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Original Research Article

Endogenous chitinase might lead to differences in growth performance and intestinal health of piglets fed different levels of black soldier fly larva meal

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ABSTRACT

This study aimed to investigate the effects of different levels of black soldier fly (BSF) replacing soybean meal (SBM) in diets on the performance and health condition of piglets. A total of 180 weaned piglets were allocated into 5 treatments: BSF0 (corn-soybean meal basal diet), BSF25 (BSF replacing 25% SBM), BSF50 (BSF replacing 50% SBM), BSF75 (BSF replacing 75% SBM) and BSF100 (BSF replacing 100% SBM). During the whole period, in comparison with BSF0, average daily gain (ADG) and average daily feed intake increased in the BSF25 and BSF50 groups, whereas ADG decreased in the BSF75 and BSF100 groups ($P < 0.05$). The result of quadratic fitting curve showed that piglets exhibited the highest ADG when BSF replaced around 20% SBM. Compared with BSF0, organic matter and dry matter digestibility improved in the BSF25 group, whereas ether extract digestibility decreased in the BSF100 group ($P < 0.05$). In comparison with BSF0, piglets from the BSF25 group showed a higher duodenal ratio of villus height to crypt depth, increased jejunal sucrase activity, serum neuropeptide Y and ghrelin levels, elevated ileal immunoglobulin (Ig) A, IgG and IgM contents and a lower leptin level, and piglets from the BSF100 group exhibited an increased relative weight of kidney ($P < 0.05$). However, no significant differences were observed in the expression level of tight junction proteins and chitin-degrading enzyme. Additionally, compared with BSF0, the abundance of short chain fatty acid producing bacteria such as Ruminococcaceae, *Faecalibacterium* and *Butyricicoccus* increased, and potential pathogenic bacteria decreased in piglets from the BSF25 group, whereas piglets from the BSF100 group had a greater abundance of harmful bacteria. In conclusion, BSF replacing 25% SBM in diets could improve digestive parameters, immune function and intestinal microbiota, and thus improved growth performance of piglets. However, BSF replacing 100% SBM showed an adverse effect on piglet performance, and the reason might be related to the limited amount of chitin-degrading enzyme.

1. Introduction

With the expansion of the livestock industry, traditional sources of protein such as soybean meal (SBM) are becoming increasingly inadequate to meet demands (FAO, 2011; OECD, 2018). Currently, insects are being considered as a potential novel protein source in animal feed due to their high protein level (FAOSTAT, 2016; IFIF, 2018; Makkar et al., 2014). Insects are also rich in amino acids, lipids, vitamins and minerals (Sánchez-Muros et al., 2014).

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Moreover, insects are proposed as a sustainable and economical protein source, because they can be easily reared in low-value organic streams (Salomone et al., 2017; Meneguz et al., 2018a,b). One particular insect, black soldier fly (BSF) (*Hermetia illucens* L) is considered a high-quality source of protein (Makkar et al., 2014; Jucker et al., 2017). The application of BSF as a protein source in fish, poultry and pig diets has been demonstrated (Dabbou et al., 2018; Renna et al., 2017; Spranghers et al., 2018).

Previous studies have reported that different levels of insect protein added to diets might exhibit different effects on the performance of animals. Li et al. (2022b) found that adding 1.4% defatted BSF to diets increased the weight gain of Tongue Sole (*Cynoglossus semilaevis*), while weight gain decreased when BSF level was increased to 5.8%. Similarly, Miah et al. (2020) showed that adding 7% full-fat silkworm chrysalis meal to broilers diets improved weight gain, while decreased weight gain was observed when the addition level was 14%. As for piglets, previous reports demonstrated that the growth of piglets increased or decreased with the increase of insect protein levels in diets (Biasato et al., 2019; Hakenasen et al., 2021; Crosbie et al., 2021). The differences might be related to the dose gradient in the experiment, and a more comprehensive gradient experiment is necessary to evaluate the effects of different levels of insect protein on piglet performance and health status. Additionally, insects are rich in chitin and piglets can secrete chitinase, which may be an important reason for the varying effects of different contents of insect protein on piglet performance, however the knowledge about chitinase secreted by piglets remains limited (Sánchez-Muros et al., 2014; Kawasaki et al., 2021).

