038413), *L. plantarum* CWLP (CGMCC 1.510) were all government-authorized probiotics in China. Pilot production of co-fermented DFRB was carried out at the Tianbang Feed Technology Co., Ltd. (Anhui, China). The basal substrate (UFR) including 80% DFRB and 20% soybean hull, was inoculated with *B. subtilis* (10^7 CFU/g), *S. cerevisiae* (10^7 CFU/g), *L. plantarum* (10^7 CFU/g) and phytase (50 U/g), and then fermented in a bed-packed incubator with 40% moisture content for 24 h, and then transferred to a plastic bag equipped with a one-way valve (Rou Duoduo Biotechnology Co., Beijing, China), sealed, and fermented at room temperature for 48 h. After the two-stage SSF, the fermentation substrate (FR) was obtained.

2.3. Animal experimental design and sample collection

Ninety finishing pigs (Duroc × Landrace × Yorkshire, body weight = 85.30 ± 0.97 kg) were randomly allocated into 3 treatments. Each treatment consisted of 3 replicates and 10 pigs per replicate: (1) basal diet (Ctrl); (2) basal diet + 10% UFR (UFR); (3) basal diet + 10% FR (FR). After 30 d of the experiment, the growth

performance was evaluated. All diets were formulated to meet the NRC (2012) nutrient requirements. The composition and nutritional value of the diets are presented in Table 1. Fecal samples were collected at the end of the experiment. One aliquot of fecal sample was kept in a sterile EP tube and stored at -80°C ; the other was placed in 10% hydrochloric acid to fix excreta nitrogen and was also kept at -80°C . After overnight starvation, blood samples (5 mL) were harvested from the anterior vena cava into 10-mL vacutainer tubes and centrifuged at $3,000 \times g$ and 4°C for 10 min to obtain serum. Then serum samples were frozen at -80°C .

2.4. Growth performance

The average daily feed intake (ADFI), average daily gain (ADG) and gain to feed ratio (G:F) were calculated to evaluate the growth performance. The ADFI was measured by recording the feed intake daily. The ADG was measured by obtaining weights on the days when the experiment started and finished.

2.5. Digestibility measurement

The apparent total tract digestibility (ATTD) of nutrients was measured according to the method reported by Liu et al. (2018). In brief, diets and fecal samples were used for the measurement of DM, CP, EE, amino acids (AAs), IDF, SDF, Ca and P according to AOAC (2007); acid insoluble ash (AIA) was chosen as the endogenous indicator. The equation for ATTD calculation was the following:

$$\text{ATTD (\%)} = 1 - \frac{(\text{AIA}_{\text{diet}} \times \text{Nutrient}_{\text{feces}})}{(\text{AIA}_{\text{feces}} \times \text{Nutrient}_{\text{diet}})} \times 100$$

Table 1

Ingredient composition and nutritional levels in experiment (% as dry matter basis).

Item	Diet ¹		
	Ctrl	UFR	FR
Ingredient			
Corn	79.10	69.50	69.50
Soybean meal	16.40	15.00	15.00
UFR	—	10	—
FR	—	—	10.00
Soy oil	1.00	2.00	2.00
Dicalcium phosphate	0.65	0.65	0.65
Sodium chloride	0.30	0.30	0.30
Limestone power	0.55	0.55	0.55
Premix ²	2.00	2.00	2.00
Total	100.00	100.00	100.00
Analyzed composition			
DE, MJ/kg	13.56	13.45	13.32
CP	13.96	13.55	13.63
EE	4.15	4.66	4.74
Ash	5.23	5.22	5.44
Ca	0.43	0.44	0.44
Total P	0.47	0.55	0.51

UFR = unfermented substrate (80% DFRB and 20% soybean hull); FR = fermented substrate (80% DFRB and 20% soybean hull); DE = digestible energy; CP = crude protein; EE = ether extract.

¹ Ctrl: basal diet; UFR: basal diet with 10% UFR; FR: basal diet supplemented with 10% FR.

² The premix provided the following nutrients per kilogram of the complete diet: 6,480 IU vitamin A as vitamin A acetate, 2,800 IU vitamin D₃ as D-activated animal sterol, 26 mg IU vitamin E as alpha tocopherol acetate, 2 mg vitamin K₃ as menadione dimethylpyrimidinol bisulfite, 50 mg thiamin as thiamine mononitrate, 4 mg riboflavin, 3 mg pyridoxine as pyridoxine hydrochloride, 0.03 mg vitamin B₁₂, 23-mg D-pantothenic acid as calcium pantothenate, 20 mg niacin, 1.2 mg folic acid, 0.2 mg biotin, and 300 mg choline as choline chloride. Also provided the following quantities of minerals per kilogram of the complete diet: 95 mg Cu as copper sulfate, 200 mg Fe as ferrous sulfate, 0.35 mg I as potassium iodate, 30 mg Mn as manganese sulfate, 0.30 mg Se as sodium selenite, and 100 mg Zn as zinc sulfate.

2.6. Serum parameters analysis

Routine blood tests including serum urea N, Ca, P, total protein (TP), albumin (ALB) and globulin (GLB) were carried out using a Mairui 5300 Blood Cell Analyzer. Serum total antioxidant capacity (TAC), superoxide dismutase (SOD) activity, catalase (CAT) activity and malonaldehyde (MDA) were measured using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. Serum parameters including IgG, IgM, TNF- α , IL- β , IFN- γ , IL-6, IL-10, IL-22, IL-23, and diamine oxidase (DAO), D-lactate (DLA) and fecal sIgA were determined using ELISA kits (Jiangsu Meibiao Biological Technology, Jiangsu, China) following the manufacturer's protocols.

2.7. Fecal short chain fatty acid (SCFA) analysis

Fecal SCFAs were determined according to our previous report (Jin et al., 2019). In brief, 1 g of fecal sample was blended in 2 mL of distilled water. Afterwards, the sample was centrifuged ($15,000 \times g$, 4 °C, 15 min) and the supernatant was collected and mixed with 0.1 mL 25% (wt/vol) phosphoric acid. The sample then was filtered and analyzed using gas chromatography (Varian CP-3800 GC, USA).

2.8. Gut microbiota analysis

The PowerSoil DNA isolation kit (MoBio Laboratories Inc., USA) was used to extract total genomic DNA from the fecal samples. The V3 to V4 gene regions of the bacterial 16S rRNA gene were amplified with primers 515F (ACTCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT). The PCR procedures were performed according to the method described by Wang et al. (2019d). PCR products were purified with a Qiagen gel extraction kit (Qiagen, Germany), and sequencing libraries were generated by using the TruSeq DNA PCR-free sample preparation kit (Illumina, USA) following the manufacturer's protocol. Illumina HiSeq2500 sequencing was conducted according to our previous report (Wang et al., 2021). Raw tags were filtered using the QIIME (V1.9.1) pipeline, and the UCHIME algorithm was applied to identify and remove chimeric sequences. Operational taxonomic units (OTUs) were clustered with a 97% similarity cutoff using UPARSE (Version 7.0.1001). Representative sequences were assigned to taxonomy using the RDP classifier (Version 2.2) based on the SILVA database. The abundance of each taxonomic group was constructed by a Perl script and visualized using SVG. The sequence data were deposited in the Sequence Read Archive (SRA) under the accession number PRJNA740149. Chao1, Simpson, Good's coverage and other alpha diversity indices were calculated in QIIME. Principal coordinate analysis (PCoA) of unweighted UniFrac distances was performed and plotted in R (Version 2.15.3). The main differentially abundant genera were selected by the LEfSe method (Segata et al., 2011). To predict metabolic functions of microbiota, the Tax4Fun was used to obtain a functional profile from the 16S rRNA data and the heatmap package of R (Version 2.15.3) was applied to generate heatmaps of genera and Level3 predicted microbial metabolic functions.

2.9. Correlation analysis of dietary fiber composition, serum parameters, fecal SCFA, gut microbiota and microbial metabolic functions

Spearman correlation analysis of dietary fiber composition, serum parameters, fecal SCFA, differential gut microbiota and microbial metabolic functions was computed by R (Version 3.2.4), and network plots were generated by Cytoscape 3.6.1 with the absolute value of the correlation coefficients > 0.45 and $P < 0.05$.

2.10. In vitro fermentation

To remove digestible components of UFR and FR, the UFR and FR were subjected to in vitro digestion prior to the bacterial fermentations. The digestion of UFR and FR were conducted based on our previous report (Jin et al., 2019). The pre-digested UFR and FR were fermented in in vitro with fecal microbiota from finishing pigs of Ctrl (Ctrl FM), UFR (UFR FM) and FR (FR FM) groups or *C. butyricum* CD (isolated from gut of pig, ATCC19398). The fermentation medium contained (per litre distilled water): 0.16 g peptone, 0.1 g yeast extract, 0.16 g Tween 80, 0.16 g NaHCO₃, 3.6 g NaCl, 1.6 g K₂HPO₄, 0.32 g L-cysteine hydrochloride, 0.36 g CaCl₂·6H₂O, 0.5 g MgSO₄·7H₂O and 0.01 g Hemin. One gram pre-digested UFR or FR was suspended in 25 mL sterile fermentation medium, boiled for 10 min and cooled down to room temperature; 5 mL sterile fermentation medium was added to 0.1 g fecal sample or 0.1 mL *C. butyricum* CD (10^5 CFU/mL); 2.5 mL of the fecal or *C. butyricum* CD suspension was mixed with 2.5 mL UFR or FR solution and incubated under anaerobic conditions at 37 °C with shaking at 125 rounds per min on a shaking incubator with a rotational radius of 10 cm; 1 mL of slurries was collected at 0, 6, 12 and 24 h fermentation for SCFA and microbial count analysis. All steps of fermentation were conducted in an anaerobic chamber (Bactron, Shel Lab, USA) with anaerobic environment (5% CO₂, 5% H₂, and 90% N₂).

