



Original Research Article

Determination of the available energy, standardized ileal digestibility of amino acids of fermented corn germ meal replacing soybean meal in growing pig diets

Tengfei He, Yuhui Zheng, Xiangshu Piao*, Shenfei Long

State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China

ARTICLE INFO

Article history:

Received 3 August 2021

Received in revised form

28 October 2021

Accepted 18 November 2021

Available online 1 February 2022

Keywords:

Available energy

Amino acid digestibility

Fermented corn germ meal

Soybean meal

Growing pig

ABSTRACT

Three experiments were conducted to compare the digestible (DE), metabolizable energy (ME), and standardized ileal digestibility (SID) of amino acids (AA) in fermented corn germ meal (FCGM) and soybean meal (SBM), and evaluate the effects of FCGM replacing SBM in growing pig diets. In Exp. 1, 18 barrows with initial body weight (BW) of 60.2 ± 3.40 kg were randomly allotted to 3 treatments with 6 replicates per treatment. The control diet used corn as the only energy ingredient, and 2 test diets were made by replacing 25.8% of corn with FCGM or SBM. The DE and ME of FCGM were less ($P < 0.01$) than those of SBM. In Exp. 2, 18 barrows (59.3 ± 2.52 kg BW) with ileal T-cannulas were randomly allotted to 3 treatments with 6 replicates per treatment. The SID of arginine, tryptophan and proline were greater ($P < 0.05$) and the tyrosine was less ($P = 0.01$) in SBM compared with FCGM. In Exp. 3, 144 growing pigs (56.1 ± 5.22 kg BW) were randomly allotted to 4 treatments with 6 pens per treatment (3 barrows and 3 gilts per pen). Four diets (FCGM0, FCGM10, FCGM20 and FCGM30) were formulated using FCGM to replace 0%, 10%, 20% and 30% of SBM, respectively. The ME and SID values of AA of SBM and FCGM were determined by Exp. 1 and 2. Results showed that increasing FCGM inclusion quadratically ($P < 0.05$) increased the average daily gain (ADG), average daily feed intake, and the levels of serum immunoglobulin G (IgG) and urea nitrogen, and linearly ($P < 0.05$) increased the serum IgM, the propanoic acid, butyric acid, total volatile fatty acid (VFA) and the Shannon index of microbiota in feces. Besides, the relative abundance of genus *Streptococcus* in FCGM0, *Lactobacillus* in FCGM10 and Lachnospiraceae in FCGM30 were increased ($P < 0.05$) compared with other treatments. In conclusion, we recommend replacing 11.80% of SBM with FCGM to obtain the optimal ADG of growing pigs. Moreover, as the ratio of FCGM replacing SBM increased in diet, the immunity, intestinal microbiota and total VFA composition of growing pigs were improved.

© 2022 Chinese Association of Animal Science and Veterinary Medicine. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Soybean meal (SBM) is an important protein feed ingredient in pig diet (Jezierny et al., 2010). However, the high cost of SBM and

shortage of protein resources highlight an urgent need for suitable complements or substitutes for SBM (Tang et al., 2012). Corn germ meal (CGM) is a by-product of corn oil production (Estrada-Restrepo, 2017), which has been used in pet feed to partly replace corn and SBM (Li et al., 2013). Research in our lab have evaluated the nutritive value of some corn co-products, such as distillers dried grains with soluble and corn gluten meal in growing pig diet (She et al., 2015; Li et al., 2017), but there are a few studies about the CGM in growing pig diet. Corn germ meal contains 20% to 25% of crude protein (CP) and less than 3% of fat, as well as a comparable amino acid (AA) composition compared with corn, making it a potential ingredient in pig diet (NRC, 2012). The main fiber components in CGM are cellulose and arabinoxylan (Jaworski et al.,

* Corresponding author.

E-mail address: piaoxsh@cau.edu.cn (X. Piao).

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



2015), which apparent total digestive tract digestibility is less than 50% (Urriola et al., 2010). The production of CGM is smaller than other corn co-products, but CGM is easily available on the market, and is cost-effective when combined with other ingredients in pig diets. However, Estrada-Restrepo (2017) reported that increasing the proportion of CGM in diet linearly decreased the carcass yield of growing-finishing pigs. Weber et al. (2010) also observed that diet containing 38.69% CGM significantly reduced feed efficiency in growing pigs. The negative impacts of CGM on pigs might due to its high fiber content, several other studies also demonstrated that high fiber content of CGM restricted its use in animal diets (Lee et al., 2012; Zhang et al., 2013).

One of the effective ways to reduce fiber content in ingredient is fermentation. Supriyati et al. (2015) found that crude fiber content of rice bran was significantly reduced after fermentation by *Bacillus amyloliquefaciens*. Wizna et al. (2009) also observed that crude fiber content was decreased when cassava waste was fermented. In addition, previous studies reported that the ferment bacteria (such as *Bacillus* and *Lactobacillus* strains in feed) could produce dietary enzymes (such as cellulase, amylase, lipase, phytase and protease) that degraded complex polysaccharides and other large nutrients into small and more digestible particles, which made feed ingredients more valuable for animals (Ouoba et al., 2003; Hu et al., 2016). However, there are almost no information about the effects of fermentation on CGM, and few researches about the application of fermented corn germ meal (FCGM) in growing pigs. Therefore, the aim of this study was to: 1) determine and compare the digestible (DE) and metabolizable energy (ME) as well as the standardized ileal digestibility (SID) of AA in FCGM and SBM; 2) evaluate the effects of partial replacement of SBM by FCGM on growth performance, serum parameters and the intestinal health of growing pigs, and obtain the appropriate ratio of FCGM to replace SBM in growing pig diet.

2. Materials and methods

The protocols of these 3 experiments were approved by the Institutional Animal Care and Use Committee of China Agricultural University (No. AW62701202-1-2, Beijing, China). The SBM and FCGM were sourced by China Oil and Foodstuffs Corporation (Beijing, China). The analyzed nutrient composition of FCGM and SBM are presented in Table 1.

2.1. Experimental design and sample collection

Exp. 1. Eighteen crossbred barrows (BW = 60.2 ± 3.40 kg) were randomly divided into 3 treatments equally, and the DE and ME contents of FCGM and SBM were determined by the difference method. The control diet included 97.0% of corn as the energy ingredient, and the other 2 diets were made by replacing 25.8% of corn in the control diet with FCGM or SBM (Table 2).

Pigs were housed in metabolic cages with an environment maintained at 24 ± 2 °C and had free access to drinking water. The feed supply was equal to 4% of BW and was divided equally into 2 parts and fed at 08:00 and 17:00 every day. The feed intake was recorded every feeding time, and the whole experiment period was divided into 7 d of adaptation to diet and 5 d of feces and urine collection. The feces were collected by plastic bags when they appeared and stored at –20 °C. Buckets with 10 mL of 6 mol/L HCl per 1,000 mL were used to collect urine. The urine volume was recorded daily and 10% of the urine was stored at –20 °C. Finally, the feces and urine of each experimental pig were separately mixed

Table 1
Composition of soybean meal and fermented corn germ meal (as-fed basis).

Item	SBM	FCGM
Common nutrient components, %		
Dry matter	89.10	85.80
Crude protein	46.56	20.40
Organic matter	93.42	90.50
Neutral detergent fiber	10.90	30.55
Acid detergent fiber	5.20	6.43
Essential amino acids, %		
Arginine	3.23	0.52
Histidine	1.66	0.69
Isoleucine	1.76	0.79
Leucine	3.18	1.83
Lysine	2.88	0.82
Methionine	0.70	0.40
Phenylalanine	2.06	0.98
Threonine	2.11	0.79
Tryptophan	0.62	0.21
Valine	2.14	1.75
Non-essential amino acids, %		
Alanine	1.93	1.74
Asparagine	4.77	1.60
Cystine	0.77	0.44
Glutamine	6.60	3.47
Glycine	1.89	1.02
Proline	2.37	1.84
Serine	2.10	1.03
Tyrosine	1.46	0.72
Antigenic proteins, mg/g		
Glycinin	36.58	–
β-conglycinin	105.34	–
Trypsin inhibitor, TIU/mg	5.76	–
Short-chain fatty acids, mmol/kg		
Acetic acid	–	110.24
Propionic acid	–	74.50
Butyric acid	–	4.37
Total	–	189.11
Live bacteria, CFU/g		
<i>Lactobacillus</i>	–	4.00 × 10 ⁶
<i>Bacillus subtilis</i>	–	6.00 × 10 ⁸

SBM = soybean meal; FCGM = fermented corn germ meal; TIU = trypsin inhibitor units; CFU = colony forming units.

Table 2
Ingredient composition of the diets in Exp. 1 (% as-fed basis).