Therefore, the present study aimed to explore different levels of BSF on weaned piglet growth performance, appetite hormones, digestive enzyme activities, level of chitin-degrading enzyme and tight junction proteins, intestinal morphology and microbiota, so as to comprehensively and systematically evaluate the effects of BSF on piglet performance and provide new knowledge on the application of BSF in pig diets.

2. Materials and methods

2.1. Animal ethics statement

The experiment was carried out after approval by the Institutional Animal Care and Use Committee of China Agricultural University (AW13303202-1-1).

2.2. Experimental design and diets

A total of 180 Duroc × Landrace × Yorkshire piglets (weaned at d 28, with initial body weight of 7.43 ± 0.84 kg) were allocated into 5 dietary treatments with 6 replicates and 6 piglets per replicate based on body weight (BW) and gender (3 barrows and 3 gilts per replicate). Piglets were raised on plastic slatted floors. Pens were also equipped with duckbill drinkers and adjustable feeders, and piglets had ad libitum access to feed and water. The humidity and temperature in the room were automatically kept at about 70% and 25 °C, respectively. A corn-soybean basal diet (BSF0) was formulated, and BSF was used to replace 25% (BSF25), 50% (BSF50), 75% (BSF75), or 100% (BSF100) of the protein provided by SBM. This experiment was divided into two stages (d 1 to 14 and d 15 to 28), and all diets in each treatment were formulated based on the nutrient requirements of piglets based on National Research Council (2012) recommendations (Table 1).

The BSF applied in this research was provided by Bennong Agricultural Technology Co., Ltd (Zhengzhou, China). The BSF larvae were raised on food waste. When the average live weight of each larva was about 200 mg, the larva was collected and then microwave-dried for 20 min. Finally, the BSF samples were ground to a meal for further analysis. The proximate nutrient composition of the BSF meal is shown in Table 2.

Table 1
Ingredients and nutrient composition of experimental diets (%, as-fed basis).

Item	Diets[1]				
	BSF0	BSF25	BSF50	BSF75	BSF100
Ingredients					
Corn	60.52	61.84	62.49	63.04	63.90
Soybean meal	16.00	12.00	8.00	4.00	0.00
Extruded soybean	7.00	7.00	7.00	7.00	7.00
Fish meal	5.90	5.90	5.90	5.90	5.90
Black soldier fly	0.00	4.50	9.10	13.70	18.30
Whey powder	2.50	2.50	2.50	2.50	2.50
Soybean oil	4.88	3.55	2.35	1.20	0.00
Dicalcium phosphate	1.10	1.00	0.90	0.85	0.53
Limestone	0.43	0.00	0.00	0.00	0.00
Salt	0.25	0.25	0.25	0.25	0.25
L-Lysine HCl	0.37	0.39	0.42	0.44	0.47
DL-Methionine	0.12	0.13	0.14	0.15	0.17
L-Threonine	0.13	0.14	0.15	0.16	0.17
L-Tryptophan	0.05	0.05	0.05	0.06	0.06
Chromic oxide	0.25	0.25	0.25	0.25	0.25
Premix[2]	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00
Analyzed nutrient composition					
GE, kcal/kg	4232	4194	4140	4161	4144
Crude protein	19.52	19.63	19.49	19.58	19.42
Ether extract	8.22	8.29	8.03	8.59	7.85
Calcium	0.64	0.71	0.90	1.03	1.15
Phosphorus	0.49	0.52	0.49	0.52	0.54
Lysine	1.40	1.46	1.38	1.43	1.52
Methionine	0.52	0.51	0.53	0.56	0.55
Threonine	0.78	0.82	0.80	0.79	0.79
Tryptophan	0.25	0.25	0.25	0.25	0.24
Chitin	–	0.57	0.77	1.02	1.38

SBM = soybean meal; BSF = black soldier fly.
[1] BSF0, control diet; BSF25, BSF replacing 25% SBM in diet; BSF50, BSF replacing 50% SBM in diet; BSF75, BSF replacing 75% SBM in diet; BSF100, BSF replacing 100% SBM in diet.
[2] Premix supplied the following per kilogram of diets: vitamin A, 12,000 IU; vitamin D_3, 2,500 IU; vitamin E, 30 IU; vitamin K_3, 3.0 mg; vitamin B_{12}, 12 μg; riboflavin, 4.0 mg; pantothenic acid, 15.0 mg; niacin, 40.0 mg; choline chloride, 400.0 mg; folacin, 0.7 mg; vitamin B_1, 1.5 mg; vitamin B_6, 3 mg; Mn, 30.0 mg; Fe, 90.0 mg; Zn, 80.0 mg; Cu, 10.0 mg; I, 0.35 mg; Se, 0.3 mg.