2.11. Statistical analysis

The assay data were analyzed using SPSS 20.0 software (SAS Inc., Chicago, IL). One-way ANOVA and Duncan's test were used to determine the difference between groups. The differences were considered significant at $P < 0.05$, and $0.05 < P < 0.10$ was deemed as a tendency. GraphPad Prism 8 (San Diego, CA, USA) was used to generate bar plots.

3. Results

3.1. Growth performance and nutrient digestibility

The growth performance of finishing pigs is presented in Table 2. The diet supplemented with FR significantly increased ADG and G:F ($P < 0.05$) compared to the UFR group. Furthermore, FR supplementation tended to increase ADFI compared with the Ctrl and UFR group ($P = 0.095$). The diet supplemented with 10% UFR tended to decrease ADFI, ADG and G:F compared to the basal diet.

The ATTD of nutrients is shown in Table 3. Compared with the Ctrl group, a significant downward trend in DM, 3 essential amino acids (Ile, Leu and Met), 2 dispensable AAs (Gly and Tyr) ($P < 0.05$) and the increase in EE, IDF and Ca ($P < 0.05$) were observed in the UFR group. The digestibility of DM, CP, SDF, IDF and Ca in FR was notably improved compared with the other 2 groups ($P < 0.05$). Moreover, the ATTD of 8 AAs, including 5 essential AAs (Ile, Leu, Lys, Met and Thr) and 3 dispensable AAs (Ala, Gly and Tyr) was significantly enhanced compared with the UFR group ($P < 0.05$). In addition, serum urea N in the FR group was significantly decreased compared with the other 2 groups ($P < 0.05$). The content of serum TP and GLB was upregulated by both UFR and FR ($P < 0.05$).

3.2. Fecal SCFA, sIgA, serum diamine oxidase and D-lactate

Based upon the increased ATTD of SDF and IDF in the FR group finishing pigs, we further measured fecal pH and SCFA content (Fig. 1A and B). No noteworthy change in pH was observed between the 3 groups ($P > 0.05$). However, the fecal acetate content and total SCFA were greatly improved in the UFR and FR group ($P < 0.05$). Additionally, fecal butyrate content in the FR group was

significantly increased compared with the Ctrl and UFR groups ($P < 0.05$). Compared to the basic diet, FR obviously improved the fecal sIgA content ($P < 0.05$) (Fig. 1C). Furthermore, finishing pigs fed FR showed lower serum level of DLA and DAO when compared with those fed Ctrl and UFR diets ($P < 0.05$) (Fig. 1D and E).

3.3. Serum immunity and antioxidant ability parameters

Serum immunity parameters of finishing pigs are presented in Fig. 2A. FR significantly promoted the level of serum IgG compared with the Ctrl group ($P < 0.05$). No significant differences were found in IgA content among the 3 groups ($P > 0.05$). For serum cytokines, FR supplementation remarkably reduced serum TNF- α , IL-1 β and INF- γ and improved the level of IL-22 and IL-23 compared with that of the Ctrl group ($P < 0.05$). Furthermore, finishing pigs fed FR also showed lower ($P < 0.05$) serum IL-1 β when compared with UFR. Fig. 2B shows the serum antioxidant ability of finishing pigs. It was found that FR notably enhanced serum TAC, CAT activity, SOD activity and decreased MDA content compared with the Ctrl group ($P < 0.05$). Moreover, compared to UFR, FR also significantly increased SOD activity and decreased MDA content ($P < 0.05$). Additionally, UFR also enhanced serum CAT activity and decreased MDA content compared to the Ctrl group ($P < 0.05$).

3.4. Gut microbiota

The bacterial diversity is presented in Table 4. The number of recovered reads ranged from 51,173 to 55,272 with Good's coverage of all samples approximately 0.99, indicating that the depth of sequencing was adequate for reliable analysis. Chao1 was a richness index of the bacterial community and showed a significant upward trend in the UFR and FR groups ($P < 0.05$). Venn analysis (Fig. 3A) revealed that there were obvious overlaps (1,481 OTUs) between the 3 groups, and 76, 90 and 86 specific OTUs were observed in Ctrl, UFR and FR, respectively. Fig. 3B shows the principal coordinate analysis (PCoA), and the result suggested that gut microbiota in FR were obviously separated from that in Ctrl and UFR.

At the phylum level, Firmicutes, Bacteroidetes and Spirochaetes were the dominant bacteria in finishing pig's gut (Fig. 3C). Fig. 3D showed that Firmicute significantly increased in FR in contrast to the Ctrl ($P < 0.05$). Bacteroidetes showed a significant stepwise decrease from the Ctrl to UFR and FR ($P < 0.05$). Of the top 35 bacterial genera (Fig. 3E), the abundances of *Alloprevotella* and *Prevotellaceae* clearly decreased in the UFR and FR groups compared with the Ctrl group ($P < 0.05$). *Streptococcus* and *Methanospaera* in the FR group significantly decreased in contrast to the Ctrl group ($P < 0.05$). Additionally, FR notably increased *Clostridiales*, *Ruminococcaceae*, *Phascolarctobacterium*, *Romboutsia*, *Turicibacter* and *Marvinbryantia* compared to UFR ($P < 0.05$). LEfSe was further applied to analyze the degree of influence of significantly different species (LDA scores > 3.0) on the overall difference in the microbial community (Fig. 3F). We found that *C. butyricum*, rumen bacterium NK4A214, *Lactobacillus amylovorus*, *Firmicutes bacterium* CAG 194 44 15 were predominant in the FR group. The abundance of *Cupriavidus pauculus* increased in the UFR group. For the Ctrl group, *Streptococcus*, *Paraprevotella clara*, *Prevotella brevis* and *Bacteroides* sp Marseille P3166 were the dominant species.

3.5. Gut microbiota metabolic function predictions

Metabolic function of the gut microbiota was predicted using Tax4Fun with the KEGG pathway database. The result of principal component analysis (PCA) of predicted functions (Fig. 4A) was

Table 2

The growth performance of finishing pigs supplied with Ctrl, UFR and FR.¹

Item	Ctrl	UFR	FR	SEM	P-value
Initial body weight, kg	85.25	84.36	86.29	1.55	0.880
Final body weight, kg	113.75	110.15	116.24	1.44	0.297
ADFI, kg/d	2.75	2.71	2.84	0.02	0.095
ADG, kg/d	0.95 ^{ab}	0.86 ^b	1.00 ^a	0.01	0.001
G:F	0.34 ^{ab}	0.32 ^b	0.35 ^a	0.00	0.025

UFR = unfermented substrate (80% DFRB and 20% soybean hull); FR = fermented substrate (80% DFRB and 20% soybean hull); SEM = standard error of the mean; ADFI = average daily feed intake; ADG = average daily gain; G:F = gain to feed ratio.

¹ Ctrl: basal diet; UFR: basal diet with 10% UFR; FR: basal diet supplemented with 10% FR. The data are the means of 6 replicates per treatment. Means without a common letter significantly differ ($P < 0.05$).

consistent with the PCoA of gut microbial community. The main predicted functional genes of gut microbiota at KEGG level 1 were assigned into metabolism, genetic information processing and environmental information processing pathways, respectively (Fig. 4B). FR significantly decreased bacterial Metabolism and Genetic information processing and improved Environmental information processing compared with Ctrl and UFR ($P < 0.05$). At level 2 and 3 (Fig. 4C and D), we found that the predicted functions of gut microbiota in the FR group were enriched in Amino acid metabolism, Carbohydrate metabolism (Butanoate metabolism and Pyruvate metabolism), Membrane transport (Secretion system, ABC transporters and Transporters) and Signal transduction. Furthermore, Glycan biosynthesis and

Table 3

Apparent total tract digestibility of nutrients and serum indices of finishing pigs.¹

Item	Ctrl	UFR	FR	SEM	P-value
ATTD, %					
DM	81.14 ^b	79.36 ^c	82.39 ^a	0.32	<0.001
CP	77.32 ^b	76.1 ^b	78.79 ^a	0.34	0.001
EE	78.89 ^b	82.98 ^a	85.08 ^a	0.79	0.001
SDF	36.71 ^b	49.75 ^b	59.79 ^a	2.48	<0.001
IDF	6.46 ^c	13.5 ^b	21.55 ^a	1.60	<0.001
Ca	31.37 ^c	36.57 ^b	38.14 ^a	1.09	0.002
P	31.71	37.35	37.94	1.99	0.426
Indispensable AA, %					
Arg	95.4	94.57	95.66	0.24	0.154
His	87.63	86.64	89.64	0.71	0.228
Ile	82.37 ^a	79.08 ^b	81.64 ^a	0.45	0.001
Leu	86.34 ^a	84.54 ^b	86.39 ^a	0.29	0.005
Lys	84.78 ^{ab}	83.14 ^b	85.32 ^a	0.30	0.003
Met	96.94 ^b	94.67 ^c	98.52 ^a	0.42	<0.001
Phe	84.59	83.08	84.37	0.48	0.401
Thr	81.81 ^b	80.51 ^b	83.62 ^a	0.40	0.002
Val	81.05	77.8	80.18	0.67	0.122
Dispensable AA, %					
Ala	78.24 ^{ab}	76.82 ^b	80.39 ^a	0.76	0.041
Asp	81.04	79.10	80.95	0.67	0.433
Cys	95.75	94.77	96.2	0.29	0.111
Glu	88.50	86.83	88.39	0.42	0.207
Gly	76.55 ^a	74.32 ^b	77.38 ^a	0.38	0.001
Pro	85.83	83.81	84.43	0.46	0.19
Ser	84.65	83.36	85.29	0.78	0.616
Tyr	81.10 ^a	75.99 ^b	81.59 ^a	0.77	0.041
Serum indices, mmol/L					
Urea N	5.37 ^a		3.88 ^b	0.12	<0.001
Ca	2.60	2.73	2.67	0.17	0.563
P	3.09	3.54	2.93	0.75	0.537

UFR = unfermented substrate (80% DFRB and 20% soybean hull); FR = fermented substrate (80% DFRB and 20% soybean hull); SEM = standard error of the mean; ATTD = apparent total tract digestibility; DM = dry matter; EE = ether extract; CP = crude protein; SDF = soluble dietary fiber; IDF = insoluble dietary fiber.