Item	Corn	SBM	FCGM
Ingredients			
Corn	97.00	72.00	72.00
SBM	–	25.00	–
FCGM	–	–	25.00
Dicalcium phosphate	1.40	1.40	1.40
Limestone	0.75	0.75	0.75
Salt	0.35	0.35	0.35
Vitamin and trace element premix ¹	0.50	0.50	0.50
Analyzed nutrient levels			
GE, MJ/kg	15.80	16.05	15.95
DM	88.76	89.05	87.64
CP	7.72	16.58	10.75
Ca	0.63	0.66	0.62

SBM = soybean meal; FCGM = fermented corn germ meal; GE = gross energy; DM = dry matter; CP = crude protein.

¹ Each kilogram of diet provided the following amounts of vitamins and minerals: Mn, 50 mg (MnO); Fe, 125 mg (FeSO₄·H₂O); Zn, 125 mg (ZnO); Cu, 150 mg (CuSO₄·5H₂O); I, 50 mg (I₂); Se, 0.30 mg (Na₂SeO₃); retinyl acetic acid, 4,500 IU; cholecalciferol, 1,350 IU; DL-α-tocopheryl acetic acid, 13.5 mg; menadione sodium bisulfite complex, 2.7 mg; niacin, 18 mg; vitamin B₁₂, 27.6 μg; thiamine, 0.6 mg; pyridoxine, 0.9 mg; riboflavin, 1.8 mg; D-calcium-pantothenate, 10.8 mg; nicotinic acid, 30.3 mg; choline chloride, 210 mg.

and then 300 g feces and 45 mL urine were taken as subsamples. Prior to analysis, the subsamples of feces were dried at 65 °C for 72 h and the urine subsamples (4 mL) were dried in a crucible with

quantitative filter paper (BKMAM, Hunan, China) at 65 °C for 8 h (Pan et al., 2016).

Exp. 2. Eighteen crossbred barrows (BW = 59.3 ± 2.52 kg) fitted with T-cannulas at the distal ileum as the method of Stein et al. (1998) were randomly divided into 3 diets equally (Table 3). A nitrogen (N)-free diet was made to calculate the endogenous AA losses, and 62% of FCGM or 27.5% of SBM as the sole AA source were contained in the other 2 test diets.

The metabolic cages, environment and feeding method were the same as those in Exp. 1. The whole experimental period was divided into 14 d of recovery, 5 d of adaptation to diet, and 2 d of ileal digesta collection. Plastic bags were used to collect the digesta from 08:00 to 17:00 and removed to –20 °C refrigerator whenever the digesta were full or at least every 30 min. Further processing of the digesta samples was as described by Pan et al. (2017). At the end of the experiment, digesta sample per pig was thawed, mixed, subsampled, and then lyophilized in a vacuum freeze-dryer (Tofflon Freezing Drying systems, Minhang District, Shanghai, China).

Exp. 3. One hundred and forty-four crossbred growing pigs (BW = 56.1 ± 5.22 kg) were allocated to 4 groups in a randomized complete way with 6 pens per group and 6 pigs per pen (3 barrows and 3 gilts). Four diets (FCGM0, FCGM10, FCGM20 and FCGM30) were formulated using FCGM to replace 0%, 10%, 20% and 30% of

Table 3
Ingredient composition of the experimental diets in Exp. 2 (% as-fed basis).

Item	N-free diet	SBM	FCGM
Ingredients			
Corn starch	73.35	57.50	23.00
SBM	–	27.50	–
FCGM	–	–	62.00
Sucrose	15.00	10.00	10.00
Soybean oil	3.00	2.00	2.00
Cellulose acetic acid ¹	4.00	–	–
Dicalcium phosphate	3.00	1.20	1.20
Limestone	–	0.70	0.70
Salt	0.45	0.30	0.30
Magnesium oxide	0.10	–	–
Potassium carbonate	0.30	–	–
Chromic oxide	0.30	0.30	0.30
Vitamin and trace element premix ²	0.50	0.50	0.50
Analyzed nutrient levels			
DM	90.86	89.25	88.02
CP	0.45	12.46	13.66
Essential amino acids			
Arginine	0.01	0.85	0.35
Histidine	–	0.42	0.41
Isoleucine	0.01	0.50	0.45
Leucine	0.04	0.83	1.10
Lysine	0.01	0.74	0.49
Methionine	0.03	0.16	0.26
Phenylalanine	0.02	0.56	0.58
Threonine	0.01	0.54	0.48
Tryptophan	–	0.15	0.15
Valine	0.02	0.60	1.11
Non-essential amino acids			
Alanine	0.02	0.53	1.08
Asparagine	0.02	1.38	0.98
Cystine	–	0.23	0.26
Glutamine	–	2.06	2.16
Glycine	0.02	0.55	0.62
Proline	–	0.59	1.12
Serine	0.01	0.61	0.68
Tyrosine	0.02	0.36	0.43

SBM = soybean meal; FCGM = fermented corn germ meal; DM = dry matter; CP = crude protein.

¹ Made by Chemical Reagents Company, Beijing, China.

² The vitamin and trace element premix compositions were the same as those in Exp. 1.

SBM in diet. The diets of Exp. 3 (Table 4) were formulated according to NRC (2012) of growing pigs and the values of ME and SID of AA of FCGM and SBM were determined in Exp. 1 and 2.

Pigs had free access to diets and water in concrete-floored pens. The pigs were weighed on d 1 and 30. The feed intake of each pen during the experiment period was recorded, and the average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (F:G) were calculated. About 1 kg fresh feces each pen was collected during d 28 to 30, mixed, and dried at 65 °C for 72 h. Diet samples (500 g) and 5 mL fresh feces (on d 28) were taken and kept at –20 °C until analysis. At 06:00 on d 30, a blood sample was collected through the jugular vein from a pig whose weight was close to the average weight per pen. The blood sample was centrifuged at 3,000 × g for 10 min at 4 °C to obtain the serum sample and was kept at –80 °C until analysis.

2.2. Chemical analysis

The dry matter (DM), CP, ether extract (EE), calcium, phosphorus or ash contents of FCGM, SBM, diets and feces samples were analyzed according to the Association of Official Analytical Chemists (AOAC, 2012). The gross energy (GE) of diets, feces and urine were measured by an automatic isoperibol oxygen bomb calorimeter (Parr 6300 calorimeter; Parr Instrument Company, Moline, IL). The detailed analysis process of AA and chromium content were described by Pan et al. (2017) and Williams et al. (1962),

Table 4
Ingredient composition and nutrient levels of the experimental diets in Exp. 3 (% as-fed basis).

Item	Dietary treatments ¹			
	FCGM0	FCGM10	FCGM20	FCGM30
Ingredients				
Corn	64.16	64.71	64.84	64.01
Soybean meal	20.00	18.00	16.00	14.00
FCGM	0.00	5.00	10.00	15.00
Wheat bran	10.00	6.50	3.40	1.30
Soybean oil	2.20	2.20	2.20	2.20
Dicalcium phosphate	1.20	0.90	0.60	0.40
Limestone	0.90	1.10	1.30	1.41
Sodium chloride	0.30	0.30	0.30	0.30
L-Lysine HCl	0.30	0.34	0.38	0.40
DL-Methionine	0.05	0.05	0.05	0.05
L-Threonine	0.08	0.08	0.10	0.10
L-Tryptophan	0.06	0.07	0.08	0.08
Vitamin and mineral premix ²	0.50	0.50	0.50	0.50
Chromic oxide	0.25	0.25	0.25	0.25
Analyzed nutrient levels				
GE, MJ/kg	16.89	16.84	16.81	16.77
CP	16.10	15.96	15.93	15.87
CF	2.81	2.88	3.00	3.41
NDF	14.76	14.87	15.03	15.39
ADF	3.87	4.02	4.16	4.32
Calculated nutrient levels³				
Metabolizable energy, MJ/kg	13.94	13.94	13.94	13.94
SID lysine	0.88	0.88	0.88	0.88
SID methionine	0.27	0.27	0.27	0.27
SID threonine	0.54	0.54	0.54	0.54
SID tryptophan	0.21	0.21	0.21	0.21

FCGM = fermented corn germ meal; GE = gross energy; CP = crude protein; CF = crude fiber; NDF = neutral detergent fiber; ADF = acid detergent fiber; SID = standard ileal digestible.

¹ FCGM0, FCGM10, FCGM20 and FCGM30 diets were made by substituting fermented corn germ meal for 0%, 10%, 20% and 30% soybean meal.

² The vitamin and trace element premix compositions were the same as those in Exp. 1.