2.3. Sample collection

At the end of each period (d 12 to 14 and d 26 to 28), approximately 1,000 g of fecal samples from all pigs in each pen were collected twice a day, then mixed completely and stored at −20 °C (Long et al., 2021). After all samples were collected, feces were thawed completely, and dried at 65 °C for 72 h. All feed and fecal samples were smashed and passed through a 40-mesh (1 mm) sieve before analysis.

According to the growth performance of piglets from different treatments, a piglet (about the average BW of the pen) from each replicate (except for the replicates with the largest or smallest initial BW) from the BSF0, BSF25, and BSF100 groups was slaughtered on d 28 to further investigate the effects of different levels of BSF on piglet performance. One piglet from each replicate from the BSF0, BSF25, and BSF100 groups also had 8 mL of blood collected from the anterior vena cava, which was then centrifuged at 3,000 × *g* for 15 min. The liver, spleen, kidney and heart of slaughtered piglets were taken out and weighed to determine the relative organ weight. Samples of stomach, pancreas, jejunum and ileum were collected, snap frozen in liquid nitrogen, and kept at −80 °C. The

Table 2
Nutrient composition of black soldier fly (BSF) and soybean meal (SBM) used in this study (%, as-fed basis).

Item	BSF	SBM
Dry matter	95.77	87.95
Crude protein	36.69	43.51
Ether extract	31.34	1.59
Ash	12.91	0.60
Digestible energy, kcal/kg	4795	3429
Calcium	5.29	0.42
Phosphorus	0.69	0.42
Chitin	3.95	–
Essential AA		
Arginine	1.73	3.07
Histidine	1.17	1.19
Isoleucine	1.58	1.93
Leucine	2.39	3.16
Lysine	2.16	2.91
Methionine	0.52	0.60
Threonine	1.35	2.02
Tryptophan	0.45	1.75
Valine	2.09	0.58
Phenylalanine	1.43	2.23
Non-essential AA		
Alanine	2.14	2.12
Asparagine	3.36	5.15
Cysteine	0.26	0.65
Glutamine	4.01	7.94
Proline	2.60	1.89
Glycine	1.86	2.25
Serine	1.38	2.44
Tyrosine	2.20	1.22

middle part of the duodenum, jejunum and ileum segments were sampled, and then fixed and stored in 4% formaldehyde solution. In addition, the contents of the ileum, cecum and colon were collected for analysis of short chain fatty acid (SCFA) concentrations and microbiota.

2.4. Growth performance, diarrhea rate and nutrient digestibility

Individual BW and feed consumption of piglets were measured on d 14 and 28 following a 12-h fast for the purpose of calculating average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). During the trial, at 09:00 and 17:00 every day, anuses of piglets were individually checked and any fecal contamination and redness were recorded. At the end of this experiment, the number of diarrheal piglets per treatment was tallied, and the diarrhea rate was calculated using the following formula:

$$\text{Diarrhea rate (\%)} = 100 \times \text{number of piglets with diarrhea}/(\text{number of total piglets} \times \text{number of days}).$$

The organic matter (OM), crude protein (CP), ether extract (EE), dry matter (DM), calcium and phosphorus contents of samples were all measured in accordance with the Association of Official Agricultural Chemists guidelines (AOAC, 2012). An automated adiabatic oxygen bomb calorimeter (Parr 6300 Calorimeter, Moline, IL, USA) was applied to measure gross energy (GE) of samples. An atomic absorption spectrometry (Z-5000; Hitachi, Tokyo, Japan) was used to measure the amount of chromium (Cr) in feed and fecal samples according to the methods described by Williams et al. (1962). The following formula was used to calculate apparent total tract digestibility (ATTD):

$$\text{ATTD (\%)} = [1 - (\text{content of Cr in diets} \times \text{content of nutrients in feces})/(\text{content of Cr in feces} \times \text{content of nutrients in diets})] \times 100$$

Except for methionine, tryptophan and cysteine, the amino acid content of feed samples was analyzed by hydrolysis in 6 mol/L HCl at 110 °C for 24 h, and then analyzed by ion-exchange chromatography with an automatic amino acid analyzer (L-8900, Hitachi, Tokyo, Japan). Cysteine and methionine levels were measured after peroxidation with performic acid and pre-column derivation using phenyl isothiocyanate. The tryptophan level was measured after hydrolysis in 4 mol/L LiOH at 110 °C for 22 h, and then analyzed by high performance liquid chromatography (Agilent1200 Series; Aligent, Santa Clara, CA, USA). The level of chitin was determined as D-Glucosamine following the methods described previously (Wang et al., 2008; Madrid et al., 2013).