¹ Ctrl: basal diet; UFR: basal diet with 10% UFR; FR: basal diet supplemented with 10% FR. The data are the means of 6 replicates per treatment. Means without a common letter significantly differ ($P < 0.05$).

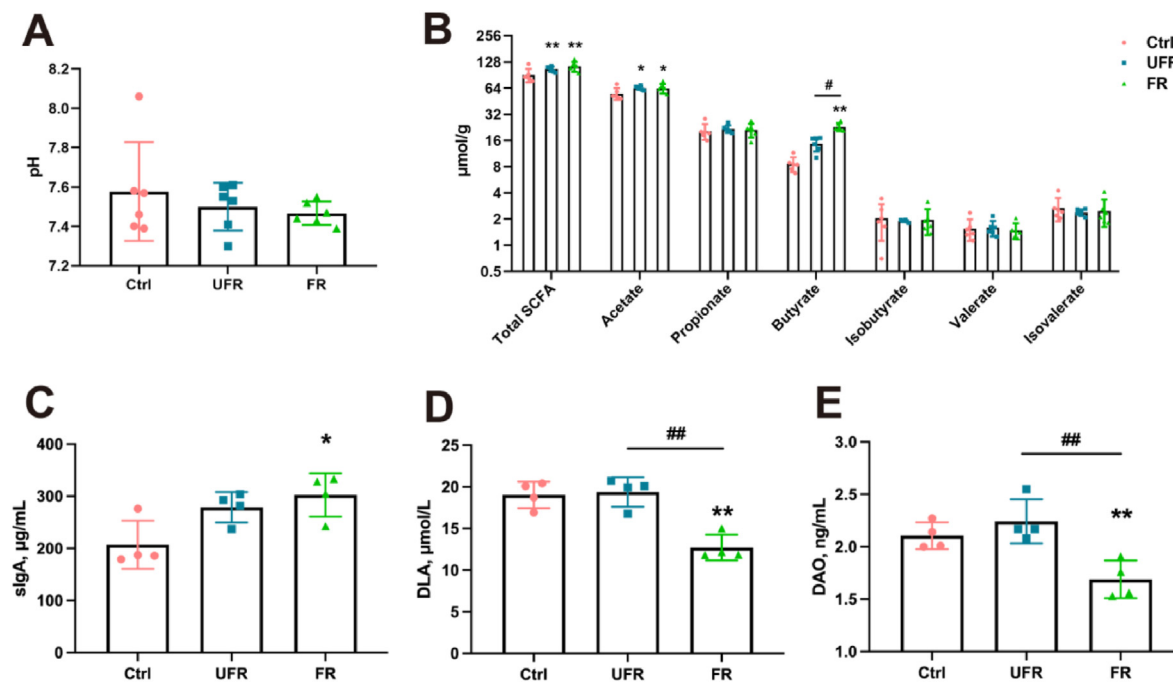


Fig. 1. The fecal pH, short chain fatty acids (SCFA), sIgA and intestinal permeability of finishing pigs. (A) Fecal pH. (B) Fecal SCFA content. (C) Fecal sIgA content. (D) Serum DAO activity. (E) Serum DLA level. UFR = unfermented substrate (80% DFRB and 20% soybean hull); FR = fermented substrate (80% DFRB and 20% soybean hull); DAO = diamine oxidase; DLA = D-lactate. Ctrl: basal diet; UFR: basal diet with 10% UFR; FR: basal diet supplemented with 10% FR. * $P < 0.05$ and ** $P < 0.01$ compared with the Ctrl. # $P < 0.05$ and ## $P < 0.01$ for UFR vs. FR.

metabolism and Immune system (IL-17 signaling pathway and Th17 cell differentiation) were downregulated in the FR group. Specifically, FR notably increased Butanoate metabolism, Secretion system and AMPK signaling pathway, HIF-1 signaling pathway and downregulated Lipopolysaccharide biosynthesis compared with the Ctrl and UFR groups ($P < 0.05$) (Fig. 4E).

3.6. Correlation analysis of dietary fiber composition, serum parameters, fecal SCFA, gut microbiota and metabolic functions

Spearman correlation analysis was further conducted to investigate the relationship among dietary fiber composition, serum parameters, fecal SCFA, gut microbiota and metabolic functions (Fig. 5A). We found that the number of significant

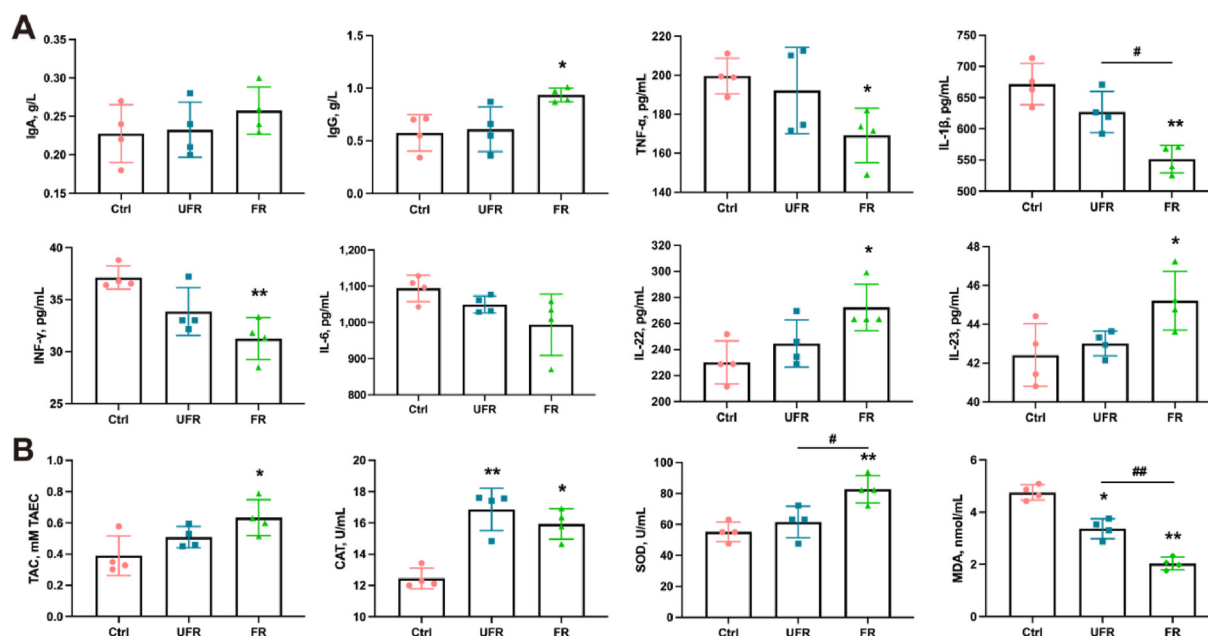


Fig. 2. Serum immunity parameters and antioxidant ability of finishing pigs. (A) Serum immunity parameters including IgA, IgG, TNF-α, IL-1β, INF-γ, IL-6, IL-10, IL-22 and IL-23. (B) Serum antioxidant ability including TAC, SOD, CAT and MDA. UFR = unfermented substrate (80% DFRB and 20% soybean hull); FR = fermented substrate (80% DFRB and 20% soybean hull); TAC = total antioxidant capacity; SOD = the activity of superoxide dismutase; CAT = the activity of catalase; MDA = malonaldehyde. Ctrl: basal diet; UFR: basal diet with 10% UFR; FR: basal diet supplemented with 10% FR. * $P < 0.05$ and ** $P < 0.01$ compared with the Ctrl. # $P < 0.05$ and ## $P < 0.01$ for UFR vs. FR.

Table 4
Alpha diversity of gut microbiota.¹

Alpha diversity	Ctrl	UFR	FR
Reads	51,173 ± 5,619	55,272 ± 4,925	54,395 ± 5,842
Good's coverage	0.99 ± 0.00	0.99 ± 0.00	0.99 ± 0.00
OTUs	1026 ± 64	1103 ± 97	1084 ± 52
Chao1	1144.22 ± 60.87 ^b	1263.73 ± 108.89 ^a	1216.25 ± 73.15 ^a
Shannon	6.05 ± 0.39	5.82 ± 0.80	6.00 ± 0.54
Simpson	0.94 ± 0.02	0.92 ± 0.07	0.94 ± 0.03

UFR = unfermented substrate (80% DFRB and 20% soybean hull); FR = fermented substrate (80% DFRB and 20% soybean hull); OTUs = operational taxonomic units.

¹ Ctrl: basal diet; UFR: basal diet with 10% UFR; FR: basal diet supplemented with 10% FR. Values were mean ± SD, *n* = 6. Means followed with different superscript letter within each line are significantly different (*P* < 0.05).

correlations between *C. butyricum* and other indicators was the most among all differential gut microbiota (the node size indicates the number of significant correlations). To show the network plots clearly, the indicators related to *C. butyricum* were selected and presented in Fig. 5B. The results showed that *C. butyricum* enriched in the FR group was positively correlated with the gut microbiota Secretion system, Butanoate metabolism and fecal butyrate (*P* < 0.05). Interestingly, *C. butyricum* and fecal butyrate both positively correlated with serum IL-22, IL-23, IgG, fecal sIgA, bacterial HIF-1 signaling pathway and negatively with serum TNF- α , IL-1 β , INF- γ , DLA and DAO (*P* < 0.05). Furthermore, fecal butyrate was positively correlated with SDF, IDF and dietary fiber (*P* < 0.05). In addition, *C. butyricum* was positively associated with the bacterial AMPK signaling pathway, *Firmicutes bacterium* CAG 194 44 15 and negatively with bacterial Lipopolysaccharide biosynthesis and the differential gut microbiota enriched in the Ctrl group.