³ The values were calculated according to feed composition and NRC (2012). Metabolizable energy and digestible amino acids of soybean meal and FCGM were obtained in Exp. 1 and 2.

respectively. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) content were determined by the procedures of Van Soest et al. (1991). In addition, the level of immunoglobulin A (IgA), IgG and IgM in serum were analyzed as the operation steps of the kit (Nanjing Jiancheng Institute of Bioengineering, Nanjing). And the serum urea nitrogen (UN), total cholesterol (TC) and triglycerides (TG) were analyzed by automatic biochemical analyzer (CX9, Beckman, California, USA).

The quantification of volatile fatty acid (VFA) in fecal samples were detected. Approximately 1.5 g of fresh fecal sample was placed in a centrifuge tube, mixed with 1.5 mL of sterile water, and centrifuged at $15,000 \times g$ for 15 min at 4 °C. The supernatant was transferred with a gas chromatography vial, mixed with 200 mL metaphosphoric acid, placed in ice for 30 min, and centrifuge at $15,000 \times g$ for 15 min at 4 °C. The VFA content in the fecal sample was measured by a Hewlett Packard 5890 gas chromatograph (HP, Pennsylvania, USA) according to the procedure mentioned by Long et al. (2018).

The microbiota community in feces was also analyzed according Ma et al. (2021). Briefly, after genomic DNA extraction, 1% agarose gel (Thermo Scientific, MA, USA) was used to detect the extracted genomic DNA. The primers, V338F (5'-ACTCCTACGGGAGGCAGCAG-3') and V806R (5'-GGACTACHVGGGTWTCTAAT-3'), targeting variable region V3 to V4 were put into use (Munyaka et al., 2015). Mastercycler Gradient (Eppendorf, Germany) was used to do PCR and Miseq platform (Allwegene, Beijing, China) was used to do deep sequencing, and Illumina Analysis Pipeline Version 2.6 (Illumina, San Diego, USA) was used to do image analysis, base calling as well as error estimation. Firstly, the raw data was screened, and the data, which the sequences less than 230 bp in length, with quality score less than 20, ambiguous bases, or do not match primer sequence and barcode label well, were eliminated. All qualified reads are clustered into operational taxonomic units (OTU) with a similarity threshold of 97% (Edgar, 2013). Sparse curves are generated, and richness and diversity indexes are calculated. Alpha and beta diversity were tested by QIIME (University of California, San Diego, USA).

2.3. Calculations

In Exp. 1, the method of Adeola (2001) was used to determine the DE and ME of SBM or FCGM as follows:

$$DE_d = (GE_i - GE_f) \times F_i$$

$$DE_f = [DE_d - (100\% - X\%) \times DE_d / 0.97] / X\%$$

$$ME_d = (GE_i - GE_f - GE_u) / F_i$$

$$ME_f = [ME_d - (100\% - X\%) \times ME_d / 0.97] / X\%$$

in which the GE_i , GE_f , GE_u and F_i represent the total GE intake, the total GE content in feces and urine, and the total feed intake over the 5-d collection period, respectively. The DE_d and DE_f represent the DE values in diet and SBM or FCGM, ME_d and ME_f represent the ME values in diet and SBM or FCGM, respectively. The X% is the percentage of energy supplied by SBM or FCGM in the basal diet.

In Exp. 2, the method of Stein et al. (2007) was used to determine the SID of AA: $AID(\%) = [1 - (AA_d/AA_f) \times (Cr_f/Cr_d)] \times 100\%$, in which the subscripts “d” and “f” represent the concentrations (g/kg DM) of the substance in ileal digesta and test diet, respectively. The endogenous loss of AA was calculated as: $IAA_{end} = [AA_d \times (Cr_f/Cr_d)]$, in which the subscript “d” represented the concentrations (g/kg DM) of the substance in ileal digesta of pigs fed N-free diet, and the subscript “f” represented the

concentrations (g/kg DM) of Cr in N-free diet. Finally, the SID was calculated as follows: $SID(\%) = AID + (IAA_{end}/AA_f) \times 100\%$.

2.4. Statistical analysis

The data of Exp. 1 and 2 were analyzed using SAS (version 9.2, 2008) with a Student's t-test for unpaired data with each pig as an experimental unit. The data except for the microbiota in Exp. 3 were analyzed using the MIXED procedure of SAS with pen as the experimental unit. The linear and quadratic effects caused by the different ratio of FCGM replacing SBM in diets were calculated by polynomial contrasts. A nonlinear regression (NLIN) model, $ADG = a \times (\text{Ratio of FCGM replacing SBM})^2 + b \times (\text{Ratio of FCGM replacing SBM}) + c$, was used to evaluate the optimal ratio of FCGM replacing SBM in diet to the ADG of growing pigs, and the a, b, c are constants (Robbins et al., 2006). Linear discriminant analysis effect size (LEfSe) used the KruskalWallis rank sum test to analyze the difference in the abundance of the microbiota in feces. The linear discriminant analysis (LDA) scores (threshold = ≥ 3) were used to indicate the size of the effect. Significant differences among groups were defined as $P \leq 0.05$, and the highly significant difference among groups was defined as $P \leq 0.01$.

3. Results

3.1. Chemical composition of SBM and FCGM

The concentration of CP, OM and the AA in SBM are higher than that in FCGM, whereas the NDF and ADF in SBM are lower than those in FCGM (Table 1). However, SBM contained glycinin (36.58 mg/g), β -conglycinin (105.34 mg/g) and trypsin inhibitor (5.76 TIU/mg), which were not found in FCGM. The fermentation process resulted in an increase of *Lactobacillus* and *Bacillus subtilis* counts in FCGM which maintained at about 4.00×10^6 and 6.00×10^8 CFU/g. In addition, the total amount of acetic acid, propionic acid and butyric acid contained in FCGM reached 189.11 mmol/kg.

3.2. Energy concentration in Exp. 1

The contents of DE and ME in diet of SBM group were greater than those of FCGM group ($P < 0.01$; Table 5). Besides, the DE and ME of FCGM or SBM were 13.75 and 12.13 MJ/kg, or 15.43 and 14.76 MJ/kg, respectively. Both DE and ME of SBM were higher than those of FCGM ($P < 0.01$).

3.3. Amino acid digestibility in Exp. 2

The SID of arginine, tryptophan and proline were greater ($P < 0.05$) in SBM than in FCGM, whereas the SID of tyrosine was lower ($P = 0.01$) in SBM compared with FCGM (Table 6).

Table 5

The digestible and metabolizable energy values in diets and ingredients in Exp. 1 (as-fed basis).

Item	Corn	SBM	FCGM	SEM ¹	P-value
Energy in diets, MJ/kg					
Digestible energy	14.19 ^b	14.39 ^a	13.97 ^c	0.06	<0.01
Metabolizable energy	13.78 ^a	13.97 ^a	13.26 ^b	0.07	<0.01
Energy in ingredients, MJ/kg					
Digestible energy	14.63 ^{ab}	15.43 ^a	13.75 ^b	0.31	0.01
Metabolizable energy	14.20 ^a	14.96 ^a	12.13 ^b	0.36	<0.01

SBM = soybean meal; FCGM = fermented corn germ meal.

Values in a row with no common superscripts differ significantly ($P < 0.05$).

¹ SEM stands for the standard error of the mean ($n = 6$).

Table 6
The standardized ileal digestibility (SID) of AA in soybean meal (SBM) and fermented corn germ meal (FCGM) in Exp. 2.

Item	SBM	FCGM	SEM ¹	P-value
Essential amino acids, %				
Arginine	89.02 ^a	86.25 ^b	0.39	0.04
Histidine	86.69	87.35	1.35	0.74
Isoleucine	87.22	86.62	0.92	0.67
Leucine	87.06	88.62	1.38	0.46
Lysine	84.59	84.63	2.63	0.99
Methionine	84.09	85.27	3.76	0.83
Phenylalanine	84.47	88.60	1.95	0.19
Threonine	83.55	85.84	1.79	0.41
Tryptophan	86.21 ^a	75.27 ^b	2.45	0.03
Valine	83.82	88.16	2.56	0.23
Non-essential amino acids, %				
Alanine	83.10	85.23	1.54	0.37
Asparagine	84.92	86.67	2.30	0.61
Cystine	82.16	83.32	2.05	0.70
Glutamine	85.80	88.50	1.20	0.17
Glycine	85.71	82.78	2.52	0.45
Proline	97.39 ^a	93.30 ^b	2.73	<0.01
Serine	83.73	84.22	1.17	0.78
Tyrosine	81.77 ^b	89.92 ^a	1.46	0.01

^{a,b}Values in a row with no common superscripts differ significantly ($P < 0.05$).
¹ SEM stands for the standard error of the mean ($n = 6$).