3.7. In vitro fermentation

After 24 h of in vitro fermentation, the pH of FR was significantly lower than UFR regardless of whether it was fermented with fecal microbiota from finishing pigs of the Ctrl (Ctrl FM), UFR (UFR FM) and FR (FR FM) groups (*P* < 0.05) (Fig. 6A). The SCFA production in response to UFR and FR are presented in Fig. 6B. Ctrl FM, UFR FM and FR FM fermented FR in vitro produced more butyrate than that of UFR (*P* < 0.05). Simultaneously, UFR FM and FR FM fermented FR in vitro produced more acetate and total SCFA than that of UFR (*P* < 0.05). Additionally, fecal microbiota from finishing pigs of the FR group showed stronger production capacity of SCFA (including acetate, propionate and butyrate) compared with those from the Ctrl and UFR groups during the in vitro fermentation of UFR and FR. Moreover, we found that the microbiological counts of *C. butyricum* CD during in vitro fermentation of pre-digested FR were notably higher than that of UFR at 12 h and 24 h (*P* < 0.05) (Fig. 6C). Correspondingly, the pH of FR was lower than that of UFR after 12 h of fermentation. The content of butyrate in FR was significantly higher than that of UFR after 24 h of in vitro fermentation (*P* < 0.05).

4. Discussion

Solid-state fermentation is an effective, economically viable technique to improve the nutrient quality and functional properties of agricultural byproducts, and is widely used in husbandry to promote growth performance and health status of animals. Based on preparation of co-fermented DFRB using *B. subtilis*, *S. cerevisiae*,

L. plantarum and phytase, our previous study found that co-fermentation effectively increased the CP, trichloroacetic acid soluble protein (TCA-SP), SDF and lactic acid content, and degraded ANF (TI and phytic acid) and IDF (Su et al., 2022). TCA-SP consists of small peptides and free amino acids, most of which can be directly absorbed by the gastrointestinal tract and elicit antioxidant and immune regulation functions (Gilbert et al., 2008). Additionally, the degradation of phytic acid and TI suggested an improvement of bioavailability of essential mineral elements and proteins (Silva and Bracarense, 2016). Similarly, Ranjan (Ranjan et al., 2019) reported that SSF using *Rhizopus oryzae* significantly reduced phytic acid and TI activity of DFRB. Higher levels of SDF suggested that co-fermented DFRB may provide more available fiber for gut microbiota (Navarro et al., 2019). Further more, our previous study found that co-fermented DFRB also contained a large amount of lactic acid and live probiotics (*B. subtilis*, *S. cerevisiae* and *L. plantarum*) (Su et al., 2022). These probiotics and their beneficial metabolites supplied to pigs may have a positive effect on growth performance and health status.

Our findings combined with previous studies highlight improvements in nutrient quality and functional properties of DFRB by SSF. Many studies have investigated the effects of DFRB on finishing pigs (Casas et al., 2018; Fan et al., 2020; Wang et al., 2019a), however, no previous experiments have evaluated the effect of fermented DFRB on finishing pigs. In the present study, diets supplemented with 10% FR increased ADG and G:F of finishing pigs compared to UFR supplementation. 10% UFR tended to have a negative effect on growth performance, which is in agreement with the results of Casas et al. (2018). SSF of the agricultural byproducts improved animal growth performance, which was mainly due to the increase in small molecules and the degradation of ANF to promote nutrient digestion and absorption. Our results suggested that FR notably improved the digestibility of DM, CP, SDF, IDF, Ca and AAs compared with UFR. In the process of co-fermentation, the macromolecular proteins in DFRB degraded to small peptides and free AAs, which partly explained the high digestibility of CP and AAs in the FR group. Additionally, the change of dietary fiber composition in DFRB during fermentation may provide more available fiber for gut microbiota to improve their digestibility. The increased digestibility of Ca may be attributed to phytase inoculated in DFRB (Dersjant-Li and Dusel, 2019). Simultaneously, the significant decrease of serum urea N and increase of serum TP in the FR group further demonstrated that co-fermented DFRB can promote the body's nitrogen deposition and hence growth performance of finishing pigs (Fabian et al., 2004).

Our previous study also found that co-fermentation enhanced the total phenol and 1,1-diphenyl-2-picrylhydrazyl (DPHH) radical scavenging activity of DFRB (Su et al., 2022). Phenolic compounds in DFRB are positively correlated with antioxidant capacity (Shin et al., 2019b). Enhanced total phenol, small peptides and DPPH radical scavenging activity indicated that co-fermented DFRB had higher antioxidant capacity. During the breeding process of finishing pigs, feed pollution, high temperature and high metabolic burden often cause excessive free radicals, which can combine with tissues, blood or cells, causing oxidative damage (Wang et al., 2006). It is widely recognized that CAT and SOD, two important endogenous antioxidant enzymes, play crucial roles in preventing oxidative damage (Sun et al., 2020). Additionally, the content of MDA in serum reflects the level of lipid peroxidation in animals (Xi et al., 2017). In this study, the increased serum TAC, CAT and SOD activities and decreased MDA content were indicative of improvements in the antioxidative status of the finishing pigs fed with co-

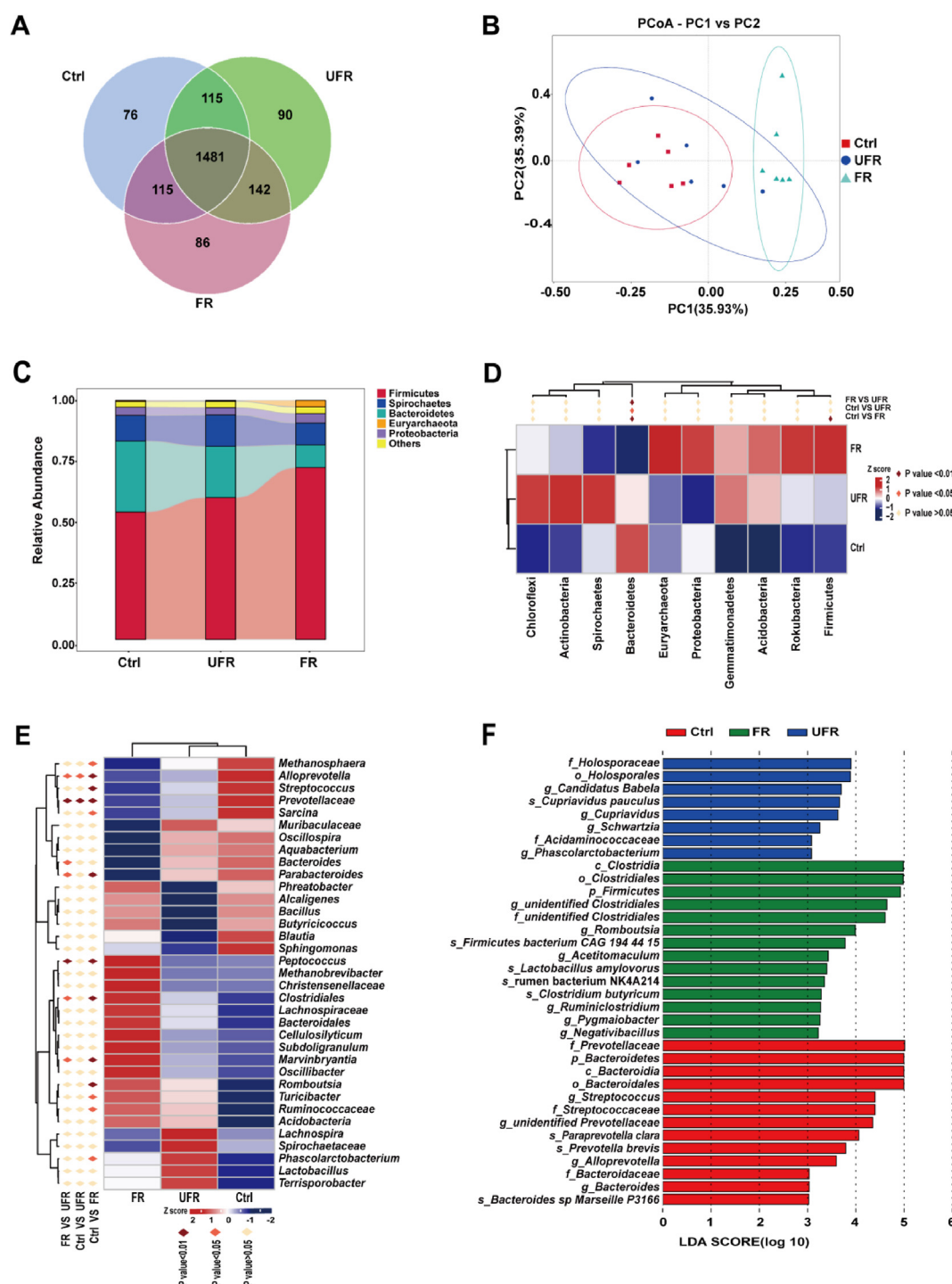


Fig. 3. Gut microbial diversity. (A) The Venn analysis of operational taxonomic units (OTUs). (B) Principal coordinate analysis (PCoA) plot of the bacterial community based on unweighted UniFrac distances. (C) Bar plots at phylum level. (D) Heatmap of significantly different phylum based on MetaStat analysis. (E) Heatmap of significantly different genera based on MetaStat analysis. (F) LEfSe histogram showing the LDA scores (>3.0) computed for features at the OTU level. UFR = unfermented substrate (80% DFRB and 20% soybean hull); FR = fermented substrate (80% DFRB and 20% soybean hull). Ctrl: basal diet; UFR: basal diet with 10% UFR; FR: basal diet supplemented with 10% FR. Letters indicate the taxonomy of the bacteria: p, phylum; c, class; o, order; f, family; g, genus; s, species.

fermented DFRB, which can be partially attributed to its antioxidant-enhancing properties.

Additionally, the increased digestibility of dietary fiber in the FR group suggested that more dietary fiber may be utilized by gut microbiota for SCFA production (Mudgil and Barak, 2013). Interestingly, our study found that the fecal acetate and total SCFA contents

were elevated in the UFR and FR groups. FR also notably increased butyrate content, which might be due to the increased SDF content in co-fermented DFRB. Chen et al. (2019) also reported that SDF-fed piglets had a higher concentration of butyrate in feces compared to IDF-fed piglets. SCFA was reported to enhance gut barrier function and modulate gut immune response (D'Souza et al., 2017). The gut

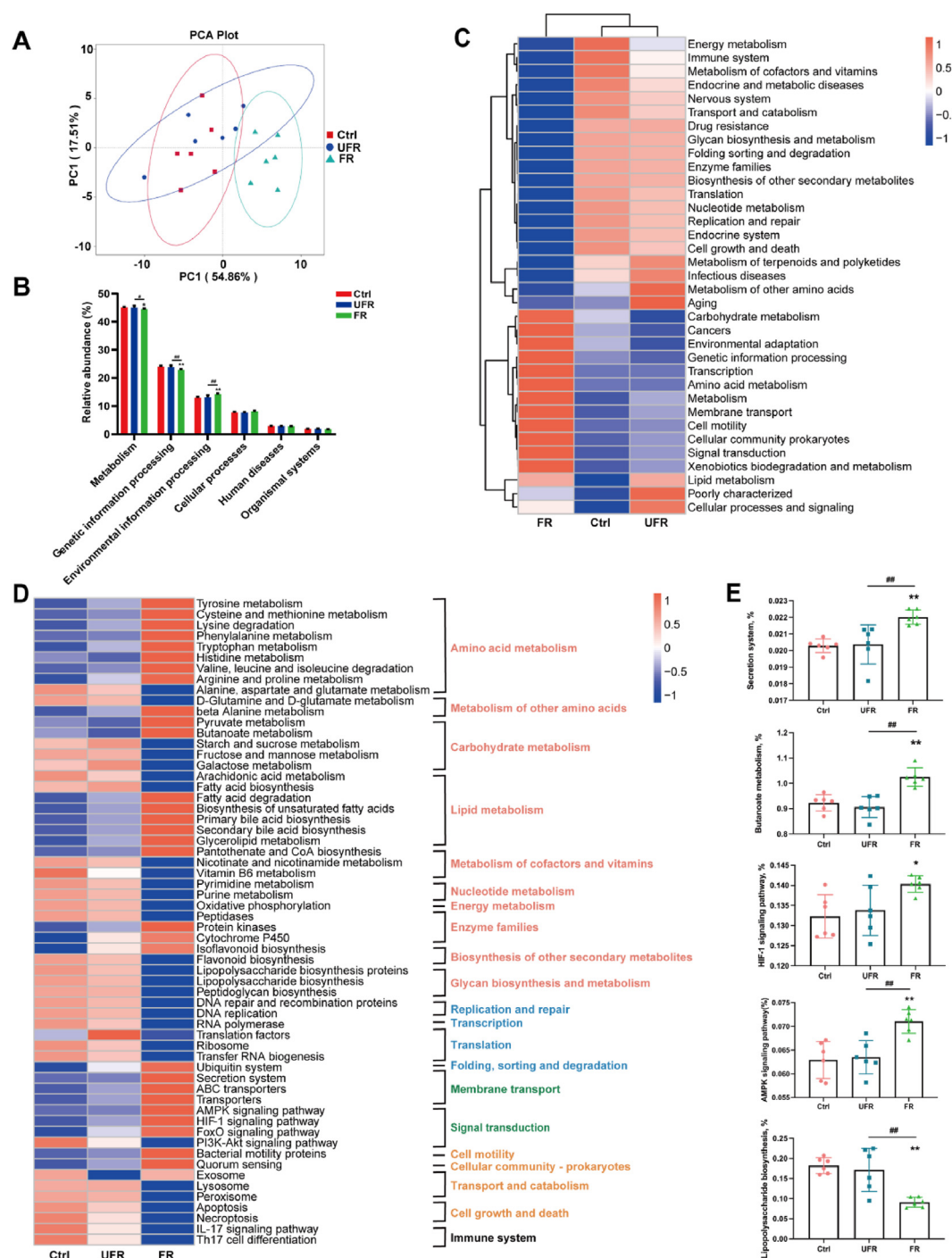


Fig. 4. Metagenomic functional predictions of gut microbiota analyzed by Tax4Fun. (A) Principal component analysis (PCA) plot of bacteria metabolic functions. (B) The metabolic pathways. (C) KEGG ortholog functional predictions. (D) KEGG ortholog functional predictions. (E) The significantly different microbial functions at level 3. UFR = unfermented substrate (80% DFRB and 20% soybean hull); FR = fermented substrate (80% DFRB and 20% soybean hull). Ctrl: basal diet; UFR: basal diet with 10% UFR; FR: basal diet supplemented with 10% FR. * $P < 0.05$ and ** $P < 0.01$ compared with the Ctrl. # $P < 0.05$ and ## $P < 0.01$ for UFR vs. FR.

barrier function is influenced by many factors, including serum DLA and DAO concentration, which increase along with intestinal permeability or injury to intestinal barrier integrity (Zhao et al., 2011). In the present study, lower serum levels of DLA and DAO in the FR group indicated that co-fermented DFRB effectively improved intestinal permeability and barrier integrity. On the contrary, DFRB tended to increase intestinal permeability. Similar results were reported by Wang et al. (2019a), who found that the DLA and DAO concentration

in serum of finishing pigs both increased with the dietary DFRB level. sIgA is the most abundant colonic antibody antigen known as “immune exclusion” (Palm et al., 2014). We found co-fermented DFRB increased the fecal sIgA concentration. Lu et al. (2020) also reported that fermented corn–soybean meal could promote intestinal sIgA in growing–finishing pigs through intestinal microbiota.

Serum immunoglobulins are important immunologically active molecules in the humoral immune response (Kong et al.,

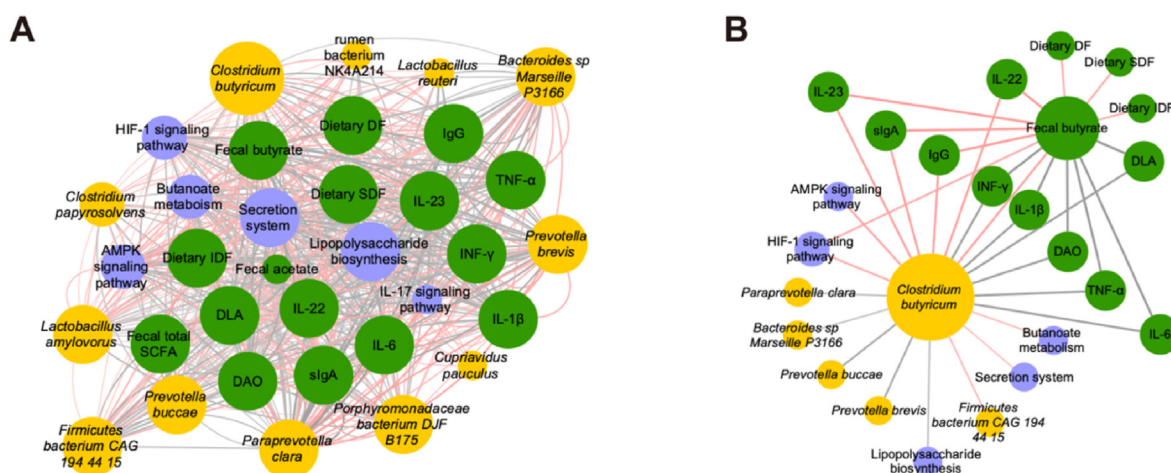


Fig. 5. Spearman correlation analysis. (A) Spearman correlation analysis of dietary fiber composition, serum parameters, fecal short chain fatty acids (SCFA), differential gut microbiota and microbial metabolic functions. (B) The indicators related to *C. butyricum*. The red lines represent positive relationships ($|r| > 0.45$, $P < 0.05$), and the gray lines indicate negative relationships ($|r| > 0.45$, $P < 0.05$). The thickness of the line represents the correlation strength of the interaction. The orange nodes represent bacteria species. The green nodes represent feed fiber, serum parameters and fecal SCFA. The purple nodes represent bacteria metabolic functions. The node size indicates number of significant correlations. DAO = diamine oxidase; DLA = D-lactate; SDF = soluble dietary fiber; IDF = insoluble dietary fiber; DF = dietary fiber.