3.4. Growth performance of growing pigs in Exp. 3

The ADG and ADFI of growing pigs showed quadratic ($P < 0.05$) effects in response to increasing the ratio of FCGM replacing SBM in diet (Table 7). Fig. 1 shows the quadratic model of ADG of growing pigs plotted against the ratio of FCGM replacing SBM in diet. The quadratic curve equation was: $ADG = -0.3075 \times (\text{Ratio of FCGM replacing SBM})^2 + 7.255 \times (\text{Ratio of FCGM replacing SBM}) + 657.55$, and the R^2 was equal to 0.98. When the ratio of FCGM replacing SBM in diet was equal to 11.80%, the ADG reached the highest point.

3.5. Apparent total tract digestibility of nutrient in growing pigs in Exp. 3

Table 8 shows the effects of increasing FCGM in diet on the apparent total tract digestibility (ATTD) of nutrient in growing pigs. The ATTD of ADF and NDF increased quadratically ($P < 0.01$) and linearly ($P = 0.02$) as the ratio of FCGM replacing SBM increased in diet, respectively.

3.6. Serum parameters of growing pigs in Exp. 3

Table 9 shows the effects of the increasing ratio of FCGM replacing SBM in diet on the serum parameters of growing pigs. Serum IgG and UN concentrations increased quadratically ($P = 0.04$), and the concentration of IgM raised linearly ($P = 0.03$) with the enhancement of FCGM level in diet.

Table 7
Effect of replacing soybean meal with fermented corn germ meal (FCGM) in the diets on growth performance of growing pigs.

Item	Dietary treatments ¹				SEM ²	P-value	
	FCGM0	FCGM10	FCGM20	FCGM30		Linear	Quadratic
Initial BW, kg	56.0	56.0	56.0	56.1	3.93	0.96	0.93
Final BW, kg	75.8	76.8	76.6	74.0	4.32	0.11	0.06
ADG, g/d	660	693	687	597	21.30	0.06	0.01
ADFI, g/d	2036	2090	2148	2017	56.50	0.46	0.03
F:G	3.08	3.01	3.13	3.38	0.09	0.11	0.57

BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; F:G = feed conversion ratio.
¹ FCGM0, FCGM10, FCGM20 and FCGM30 diets were made by substituting fermented corn germ meal for 0%, 10%, 20% and 30% soybean meal.
² SEM stands for the standard error of the mean ($n = 6$).

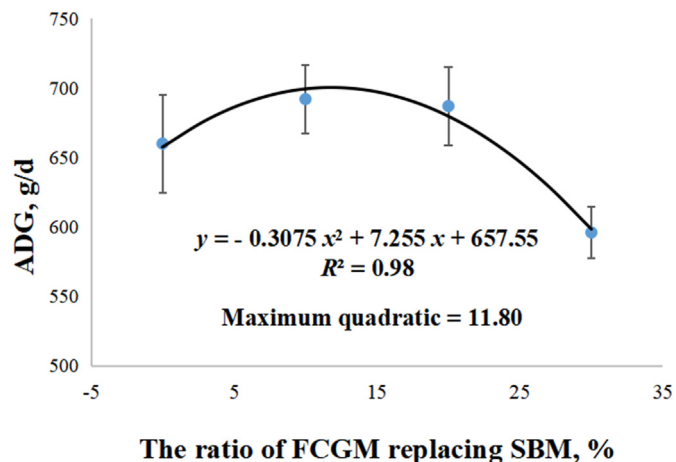


Fig. 1. Quadratic model of average daily gain (ADG) of growing pigs plotted against the ratio of fermented corn germ meal (FCGM) replacing soybean meal (SBM) in diet. The quadratic curve equation was: $ADG = -0.3075 \times (\text{Ratio of FCGM replacing SBM})^2 + 7.255 \times (\text{Ratio of FCGM replacing SBM}) + 657.55$, and the R^2 was equal to 0.98. When the ratio of FCGM replacing SBM in diet was equal to 11.80%, the ADG (699.88 g/d) reached the highest point.

Table 8
Effect of replacing soybean meal with fermented corn germ meal (FCGM) in the diets on nutrient utilization of growing pigs (%).

Item	Dietary treatments ¹				SEM ²	P-value	
	FCGM0	FCGM10	FCGM20	FCGM30		Linear	Quadratic
GE	81.7	81.9	82.4	80.4	0.54	0.15	0.16
CP	77.7	79.2	79.8	78.8	0.74	0.27	0.09
OM	82.4	81.9	81.7	81.6	0.03	0.14	0.56
ADF	41.5	40.5	46.2	44.5	1.81	0.08	<0.01
NDF	50.5	45.9	54.8	54.8	1.91	0.02	0.26

GE = gross energy; CP = crude protein; OM = organic matter; ADF = acid detergent fiber; NDF = and neutral detergent fiber.
¹ FCGM0, FCGM10, FCGM20 and FCGM30 diets were made by substituting fermented corn germ meal for 0%, 10%, 20% and 30% soybean meal.
² SEM stands for the standard error of the mean ($n = 6$).

3.7. The VFA in fecal samples of growing pigs in Exp. 3

The results of VFA in feces showed that the propanoic acid, butyric acid and total VFA increased linearly ($P < 0.05$) as the ratio of FCGM replacing SBM increased in diet (Table 10).

3.8. The 16S rRNA gene sequence in feces of growing pigs in Exp. 3

There were 871 common OTU and 21 unique OTU among 4 treatments in Exp. 3 (Fig. 2). Table 11 shows the results of alpha diversity analysis of which the Shannon index increased linearly with the ratio of FCGM replacing SBM in diet raised ($P < 0.01$).

Table 9
Effect of replacing soybean meal with fermented corn germ meal (FCGM) in the diets on serum parameters of growing pigs.

Item	Dietary treatments ¹				SEM ²	P-value	
	FCGM0	FCGM10	FCGM20	FCGM30		Linear	Quadratic
IgA, g/L	0.90	1.11	1.04	0.91	0.08	0.92	0.16
IgG, g/L	8.41	9.26	9.12	8.28	0.37	0.74	0.04
IgM, g/L	0.74	0.80	0.76	0.94	0.05	0.03	0.24
UN, mmol/L	3.28	2.84	2.14	2.79	0.23	0.06	0.04
TC, mmol/L	2.34	2.18	2.46	2.23	0.09	0.90	0.70
TG, mmol/L	0.39	0.43	0.52	0.47	0.04	0.13	0.32

IgA = immunoglobulin A; IgG = immunoglobulin G; IgM = immunoglobulin M; UN = urea nitrogen; TC = total cholesterol; TG = total triglycerides.

¹ FCGM0, FCGM10, FCGM20 and FCGM30 diets were made by substituting fermented corn germ meal for 0%, 10%, 20% and 30% soybean meal.

² SEM stands for the standard error of the mean (n = 6).

Table 10
Effect of replacing soybean meal with fermented corn germ meal (FCGM) in the diets on fecal volatile fatty acids of growing pigs (mmol/kg).

Item	Dietary treatments ¹				SEM ²	P-value	
	FCGM0	FCGM10	FCGM20	FCGM30		Linear	Quadratic
Acetic acid	68.41	76.26	79.52	84.68	5.95	0.08	0.83
Propionic acid	24.15	29.45	30.00	32.27	2.35	0.04	0.53
Isobutyric acid	1.56	1.62	1.92	1.60	0.18	0.65	0.33
Butyric acid	9.36	13.01	16.30	17.74	1.85	<0.01	0.57
Isovaleric acid	4.55	5.03	5.78	5.03	0.49	0.34	0.24
Valeric acid	1.44	2.66	2.54	2.63	0.54	0.19	0.32
Total volatile fatty acid	109	128	136	144	8.23	0.01	0.53

¹ FCGM0, FCGM10, FCGM20 and FCGM30 diets were made by substituting fermented corn germ meal for 0%, 10%, 20% and 30% soybean meal.

² SEM stands for the standard error of the mean (n = 6).