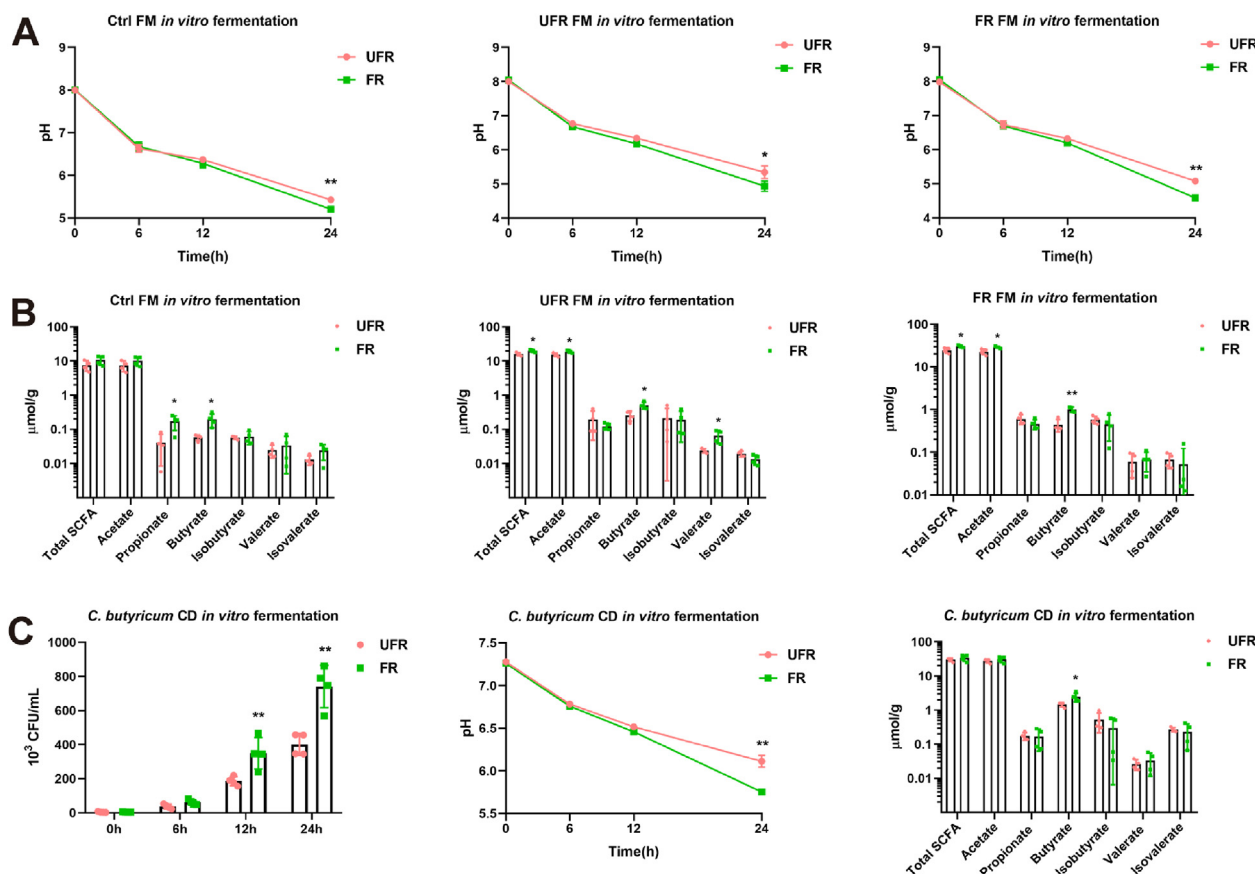


Fig. 6. In vitro fermentation with fecal microbiota and *C. butyricum*. (A) The pH of UFR and FR during in vitro fermentation with fecal microbiota from finishing pigs of Ctrl (Ctrl FM), UFR (UFR FM) and FR (FR FM) groups. (B) The short chain fatty acids (SCFA) content of UFR and FR during in vitro fermentation with fecal microbiota from finishing pigs of Ctrl, UFR, FR groups. (C) The microbiological counts, pH and SCFA content of UFR and FR during in vitro fermentation with *C. butyricum* CD. UFR = pre-digested unfermented substrate (80% DFRB and 20% soybean hull); FR = pre-digested fermented substrate (80% DFRB and 20% soybean hull); Ctrl FM = fecal microbiota from finishing pigs of Ctrl group; UFR FM = fecal microbiota from finishing pigs of UFR group; FR FM = fecal microbiota from finishing pigs of FR group. * $P < 0.05$ and ** $P < 0.01$ compared with the Ctrl FM. # $P < 0.05$ and ## $P < 0.01$ for UFR FM vs. FR FM.

2007). In this study, the serum GLB and IgG content in the FR group significantly increased compared with the Ctrl group. This result was similar to those of Lu et al. (2019), who reported that

finishing pigs fed fermented corn–soybean meal had higher serum concentration of IgG. For serum cytokines, FR reduced the serum level of TNF- α , IL-1 β and INF- γ and improved the level of IL-

22 and IL-23 compared with Ctrl. TNF- α , IL-1 β and INF- γ are indicators of pro-inflammatory reactions, whereas IL-22 and IL-23 exert an anti-inflammatory effect (Mizoguchi et al., 2018; Zhou et al., 2019; Zong et al., 2021a). Thus, the presence of serum cytokines indicated that FR protected finishing pigs from potential inflammatory reactions. Interestingly, UFR also tended to decrease these pro-inflammatory cytokines and enhance anti-inflammatory cytokines. Similar results were reported by Casas and Stein, who found that serum TNF- α tended to decrease with the inclusion of full fat rice bran in the weanling pig diet. One possible reason is that the increased dietary fiber content in UFR and FR diets participates in the regulation of gut microbiota and their metabolites, especially SCFA.

Many studies have reported that gut microbiota interact with gut barrier function and inflammation (Yang et al., 2018; Zong et al., 2020). Changing the content of the dietary fiber, as the main energy source for gut microbiota, is an effective way of adjusting gut bacteria. In this study, UFR and FR effectively enhanced the richness of the gut microbiota of finishing pigs. The results were similar to those of Pu et al. (2020), who reported that finishing pigs fed with DFRB increased gut microbial richness. Noteworthy, gut microbiota in FR were clearly different from those in Ctrl and UFR, which indicated that co-fermentation may play an important role in the regulation of gut microbiota of finishing pigs. Firmicutes has been proven to comprise a range of cellulolytic microbiota, which are advantageous to cellulose degradation and SCFA production (Zhu et al., 2015). Finishing pigs supplemented with FR increased the abundance of Firmicutes, which suggested that FR may enhance the ability to digest fiber in the hindgut. *Clostridiales* and *Ruminococcaceae* also showed a great boost in cellulose decomposition (Bensoussan et al., 2017; Hu et al., 2016). Our investigation found that *C. butyricum*, rumen bacterium NK4A214 and *L. amylovorus* were the dominant species in the FR group. *C. butyricum* is a pivotal mediator for fiber fermentation, butyrate production, gut immunity and barrier function (Zhang et al., 2018). Simultaneously, *C. butyricum* was reported to promote rumen bacterium NK4A214 (Liang et al., 2021). *L. amylovorus* has been associated with diets including fermentable dietary fiber and oligosaccharides, which in turn favored a more diverse gut microbiome and prevented pathogen-induced damage to the porcine intestinal barrier (Trevisi et al., 2018). In summary, FR increased the richness of finishing pigs' gut microbiota and increased fiber-utilizing bacteria.

Changes in gut microbiota caused by the supplementation of co-fermented DFRB lead to the alteration of microbial metabolic function. The increase of Amino acid metabolism and Carbohydrate metabolism of gut microbiota in the FR group demonstrated improved digestibility of protein and dietary fiber in finishing pigs. In particular, the increase in Butanoate metabolism and Pyruvate metabolism in the FR group suggested that FR provided more butyrate to participate in energy supply for intestinal epithelial cells (Cao et al., 2019). Additionally, enhanced Membrane transport suggested that the synthesis and secretion of metabolites (such as SCFA) and enzymes (such as cellulase) that gut microbiota in the FR group participated in were improved (Cerisy et al., 2019). Previous studies reported that microbiota-derived butyrate augmented the distal gut barrier function via the HIF-1 and AMPK signaling pathway (Kelly et al., 2015; Peng et al., 2009). The increase in the two signaling pathways in the FR group indicated an improvement in gut barrier function of finishing pigs. Moreover, Lipopolysaccharide biosynthesis and the IL-17 signaling pathway, both pro-inflammatory effects, were downregulated in the FR group (Amatya et al., 2017; Wang et al., 2019b; Zong et al., 2021b), which suggested that gut microbiota in FR mitigated potential inflammatory reactions.

Spearman correlation analysis further revealed that *C. butyricum* played an important role in regulating the immune and gut barrier function of finishing pigs. Specifically, dietary fiber in FR may improve the immune and gut barrier function by regulating the secretion of butyrate by *C. butyricum* and Butanoate metabolism. Concurrently, the HIF-1 signaling pathway may be involved in the process. Studies have reported that butyrate produced by *C. butyricum* of the distal gut reduced gut permeability by activating the HIF-1 signaling pathway to upregulate the expression of gut tight-junction proteins (Fachi et al., 2019; Shin et al., 2019a). Additionally, Liu et al. (2021) reported that dietary fiber reduced the secretion of pro-inflammatory cytokines and increased anti-inflammatory cytokines by regulating the secretion of butyrate to improve the immune function of sows. Butyrate was also reported to upregulate IL-22 production by promoting HIF-1 α expression in the gut of mice (Yang et al., 2020). However, the potential mechanism in finishing pigs needs to be further investigated.

In vitro fermentation results demonstrated that co-fermented DFRB, compared with DFRB, was more conducive to the proliferation of distal gut bacteria of finishing pigs to produce SCFA, especially acetate and butyrate. Additionally, fecal microbiota from the FR group can more efficiently utilize DFRB and co-fermented DFRB to produce SCFA than from the Ctrl and UFR groups. Furthermore, co-fermented DFRB was more effective in promoting the proliferation of *C. butyricum* isolated from pigs to produce butyrate. The physicochemical structure of dietary fiber is a critical factor that determines fermentation rate, SCFA profiles and growth of different bacterial groups (Wang et al., 2019c). Co-fermentation may change the physicochemical structure of fiber in DFRB, such as increasing the proportion SDF, thereby modulating hindgut microbiota, for example increasing *C. butyricum*, to improve the digestibility of fiber and the production of butyrate. Similarly, Chen et al. (2019) reported that SDF-fed piglets had a higher concentration of fecal butyrate compared to IDF-fed piglets. In general, *in vitro* fermentation further verified that the physicochemical structure of fiber in co-fermented DFRB enhanced the abundance of *C. butyricum* and butyrate secretion in the hindgut of finishing pigs.

5. Conclusions

In conclusion, our findings demonstrated that diets supplemented with co-fermented DFRB improved growth performance, antioxidant activity, immune status and intestinal permeability of finishing pigs. Co-fermented DFRB also increased gut microbial richness and regulated microbiota with increasing fiber-degrading bacteria, such as *C. butyricum* and *L. amylovorus*. Furthermore, correlation analyses indicated that dietary fiber in co-fermented DFRB might improve the immune and gut barrier function of finishing pigs by regulating the secretion of butyrate of *C. butyricum*. *In vitro* fermentation further verified the fiber in co-fermented DFRB enhanced *C. butyricum* and butyrate secretion. These findings provided an experimental and theoretical basis for the application of co-fermented DFRB in finishing pigs.

Author contributions

Weifa Su: Conceptualization, Methodology, Investigation, Writing - original draft. **Tao Gong, Zipeng Jiang:** Investigation, Visualization. **Yu Zhang and Cheng Wang:** Formal analysis, Visualization. **Mingliang Jin:** Writing - review & editing. **Fengqin Wang:** Methodology. **Zeqing Lu and Yizhen Wang:** Resources, Writing - review and editing, Supervision.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgments

The authors thank the specialized research fund from Science and Technology Projects of Zhejiang (2022C02043, 2021C02008, CTZB-2020080127), China Agriculture Research System (CARS-35), National Center of Technology Innovation for Pigs.

References

- Official methods of analysis of AOAC International. Current Through Revision 4. 18th ed Gaithersburg (MD); 2007.
- Bensoussan L, Morais S, Dassa B, Friedman N, Henrissat B, Lombard V, et al. Broad phylogeny and functionality of cellulosomal components in the bovine rumen microbiome. *Environ Microbiol* 2017;19(1):185–97. <https://doi.org/10.1111/1462-2920.13561>.
- Cao G, Tao F, Hu Y, Li Z, Zhang Y, Deng B, et al. Positive effects of a clostridium butyricum-based compound probiotic on growth performance, immune responses, intestinal morphology, hypothalamic neurotransmitters, and colonic microbiota in weaned piglets. *Food Funct* 2019;10(5):2926–34. <https://doi.org/10.1039/c8fo02370k>.
- Casas GA, Overholt MF, Dilger AC, Boler DD, Stein HH. Effects of full fat rice bran and defatted rice bran on growth performance and carcass characteristics of growing-finishing pigs. *J Anim Sci* 2018;96(6):2293–309. Go to ISI>://WOS:000434415300021.
- Casas GA, Stein HH. Effects of full fat or defatted rice bran on growth performance and blood characteristics of weanling pigs. *J Anim Sci* 2016;94(10):4179–87. Go to ISI>://WOS:000388955400013.
- Cerisy T, Iglesias A, Rostain W, Boutard M, Pelle C, Perret A, et al. ABC transporters required for hexose uptake by clostridium phytofermentans. *J Bacteriol* 2019;201(15). Go to ISI>://WOS:000474755100010.
- Chen TT, Chen DW, Tian G, Zheng P, Mao XB, Yu J, et al. Soluble fiber and insoluble fiber regulate colonic microbiota and barrier function in a piglet model. *BioMed Res Int* 2019;2019. Go to ISI>://WOS:000505936600002.
- D'Souza WN, Douangpanya J, Mu SR, Jaekel P, Zhang M, Maxwell JR, et al. Differing roles for short chain fatty acids and gpr43 agonism in the regulation of intestinal barrier function and immune responses. *PLoS One* 2017;12(7). Go to ISI>://WOS:000406634500017.
- Daou C, Zhang H. Functional and physiological properties of total, soluble, and insoluble dietary fibres derived from defatted rice bran. *J Food Sci Tech Mys* 2014;51(12):3878–85. Go to ISI>://WOS:000345916400035.
- Dersjant-Li Y, Dusel G. Increasing the dosing of a butyriauxella phytase improves phytate degradation, mineral, energy, and amino acid digestibility in weaned pigs fed a complex diet based on wheat, corn, soybean meal, barley, and rapeseed meal. *J Anim Sci* 2019;97(6):2524–33. Go to ISI>://WOS:000473740300023.
- Fabian J, Chiba LI, Frobish LT, McElhenney WH, Kuhlers DL, Nadarajah K. Compensatory growth and nitrogen balance in grower-finisher pigs. *J Anim Sci* 2004;82(9):2579–87. Go to ISI>://WOS:000223743800008.
- Fachi JL, Felipe JS, Pral LP, da Silva BK, Correa RO, de Andrade MCP, et al. Butyrate protects mice from clostridium difficile-induced colitis through an hif-1-dependent mechanism. *Cell Rep* 2019;27(3):750–61. <https://doi.org/10.1016/j.celrep.2019.03.054>. e757.
- Fan LJ, Huang RH, Wu CW, Cao Y, Du TR, Pu G, et al. Defatted rice bran supplementation in diets of finishing pigs: effects on physiological, intestinal barrier, and oxidative stress parameters. *Animals-Basel* 2020;10(3). Go to ISI>://WOS:000529378800082.
- Ganda Mall JP, Lofvendahl L, Lindqvist CM, Brummer RJ, Keita AV, Schoultz I. Differential effects of dietary fibres on colonic barrier function in elderly individuals with gastrointestinal symptoms. *Sci Rep* 2018;8(1):13404. <https://doi.org/10.1038/s41598-018-31492-5>.
- Gilbert ER, Wong EA, Webb KE. Board-invited review: peptide absorption and utilization: implications for animal nutrition and health. *J Anim Sci* 2008;86(9):2135–55. Go to ISI>://WOS:000258851500010.
- Hu J, Nie Y, Chen J, Zhang Y, Wang Z, Fan Q, et al. Gradual changes of gut microbiota in weaned miniature piglets. *Front Microbiol* 2016;7:1727. <https://doi.org/10.3389/fmicb.2016.01727>.
- Huang BB, Huang CF, Lyu ZQ, Chen YF, Li PL, Liu L, et al. Available energy and amino acid digestibility of defatted rice bran fed to growing pigs. *J Anim Sci* 2018;96(8):3138–50. Go to ISI>://WOS:000443953300012.
- Jin ML, Kalainy S, Baskota N, Chiang DN, Deehan EC, McDougall C, et al. Faecal microbiota from patients with cirrhosis has a low capacity to ferment non-digestible carbohydrates into short-chain fatty acids. *Liver Int* 2019;39(8):1437–47. Go to ISI>://WOS:000478649000009.
- Kelly CJ, Zheng L, Campbell EL, Saeedi B, Scholz CC, Bayless AJ, et al. Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial hif augments tissue barrier function. *Cell Host Microbe* 2015;17(5):662–71. <https://doi.org/10.1016/j.chom.2015.03.005>.
- Kong XF, Yin YL, Wu GY, Liu HJ, Yin FG, Li TJ, et al. Dietary supplementation with acanthopanax senticosus extract modulates cellular and humoral immunity in weaned piglets. *Asian Austral J Anim* 2007;20(9):1453–61. Go to ISI>://WOS:000249166400019.
- Kumar A, Henderson A, Forster GM, Goodyear AW, Weir TL, Leach JE, et al. Dietary rice bran promotes resistance to salmonella enterica serovar typhimurium colonization in mice. *BMC Microbiol* 2012;12:71. <https://doi.org/10.1186/1471-2180-12-71>.
- Li YK, Li ZC, Liu H, Noblet J, Liu L, Li DF, et al. Net energy content of rice bran, corn germ meal, corn gluten feed, peanut meal, and sunflower meal in growing pigs. *Asian Austral J Anim* 2018;31(9):1481–90. <https://doi.org/10.5713/ajas.17.0829>.
- Liang J, Kou S, Chen C, Raza SHA, Wang S, Ma X, et al. Effects of clostridium butyricum on growth performance, metabonomics and intestinal microbial differences of weaned piglets. *BMC Microbiol* 2021;21(1):85. <https://doi.org/10.1186/s12866-021-02143-z>.
- Liu B, Zhu X, Cui Y, Wang W, Liu H, Li Z, et al. Consumption of dietary fiber from different sources during pregnancy alters sow gut microbiota and improves performance and reduces inflammation in sows and piglets. *mSystems* 2021;6(1). <https://doi.org/10.1128/mSystems.00591-20>.
- Liu JB, Yan HL, Cao SC, Liu J, Zhang HF. Effect of feed intake level on the determination of apparent and standardized total tract digestibility of phosphorus for growing pigs. *Anim Feed Sci Technol* 2018;246:137–43. Go to ISI>://WOS:000452342600015.
- Liu L, Zhang RF, Deng YY, Zhang Y, Xiao J, Huang F, et al. Fermentation and complex enzyme hydrolysis enhance total phenolics and antioxidant activity of aqueous solution from rice bran pretreated by steaming with alpha-amylase. *Food Chem* 2017;221:636–43. Go to ISI>://WOS:000389909100080.
- Lu JF, Zhang XY, Liu YH, Cao HG, Han QC, Xie BC, et al. Effect of fermented corn-soybean meal on serum immunity, the expression of genes related to gut immunity, gut microbiota, and bacterial metabolites in grower-finisher pigs. *Front Microbiol* 2019;10. Go to ISI>://WOS:000501292000001.
- Lu JF, Zhu MQ, Cao HG, Zhang X, Wang ZL, Zhang XY, et al. Impact of fermented corn-soybean meal on gene expression of immunity in the blood, level of secretory immunoglobulin a, and mucosa-associated bacterial community in the intestine of grower-finisher pigs. *Front Vet Sci* 2020;7. Go to ISI>://WOS:000544748400001.
- Mizoguchi A, Yano A, Himuro H, Ezaki Y, Sadanaga T, Mizoguchi E. Clinical importance of il-22 cascade in ibd. *J Gastroenterol* 2018;53(4):465–74. Go to ISI>://WOS:000428328200001.
- Mudgil D, Barak S. Composition, properties and health benefits of indigestible carbohydrate polymers as dietary fiber: a review. *Int J Biol Macromol* 2013;61:1–6. Go to ISI>://WOS:000326660700001.
- Navarro DMDL, Bruininx EMAM, de Jong L, Stein HH. Effects of inclusion rate of high fiber dietary ingredients on apparent ileal, hindgut, and total tract digestibility of dry matter and nutrients in ingredients fed to growing pigs. *Anim Feed Sci Technol* 2019;248:1–9. Go to ISI>://WOS:000458714400001.
- Ni KK, Wang FF, Zhu BG, Yang JX, Zhou GA, Pan Y, et al. Effects of lactic acid bacteria and molasses additives on the microbial community and fermentation quality of soybean silage. *Bioresour Technol* 2017;238:706–15. Go to ISI>://WOS:000402485500084.
- NRC. Nutrient requirements of Swine. 11th revised ed. Washington(DC): National Academic Press; 2012.
- Palm NW, de Zoete MR, Cullen TW, Barry NA, Stefanowski J, Hao LM, et al. Immunoglobulin a coating identifies colitogenic bacteria in inflammatory bowel disease. *Cell* 2014;158(5):1000–10. Go to ISI>://WOS:000340945000007.
- Peng L, Li ZR, Green RS, Holzman IR, Lin J. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of amp-activated protein kinase in caco-2 cell monolayers. *J Nutr* 2009;139(9):1619–25. <https://doi.org/10.3945/jn.109.104638>.
- Pu G, Li PH, Du TR, Niu Q, Fan LJ, Wang H, et al. Adding appropriate fiber in diet increases diversity and metabolic capacity of distal gut microbiota without altering fiber digestibility and growth rate of finishing pig. *Front Microbiol* 2020;11. Go to ISI>://WOS:000529967100001.
- Ranjan A, Sahu NP, Deo AD, Kumar S. Solid state fermentation of de-oiled rice bran: effect on in vitro protein digestibility, fatty acid profile and anti-nutritional factors. *Food Res Int* 2019;119:1–5. Go to ISI>://WOS:000464088700001.
- Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. *Genome Biol* 2011;12(6). Go to ISI>://WOS:000296646600008.
- Shin D, Chang SY, Boger P, Won K, Choi JY, Choi YJ, et al. Beneficial roles of probiotics on the modulation of gut microbiota and immune response in pigs. *PLoS One* 2019a;14(8). Go to ISI>://WOS:000485052300017.