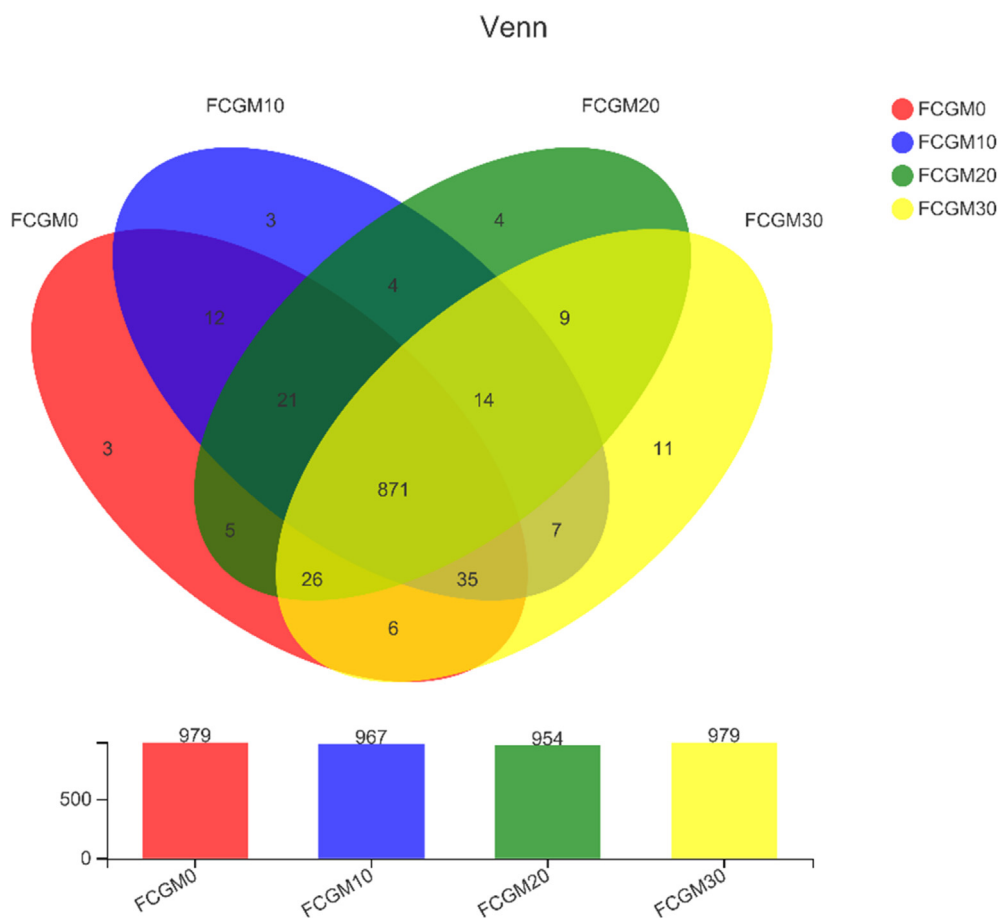


Fig. 2. Microbial OTU Venn diagram in fecal samples of growing pigs as affected by different ratio of fermented corn germ meal (FCGM) replacing soybean meal in diet. FCGM0, FCGM10, FCGM20 and FCGM30 diets were made by substituting FCGM for 0%, 10%, 20% and 30% soybean meal (n = 6). OUT = operational taxonomic units.

Table 11
Effect of replacing soybean meal with fermented corn germ meal (FCGM) in the diets on the diversity of bacterial community in feces of growing pigs.

Item	Dietary treatments ¹				SEM ²	P-value	
	FCGM0	FCGM10	FCGM20	FCGM30		Linear	Quadratic
Sobs	676	702	709	700	29.8	0.56	0.57
Shannon	4.41	4.57	4.80	4.93	0.12	<0.01	0.94
Simpson	0.04	0.04	0.05	0.06	0.01	0.16	0.98
Ace	811	733	793	807	31.2	0.74	0.17
Chao	820	735	803	820	32.1	0.64	0.14

¹ FCGM0, FCGM10, FCGM20 and FCGM30 diets were made by substituting fermented corn germ meal for 0%, 10%, 20% and 30% soybean meal.

² SEM stands for the standard error of the mean ($n = 6$).

The relative abundances of the 4 most common taxa in different treatments at the phylum level are shown in Fig. 3. Firmicutes and Bacteroidetes were the most abundant, followed by Spirochaetes and Proteobacteria, and no significant difference was found at the phylum level among treatments. Figs. 4 and 5 show the top 10 genus-level bacterial communities in relative abundance and the dominant microorganisms with taxa LDA value higher than 3.0 analyzed by LEfSe, respectively. The FCGM0 group had abundant *Streptococcus* ($P < 0.05$) genus, the FCGM10 group was abundant in *Lactobacillus* ($P < 0.05$) genus, group FCGM20 had high *Clostridium_sensu_stricto* ($P < 0.05$) and group FCGM30 had high *Lachnospiraceae*, *Ruminiclostridium* and *Ruminococcus* genus ($P < 0.05$).

4. Discussion

As expected, Exp. 1 showed that the DE and ME were significantly lower in FCGM than in SBM, which might be related to the difference in chemical composition of SBM and FCGM. From Table 1, we could find that the CP content of SBM higher than that of FCGM, and the content of NDF and ADF of SBM were lower than that of FCGM, respectively. Noblet and Goff (2001) summarized the factors affecting the energy value of pig diets, they found that the DE and ME in ingredient were negatively correlated with the content of ADF and NDF, but positively correlated with the CP content of ingredient. Besides, previous research also reported the content of ADF in CGM was negatively correlated with its DE and ME (Shi et al., 2019). Therefore, the higher NDF and ADF as well as lower CP content in FCGM might be the main reason that caused the energy value of FCGM significantly lower than that of SBM. Besides, the available energy content of FCGM in this study can be used as a reference for

pig diet formulations in production because these values were absent in NRC (2012). Although the content of AA in FCGM was lower than that in SBM, the SID values of most AA (except arginine, tryptophan, and proline) of FCGM were not significantly different from those of SBM in Exp. 2, which indicated that FCGM had a certain similarity with SBM in terms of the digestibility of AA. Besides, compared with SBM, FCGM did not contain antigen proteins (such as glycinin and β -conglycinin) or trypsin inhibitors which were harmful to the nutrient utilization and intestinal health of pigs (Dunsoford et al., 1989; Li et al., 1991). Furthermore, FCGM contains probiotics (*Lactobacillus* and *B. subtilis*) and total VFA which were beneficial to animals (Scholten et al., 1999). Therefore, although the available energy value of FCGM was lower than that of SBM, FCGM has the potential to partially replace SBM in a traditional corn–soybean meal diets and may have better feeding effects for pigs.

The effects of fermented feed in pigs have been studied for decades, however, almost no research on the effects of FCGM in diet of growing pigs. The results of Exp. 3 showed that the ADG and ADFI were increased quadratically with the ratio of FCGM replacing SBM raised in diet, which indicated that a suitable ratio of FCGM replacing SBM provided a better growth performance of growing pigs. The increased ADFI and ADG were observed when the ratio of FCGM replacing SBM was less than 20%, which might be associated with the increased levels of fiber content in diets. From the analyzed diet nutrient levels of Exp. 3, we found that the CF, NDF and ADF of diet all increased as the ratio of FCGM replacing SBM increased. Supriyati et al. (2015) found that the feed intake of animals would improve as the crude fiber raised in diet, and another research also reported that appropriate amount of fiber (below 5%) addition was beneficial for the growth performance of animals (Mateos et al., 2002). Interestingly, the ADG and ADFI of growing pigs dropped sharply when the ratio of FCGM replacing SBM in diet reached 30%, which might be related to the poor palatability of the FCGM30 diet. The palatability of fermented feed ingredients was affected by the pH value and the concentration of organic acids produced during the fermentation process (Le et al., 2016). The high concentrations of acetic acid, butyric acid and biogenic amines produced by fermentation would reduce the palatability of the diet (Missotten et al., 2015). Thus, the FCGM used in the current study contained acetic acid (110.24 mmol/kg) and butyric acid (4.37 mmol/kg), which might reduce the palatability of the diet of FCGM30 as the FCGM replacing SBM reached a large proportion (30%), and further caused a decrease in the ADFI and ADG of growing pigs.

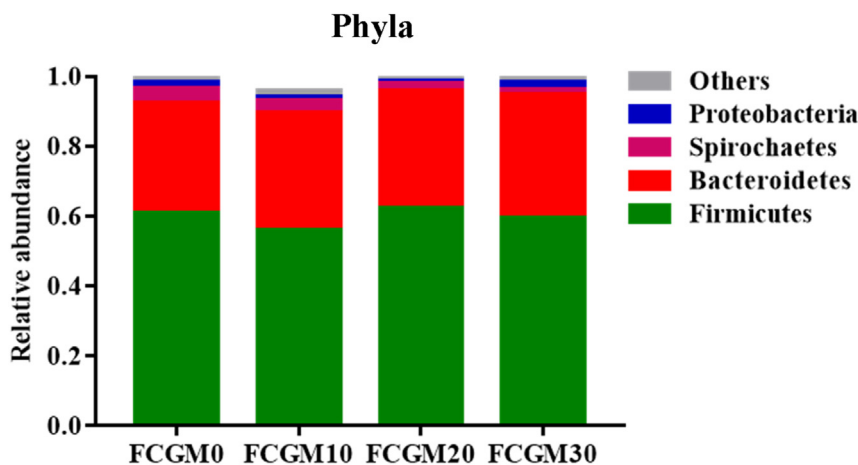


Fig. 3. Fecal microbial community at the phylum level of growing pigs as affected by different ratio of fermented corn germ meal (FCGM) replacing soybean meal in diet (relative abundances below 0.05% were grouped as “others”). FCGM0, FCGM10, FCGM20 and FCGM30 diets were made by substituting FCGM for 0%, 10%, 20% and 30% soybean meal ($n = 6$).