- Shin HY, Kim SM, Lee JH, Lim ST. Solid-state fermentation of black rice bran with *aspergillus awamori* and *aspergillus oryzae*: effects on phenolic acid composition and antioxidant activity of bran extracts. *Food Chem* 2019b;272:235–41. <https://doi.org/10.1016/j.foodchem.2018.07.174>.
- Silva EO, Bracarense AP. Phytic acid: from antinutritional to multiple protection factor of organic systems. *J Food Sci* 2016;81(6):R1357–62. <https://doi.org/10.1111/1750-3841.13320>.
- Sohail M, Rakha A, Butt MS, Iqbal MJ, Rashid S. Rice bran nutraceuticals: a comprehensive review. *Crit Rev Food Sci Nutr* 2017;57(17):3771–80. <https://doi.org/10.1080/10408398.2016.1164120>.
- Sun JJ, Wang P, Chen GP, Luo JY, Xi QY, Cai GY, et al. Effect of moringa oleifera supplementation on productive performance, colostrum composition and serum biochemical indexes of sow. *J Anim Physiol an N* 2020;104(1):291–9. Go to ISI>://WOS:000493092000001.
- Su W, Jiang Z. Dynamics of defatted rice bran in physicochemical characteristics, microbiota and metabolic functions during two-stage co-fermentation. *Int J Food Microbiol* 2022;362(6):109489. <https://doi.org/10.1016/j.ijfoodmicro.2021.109489>.
- Trevi P, Priori D, Jansman AJM, Luise D, Koopmans SJ, Hynonen U, et al. Molecular networks affected by neonatal microbial colonization in porcine jejunum, lumenally perfused with enterotoxigenic *escherichia coli*, f4ac fimbria or *lactobacillus amylovorus*. *PLoS One* 2018;13(8):e0202160. <https://doi.org/10.1371/journal.pone.0202160>.
- Wang C, Wei S, Chen N, Xiang Y, Wang Y, Jin M. Characteristics of gut microbiota in pigs with different breeds, growth periods and genders. *Microb Biotechnol* 2021. <https://doi.org/10.1111/1751-7915.13755>.
- Wang F, Xu ZR, Su JH. Effect of hch contamination of diet on the growth performance and immune and antioxidant ability in growing/finishing pigs. *Vet Res Commun* 2006;30(6):645–54. Go to ISI>://WOS:000239023500007.
- Wang H, Li PH, Du TR, Pu G, Fan LJ, Gao C, et al. Effects of increasing levels of defatted rice bran on intestinal physical barrier and bacteria in finishing pigs. *Animals-Basel* 2019a;9(12). Go to ISI>://WOS:000506636400039.
- Wang K, Chen G, Cao G, Xu Y, Wang Y, Yang C. Effects of *clostridium butyricum* and *enterococcus faecalis* on growth performance, intestinal structure, and inflammation in lipopolysaccharide-challenged weaned piglets. *J Anim Sci* 2019b;97(10):4140–51. <https://doi.org/10.1093/jas/skz235>.
- Wang M, Wichienchot S, He X, Fu X, Huang Q, Zhang B. In vitro colonic fermentation of dietary fibers: fermentation rate, short-chain fatty acid production and changes in microbiota. *Trends Food Sci Technol* 2019c;88:1–9. <https://doi.org/10.1016/j.tifs.2019.03.005>.
- Wang Y, He LW, Xing YQ, Zhou W, Pian RQ, Yang FY, et al. Bacterial diversity and fermentation quality of moringa oleifera leaves silage prepared with lactic acid bacteria inoculants and stored at different temperatures. *Bioresour Technol* 2019d;284:349–58. Go to ISI>://WOS:000464942500041.
- Webber DM, Hettiarachchy NS, Li RQ, Horax R, Theivendran S. Phenolic profile and antioxidant activity of extracts prepared from fermented heat-stabilized defatted rice bran. *J Food Sci* 2014;79(11):H2383–91. Go to ISI>://WOS:000344796500032, <https://onlinelibrary.wiley.com/doi/full/10.1111/1750-3841.12658>.
- Xi QY, Jiang Y, Zhao S, Zeng B, Wang F, Wang LN, et al. Effect of ginseng polysaccharides on the immunity and growth of piglets by dietary supplementation during late pregnancy and lactating sows. *Anim Sci J* 2017;88(6):863–72. Go to ISI>://WOS:000403020300005.
- Yang J, Qian K, Wang C, Wu Y. Roles of probiotic lactobacilli inclusion in helping piglets establish healthy intestinal inter-environment for pathogen defense. *Probiotics Antimicrob Proteins* 2018;10(2):243–50. <https://doi.org/10.1007/s12602-017-9273-y>.
- Yang W, Yu T, Huang X, Bilotta AJ, Xu L, Lu Y, et al. Intestinal microbiota-derived short-chain fatty acids regulation of immune cell il-22 production and gut immunity. *Nat Commun* 2020;11(1):4457. <https://doi.org/10.1038/s41467-020-18262-6>.
- Zhang J, Sun J, Chen X, Nie C, Zhao J, Guan W, et al. Combination of *clostridium butyricum* and corn bran optimized intestinal microbial fermentation using a weaned pig model. *Front Microbiol* 2018;9:3091. <https://doi.org/10.3389/fmicb.2018.03091>.
- Zhao Y, Qin GX, Sun ZW, Che DS, Bao N, Zhang XD. Effects of soybean agglutinin on intestinal barrier permeability and tight junction protein expression in weaned piglets. *Int J Mol Sci* 2011;12(12):8502–12. Go to ISI>://WOS:000298405100014.
- Zhou J, Xiong X, Yin J, Zou LJ, Wang KX, Shao YR, et al. Dietary lysozyme alters sow's gut microbiota, serum immunity and milk metabolite profile. *Front Microbiol* 2019;10. Go to ISI>://WOS:000458046800001.
- Zhu Y, Wang C, Li F. Impact of dietary fiber/starch ratio in shaping caecal microbiota in rabbits. *Can J Microbiol* 2015;61(10):771–84. <https://doi.org/10.1139/cjm-2015-0201>.
- Zong X, Fu J, Xu BC, Wang YZ, Jin ML. Interplay between gut microbiota and antimicrobial peptides. *Animal Nutrition* 2020;6(4):389–96. Go to ISI>://WOS:000599751400002.
- Zong X, Xiao X, Jie F, Cheng Y, Jin M, Yin Y, et al. Ythdf1 promotes nlrp3 translation to induce intestinal epithelial cell inflammatory injury during endotoxin shock. *Sci China Life Sci* 2021a;64(11):1988–91. <https://doi.org/10.1007/s11427-020-1909-6>.
- Zong X, Xiao X, Shen B, Jiang Q, Wang H, Lu Z, et al. The n6-methyladenosine rna-binding protein ythdf1 modulates the translation of traf6 to mediate the intestinal immune response. *Nucleic Acids Res* 2021b;49(10):5537–52. <https://doi.org/10.1093/nar/gkab343>.