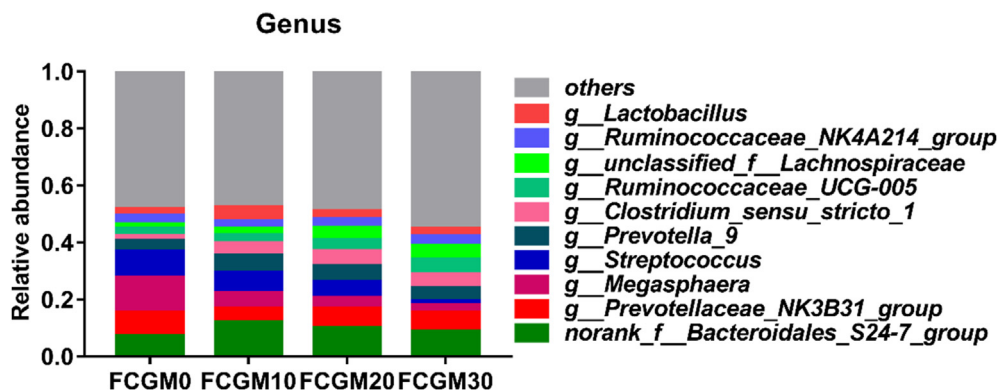


Fig. 4. Fecal microbial community at the genus level of growing pigs as affected by different ratio of fermented corn germ meal (FCGM) replacing soybean meal in diet (relative abundances below 0.05% were grouped as “others”). FCGM0, FCGM10, FCGM20 and FCGM30 diets were made by substituting FCGM for 0%, 10%, 20% and 30% soybean meal (n = 6).

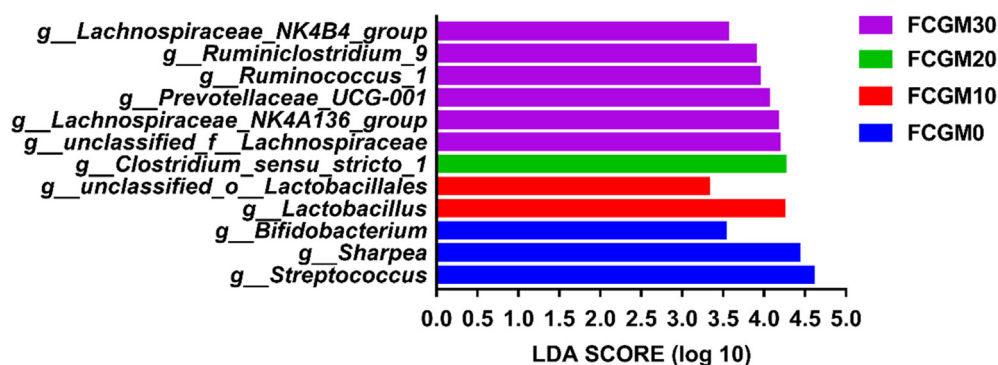


Fig. 5. Fecal microbial community at the genus level of growing pigs as affected by different ratio of fermented corn germ meal (FCGM) replacing soybean meal in diet using LEfSe analyses (linear discriminant analysis score above 3, P < 0.05). FCGM0, FCGM10, FCGM20 and FCGM30 diets were made by substituting FCGM for 0%, 10%, 20% and 30% soybean meal (n = 6).

The ATTD of ADF and NDF were increased quadratically and linearly, respectively, and the ATTD of CP tended to increase quadratically with the increase of the ratio of FCGM replacing SBM. In partial consistent with our results, Xu et al. (2019) found that the digestibility of DM, CP and GE of growing pigs fed fermented feed was increased by the method of meta-analysis. Similarly, Lee et al. (2014) also reported that increasing the content of fermented biomass of *B. subtilis* LS 1–2 in diet could linearly increase the digestibility of CP and GE in weaned piglets. Fermented feed could improve the digestibility of nutrients in animals, possibly because fermented feed contains the probiotics (such as *Bacillus* and *Lactobacillus plantarum*), which could produce dietary enzymes, such as cellulase and amylase, which degraded CP and carbohydrates into small peptides, free AA and other small molecular nutrients (Ouoba et al., 2003; Hu et al., 2016). Besides, fermentation also produced growth factors which could improve the activity of protease, amylase, and probiotic (Ouoba et al., 2003; Feng et al., 2007). In the current study, the FCGM analyzed to contain 4.00×10^6 CFU/g *Lactobacillus* and 6.00×10^8 CFU/g *B. subtilis* on as-fed basis, which might be the main reason that the digestibility of ADF and NDF increased with the levels of FCGM raised in diets. On the other hand, we also found the abundant Lachnospiraceae genus in feces of FCGM15 group, which could degrade non-starch polysaccharides and improve the utilization of dietary fiber (Reichardt et al., 2014). Therefore, the higher digestibility of ADF and NDF in pigs fed FCGM diets may be due to the live *Lactobacillus* and *B. subtilis* strains contained in FCGM.

It was reported that the composition of microorganisms in the intestines of animals has a strong correlation with their immune

systems (Vrese and Marteau, 2007). In this research, the levels of serum IgG and IgM increased quadratically or linearly with the ratio of FCGM replacing SBM raised in diet, which might be caused by the improvement of the pig intestinal microflora. Previous studies showed that probiotics or organic acids in diet could inhibit *Escherichia coli* and *Salmonella* in the large intestine, and contribute to improve the concentrations of serum IgM and IgG of pigs (Zhu et al., 2017; Long et al., 2018). The FCGM used in the present study contained probiotics and VFA, which could favor the beneficial microorganisms and improve the intestinal balance of pigs (Peng et al., 2021). Besides, we also found that increasing the level of FCGM in diet could lead to a significant linear increase in butyric acid and total VFA in feces, which could reduce the disease sensitivity by lowering intestinal pH and modifying the immune system of pigs (Dibner and Buttin, 2002). In line with our results, Mizumachi et al. (2009) found that a fermented liquid diet prepared with *L. plantarum* LQ80 could improve the IgM and IgG levels in the serum of piglets. Our results indicated that the probiotics contained in FCGM as well as the high levels of the VFA in the large intestine of pigs fed FCGM diets might be the main reasons for the enhanced immunity of growing pigs.

The VFA are the main products of dietary fiber fermented by microorganisms in the large intestine, which can be rapidly absorbed by intestinal cells and have beneficial effects on animals (Bergman, 1990; Long et al., 2021). We found that increasing the ratio of FCGM replacing SBM in diet resulted in a significant linear increase in propionic acid, butyric acid and total VFA contents of feces, which was consistent with the increase in ATTD of ADF and NDF, indicating that fermented feed promoted microbial utilization

of carbohydrates to produce VFA. Interestingly, the relative abundance of Lachnospiraceae was significantly higher in treatment FCGM30 than in other treatments, which could degrade non-starch polysaccharides to produce propionic acid and butyric acid (Reichardt et al., 2014; Louis et al., 2004). The VFA, especially butyric acid, was beneficial on the epithelial absorption function and cell growth of pigs (Bergman, 1990), which was also consistent with the highest digestibility of NDF and ADF as well as ADG of growing pigs appeared in diets with FCGM.

Alpha diversity is the diversity of species richness in a specific ecosystem (Xu et al., 2020). In Exp. 3, the Shannon index was increased linearly with the ratio of FCGM replacing SBM in diet raised, which could be explained by the suitable growth environment for the microorganisms due to the probiotic and organic acid of FCGM in diet. And our results also showed that FCGM in diet did not affect the relative abundance of microbiota at the phylum level, but at the genus level, the relative abundance of Lachnospiraceae in diet FCGM30 and *Lactobacillus* in diet FCGM10 were significantly increased compared with diet FCGM0, and the relative abundance of *Streptococcus* in diet FCGM0 was significantly higher than other groups. The increase of Lachnospiraceae in feces might be related to the increment in dietary fiber content (Jackson and Jewell, 2019). Biddle et al. (2013) found that Lachnospiraceae specializes in degrading fiber in the diet, which was also corresponding with the improvement of VFA content in feces. Additionally, it exhibited a significant increment in the relative abundance of *Lactobacillus* in diet FCGM10. The increment in *Lactobacillus* can produce lactic acid, which could delay the proliferation of potential pathogens including *E. coli* by reducing the pH of gastrointestinal and effectively improving the growth performance of pigs (Suiryanrayna and Ramana, 2015). *Streptococcus*, one of the major pathogens in swine, has a strong virulence and drug resistance due to the ability to form biofilms (Wang et al., 2018). Researches showed that *Lactobacillus* and *Bacillus* can inhibit the growth and activity of intestinal pathogens, such as *Streptococcus* (Lan et al., 2016; Sirichokchatchawan et al., 2018). Therefore, the microbiological results showed that appropriate addition of FCGM in diets could inhibit the intestinal pathogens (such as Lachnospiraceae) and enrich beneficial bacteria (such as *Lactobacillus*) and further promote the immunity and health status of growing pigs.

5. Conclusion

It was concluded that the DE and ME as well as the SID of arginine, tryptophan and proline were lower in FCGM than in SBM. A low ratio of FCGM replacing SBM in diet could improve the growth performance of growing pigs, and we recommend replacing 11.80% of SBM with FCGM to obtain the optimal ADG of growing pigs. Moreover, as the ratio of FCGM replacing SBM increased in diet, the immunity, intestinal microbiota and VFA composition of growing pigs were improved.

Author contributions

Tengfei He: Conceptualization, Methodology, Software; Data curation, Writing – Original draft preparation; **Yuhui Zheng:** Visualization, Investigation; **Shenfei Long:** Software, Validation; **Xiangshu Piao:** Supervision; Writing – Reviewing and Editing.

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

This study is supported by the National Natural Science Foundation of China (31772612), the Beijing Natural Science Foundation (6202019) and the Key Research & Developmental Program of Shandong Province (2019JZZY020308).

References

- Adeola O. Digestion and balance techniques in pigs. In: Lewis J, Southern LL, editors. Swine nutrition. Washington, DC: CRC Press; 2001. p. 903–16. <https://doi.org/10.1201/9781420041842.ch40>.
- AOAC. Official methods of analysis. 19th ed. Arlington, VA: Off. Assoc. Anal. Chem.; 2012.
- Bergman EN. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol Rev* 1990;70:567–90. <https://doi.org/10.1152/physrev.1990.70.2.567>.
- Biddle A, Stewart L, Blanchard J, Leschine S. Untangling the genetic basis of fibrolytic specialization by Lachnospiraceae and Ruminococcaceae in diverse gut communities. *Diversity* 2013;5:627–40. <https://doi.org/10.3390/d5030627>.
- Dibner JJ, Buttin P. Use of organic acid as a model to study the impact of gut microflora on nutrition and metabolism. *J Appl Poult Res* 2002;11:453–63. <https://doi.org/10.3109/00016347909154051>.
- Dunsoford BR, Knabe DA, Hacnsly WE. Effect of dietary soybean meal on the microscopic anatomy of the small intestine in the early-weaned pig. *J Anim Sci* 1989;72:1855–63. <https://doi.org/10.2527/jas1989.6771855x>.
- Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* 2013;10:996–8. <https://doi.org/10.1038/nmeth.2604>.
- Estrada-Restrepo JE. Effects of body weight and research conditions on the productive energy content of corn germ meal fed to growing-finishing pigs [Doctoral degree thesis Dissertation]. University of Illinois at Urbana-Champaign; 2017.
- Feng J, Liu X, Xu ZR, Wang YZ, Liu JX. Effects of fermented soybean meal on digestive enzyme activities and intestinal morphology in broilers. *Poult Sci* 2007;86(6):1149–54. <https://doi.org/10.1093/ps/86.6.1149>.
- Hu YN, Wang YW, Li AK, Wang ZS, Zhang XL, Yun TT, et al. Effects of fermented rapeseed meal on antioxidant functions, serum biochemical parameters and intestinal morphology in broilers. *Food Agric Immunol* 2016;27:182–93. <https://doi.org/10.1080/09540105.2015.1079592>.
- Jackson MI, Jewell DE. Balance of saccharolysis and proteolysis underpins improvements in stool quality induced by adding a fiber bundle containing bound polyphenols to either hydrolyzed meat or grain-rich foods. *Gut Microbes* 2019;10:298–320. <https://doi.org/10.1080/19490976.2018.1526580>.
- Jaworski NW, Lærke HN, Bach Knudsen KE, Stein HH. Carbohydrate composition and in vitro digestibility of dry matter and nonstarch polysaccharides in corn, sorghum, and wheat and coproducts from these grains. *J Anim Sci* 2015;93:1103–13. <https://doi.org/10.2527/jas.2014-8147>.
- Jezierny D, Mosenthin R, Bauer E. The use of grain legumes as a protein source in pig nutrition: a review. *Anim Feed Sci Technol* 2010;157:111–28. <https://doi.org/10.1016/j.anifeedsci.2010.03.001>.
- Lan RX, Lee SL, Kim IH. Effects of multistrain probiotics on growth performance, nutrient digestibility, blood profiles, faecal microbial shedding, faecal score and noxious gas emission in weaning pigs. *J Anim Physiol Anim Nutr* 2016;100:1130–8. <https://doi.org/10.1111/jpn.12501>.
- Le MH, Galle S, Yang Y, Landero JL, Beltranena E, Gänzle MG, et al. Effects of feeding fermented wheat with *Lactobacillus reuteri* on gut morphology, intestinal fermentation, nutrient digestibility, and growth performance in weaned pigs. *J Anim Sci* 2016;94:4677–87. <https://doi.org/10.2527/jas.2016-0693>.
- Lee JW, Mckeith FK, Stein HH. Up to 30% corn germ may be included in diets fed to growing-finishing pigs without affecting pig growth performance, carcass composition, or pork fat quality. *J Anim Sci* 2012;90:4933–42. <https://doi.org/10.2527/jas.2012-5129>.
- Lee SH, Ingale SL, Kim JS, Kim KH, Lokhande Anushka, Kim EK, et al. Effects of dietary supplementation with *Bacillus subtilis* LS 1–2 fermentation biomass on growth performance, nutrient digestibility, cecal microbiota and intestinal morphology of weanling pig. *Anim Feed Sci Technol* 2014;188:102–10. <https://doi.org/10.1016/j.anifeedsci.2013.12.001>.
- Li DF, Nelssen JL, Reddy PG, Blecha F, Klemm RD, Giesting DW, et al. Measuring suitability of soybean products for early-weaned pigs with immunological criteria. *J Anim Sci* 1991;69:3299–307. <https://doi.org/10.2527/1991.6983299x>.
- Li MH, Robinson EH, Oberle DF, Lucas PM, Bosworth BG. Use of corn meal in diet for pond-raised channel catfish, *Ictalurus punctatus*. *J World Aquacult Soc* 2013;44:282–7. <https://doi.org/10.1111/jwas.12019>.
- Li ZC, Li P, Liu DW, Li DF, Wang FL, Su YB, et al. Determination of the energy value of corn distillers dried grains with solubles containing different oil levels when fed to growing pigs. *J Anim Physiol Anim Nutr* 2017;101:339–48. <https://doi.org/10.1111/jpn.12445>.

- Long SF, Xu YT, Pan L, Wang QQ, Wang CL, Wu JY, et al. Mixed organic acids as antibiotic substitutes improve performance, serum immunity, intestinal morphology and microbiota for weaned piglets. *Anim Feed Sci Technol* 2018;235:23–32. <https://doi.org/10.1016/j.anifeedsci.2017.08.018>.
- Long SF, Liu SJ, Wang J, Mahfuz S, Piao XS. Natural capsicum extract replacing chlortetracycline enhances performance via improving digestive enzyme activities, antioxidant capacity, anti-inflammatory function, and gut health in weaned pigs. *Anim Nutr* 2021;7:305–14. <https://doi.org/10.1016/j.aninu.2020.12.004>.
- Louis P, Duncan SH, McCrae SI, Millar J, Jackson MS, Flint HJ. Restricted distribution of the butyric acid kinase pathway among butyric acid-producing bacteria from the human colon. *J Bacteriol* 2004;186:2099–106. <https://doi.org/10.1128/JB.186.7.2099-0106.2004>.
- Ma JY, Piao XS, Shang QH, Long SF, Liu SJ, Mahfuz S. Mixed organic acids as an alternative to antibiotics improve serum biochemical parameters and intestinal health of weaned piglets. *Anim Nutr* 2021;7:737–49. <https://doi.org/10.1016/j.aninu.2020.11.018>.
- Mateos GG, Lazaro R, Gracia MI. The feasibility of using nutritional modifications to replace drugs in poultry feeds. *J Appl Poult Res* 2002;11:437–52. <https://doi.org/10.1093/japr/11.4.437>.
- Missotten JA, Michiels J, Degroote J, De Smet S. Fermented liquid feed for pigs: an ancient technique for the future. *J Anim Sci Biotechnol* 2015;6:4. <https://doi.org/10.1186/2049-1891-6-4>.
- Mizumachi K, Aoki R, Ohmori H, Saeki M. Effect of fermented liquid diet prepared with *Lactobacillus plantarum* LQ80 on the immune response in weaning pigs. *Animal* 2009;3:670–6. <https://doi.org/10.1017/S1751731109003978>.
- Munyaka PM, Eissa N, Bernstein CN, Khafipour E, Chia JE. Antepartum antibiotic treatment increases offspring susceptibility to experimental colitis: a role of the gut microbiota. *PLoS One* 2015;10:536–58. <https://doi.org/10.1371/journal.pone.0142536>.
- Noblet J, Goff GL. Effect of dietary fibre on the energy value of feeds for pigs. *Anim Feed Sci Technol* 2001;90:35–52. [https://doi.org/10.1016/S0377-8401\(01\)00195-x](https://doi.org/10.1016/S0377-8401(01)00195-x).
- NRC. Nutrient requirements of swine. 11th revised ed. Washington, DC: National Academy Press; 2012.
- Ouoba LI, Rechinger KB, Barkholt V, Diawara B, Traore AS, Jakobsen M. Degradation of proteins during the fermentation of African locust bean (*Parkia biglobosa*) by strains of *Bacillus subtilis* and *Bacillus pumilus* for production of Soumbala. *J Appl Microbiol* 2003;94(3):396–402. <https://doi.org/10.1046/j.1365-2672.2003.01845.x>.
- Pan L, Li P, Ma XK, Xu YT, Tian QY, Liu L, et al. Tannin is a key factor in the determination and prediction of energy content in sorghum grains fed to growing pigs. *J Anim Sci* 2016;94:2879–89. <https://doi.org/10.2527/jas.2016-0457>.
- Pan L, Shang QH, Wu Y, Ma XK, Long SF, Liu L, et al. Concentration of digestible and metabolizable energy, standardized ileal digestibility, and growth performance of pigs fed diets containing sorghum produced in the United States or corn produced in China. *J Anim Sci* 2017;95:4880–92. <https://doi.org/10.2527/jas2017.1859>.
- Peng J, Tang YM, Huang YH. Gut health: the results of microbial and mucosal immune interactions in pigs. *Anim Nutr* 2021;7(2):282–94. <https://doi.org/10.1016/j.aninu.2021.01.001>.
- Reichardt N, Duncan SH, Young P, Belenguer A, McWilliam Leitch C, Scott KP, Flint HJ, Louis P. Phylogenetic distribution of three pathways for propionic acid production within the human gut microbiota. *ISME J* 2014;8:1323–35. <https://doi.org/10.1038/ismej.2014.14>.
- Robbins KR, Saxton AM, Southern LL. Estimation of nutrient requirements using broken-line regression analysis. *J Anim Sci* 2006;84:155–65. https://doi.org/10.2527/2006.8413_supplE155x.
- Scholten RHJ, van der Peet-Schwering CMC, Verstegen MWA, den Hartog LA, Schrama JW, Vesseur PC. Fermented co-products and fermented compound diets for pigs: a review. *Anim Feed Sci Technol* 1999;82:1–19. [https://doi.org/10.1016/S0377-8401\(99\)00096-6](https://doi.org/10.1016/S0377-8401(99)00096-6).
- She Y, Su YB, Liu L, Huang CF, Li JT, Li P, et al. Effects of microbial phytase on coefficient of standardized total tract digestibility of phosphorus in growing pigs fed corn and corn co-products, wheat and wheat co-products and oilseed meals. *Anim Feed Sci Technol* 2015;208:132–44. <https://doi.org/10.1016/j.anifeedsci.2015.07.011>.
- Shi M, Liu ZY, Wang HL, Shi CX, Liu L, Wang JJ, et al. Determination and prediction of the digestible and metabolizable energy contents of corn germ meal in growing pigs. *Asian Australas J Anim Sci* 2019;32:405–12. <https://doi.org/10.5713/ajas.17.0891>.
- Sirichokhachawan W, Pupa P, Praechansri P, Am-In N, Tanasupawat S, Sonthayanon P, Prapasarakul N. Autochthonous lactic acid bacteria isolated from pig faeces in Thailand show probiotic properties and antibacterial activity against enteric pathogenic bacteria. *Microb Pathog* 2018;119:208–15. <https://doi.org/10.1016/j.micpath.2018.04.031>.
- Stein HH, Shipley CF, Easter RA. Technical note: a technique for inserting a T-cannula into the distal ileum of pregnant sows. *J Anim Sci* 1998;76:1433–6. <https://doi.org/10.2527/1998.7651433x>.
- Stein HH, Sève B, Fuller MF, Moughan PJ, de Lange CF. Committee on terminology to report AA bioavailability and digestibility. invited review: amino acid bioavailability and digestibility in pig feed ingredients: terminology and application. *J Anim Sci* 2007;85:172–80. <https://doi.org/10.2527/jas.2005-742>.
- Suiryanrayna MV, Ramana JV. A review of the effects of dietary organic acids fed to swine. *J Anim Sci Biotechnol* 2015;6:45. <https://doi.org/10.1186/s40104-015-0042-z>.
- Supriyati TH, Susanti T, Susana IWR. Nutritional value of rice bran fermented by *Bacillus amyloliquefaciens* and humic substances and its utilization as a feed ingredient for broiler chickens. *Asian Australas J Anim Sci* 2015;28:231–8. <https://doi.org/10.5713/ajas.14.0039>.
- Tang JW, Sun H, Yao XH, Wu YF, Wang X, Feng J. Effects of replacement of soybean meal by fermented cottonseed meal on growth performance, serum biochemical parameters and immune function of yellow-feathered broilers. *Asian Australas J Anim Sci* 2012;25:393–400. <https://doi.org/10.5713/ajas.2011.11381>.
- Urriola PE, Shurson GC, Stein HH. Digestibility of dietary fiber in distillers co-products fed to growing pigs. *J Anim Sci* 2010;88:2373–81. <https://doi.org/10.2527/jas.2009-2227>.
- Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci* 1991;74:3583–97. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2).
- Vrese MD, Marteau PR. Probiotics and prebiotics: effects on diarrhea. *J Nutr* 2007;137:803–11. <https://doi.org/10.1089/jmf.2005.067>.
- Wang Y, Wang YX, Sun LY, Grenier D, Yi L. Streptococcus suis biofilm: regulation, drug-resistance mechanisms, and disinfection strategies. *Appl Microbiol Biotechnol* 2018;102:9121–9. <https://doi.org/10.1007/s00253-018-9356-z>.
- Weber TE, Trabue SL, Ziemer CJ, Kerr BJ. Evaluation of elevated dietary corn fiber from corn germ meal in growing female pigs. *J Anim Sci* 2010;88:192–201. <https://doi.org/10.2527/jas.2009-1896>.
- Williams CH, David DJ, Iismaa O. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. *J Agric Sci* 1962;59:381–5. <https://doi.org/10.1017/S002185960001546X>.
- Wizna Abbas H, Rizal Y, Dharma A, Kompiang IP. Improving the quality of tapioca by-products (onggok) as poultry feed through fermentation by *Bacillus amyloliquefaciens*. *Paki J Nutr* 2009;8:1636–40. <https://doi.org/10.3923/pjn.2009.1636.1640>.
- Xu BC, Zhu LY, Fu J, Li Z, Wang YZ, Jin ML. Overall assessment of fermented feed for pigs: a series of meta-analyses. *J Anim Sci* 2019;97:4810–21. <https://doi.org/10.1093/jas/skz2350>.
- Xu YT, Lahaye L, He ZX, Zhang JX, Yang CB, Piao XS. Micro-encapsulated essential oils and organic acids combination improves intestinal barrier function, inflammatory responses and microbiota of weaned piglets challenged with enterotoxigenic *Escherichia coli* F4 (K88+). *Anim Nutr* 2020;6:269–77. <https://doi.org/10.1016/j.aninu.2020.04.004>.
- Zhang HY, Yi JQ, Piao XS, Li PF, Zeng ZK, Wang D, et al. The metabolizable energy value, standardized ileal digestibility of amino acids in soybean meal, soy protein concentrate and fermented soybean meal, and the application of these products in early-weaned piglets. *Asian Australas J Anim Sci* 2013;26:691–9. <https://doi.org/10.5713/ajas.2012.12429>.
- Zhu JJ, Gao MX, Zhang RL, Sun ZJ, Wang CM, Yang FF, et al. Effects of soybean meal fermented by *L. plantarum*, *B. subtilis* and *S. cerevisiae* on growth, immune function and intestinal morphology in weaned piglets. *Microb Cell Fact* 2017;16:191–201. <https://doi.org/10.1186/s12934-017-0809-3>.