2.5. Digestive enzyme and acidic mammalian chitinase (AMCase) activities

The samples of pancreas, stomach, jejunum and ileum were homogenized in 9 volumes of ice-cold saline. Tissue homogenates were centrifuged at 15,000 × *g* for 15 min at 4 °C to collect the supernatant for analyzing enzyme activities. The activities of α-amylase, trypsin, chymotrypsin, maltase, sucrase, lactase and AMCase were determined using respective kits (Nanjing Jiancheng Institute of Bioengineering, Nanjing, China) and a UV-VIS spectrophotometer (UV1100, MAPADA, Shanghai, China).

2.6. Intestinal morphology

The intestinal sample preparation process included dehydration, xylene clearing, and paraffin wax embedding. Hematoxylin and eosin were used to stain serial slices which were 5 μm thick. At least 15 intact and well oriented villi, and their corresponding crypts from each segment were measured under a light microscope.

2.7. Serum parameters and immune immunoglobulin

The levels of serum leptin, ghrelin, peptide YY (PYY), neuropeptide Y (NPY), cholecystokinin (CCK), 5-hydroxytryptamine (5-HT), diamine oxidase (DAO), endotoxin and D-lactate, ileal Immunoglobulin A (IgA), Immunoglobulin M (IgM) and Immunoglobulin G (IgG) were measured using ELISA kits (Laibo Tairui Technology Development Co., Ltd, Beijing, China). A radioimmunoassay (Sn-96513, Shanghai, China) was used to evaluate the level of growth hormone (GH) in serum samples.

2.8. Quantitative real-time PCR analysis

Quantitative real-time PCR was used to evaluate the relative mRNA expression of tight junction proteins including occludin, claudin-1 and zonula occludens-1 (*ZO-1*) in the ileum and *AMCase* in the stomach, jejunum and ileum. Total RNA was extracted using TRIZOL reagent (Invitrogen, Carlsbad, CA) as directed by the manufacturer. A spectrophotometer (NanoDrop ND-1000; Thermo Fisher Scientific, DE) was used to determine the quality and quantity of RNA. Agarose gel electrophoresis was used to assess RNA integrity. Following DNase I (TaKaRa Biotechnology, Dalian, China) instructions, RNA was used for reverse transcription and polymerase chain reaction. A reverse transcription kit (Invitrogen) was used to synthesize first-strand cDNA. Primers were designed using software (Oligo 6.0; Molecular Biology Insights, Cascade, CO), which are presented in Table 3. A 10-μL volume containing 1 μL of cDNA template, 5 μL of SYBR Green mix, 0.2 μL of ROX Reference Dye (50 times), and 0.2 μL of forward and reverse primers was used to conduct real-time PCR. Predenaturation (10 s at 95 °C), 40 cycles of amplification (5 s at 95 °C and 20 s at 60 °C), melting curve

Table 3
Primer sequences of target genes concerned with intestinal barrier function and acidic mammalian chitinase.

Item	Sequences (5′ to 3′)	Length, bp
AMCase	F: TGGTTTGGGCCATTGATCT	81
	R: ACTTCAGCTTGCTTATGAGTG	
Occludin	F: GTGGGACAAGGAACGTATT	115
	R: TCTCTCCGCATAGTCCGAA	
ZO-1	F: GCTCAGCCCTATCCATCT	90
	R: GGACGGGACCTGCTCATAA	
Claudin-1	F: ATACAGGAGGGAAGCCAT	87
	R: ATATTTAAGGACCGCCCTCTC	

AMCase = acidic mammalian chitinase; *ZO-1* = zonula occludens-1; F = forward primer; R = reverse primer.

construction (60 to 99 °C with heating rate of 0.1 °C/s, and fluorescence measurements) were the thermal cycling conditions. Using the $2^{-\Delta\Delta Ct}$ technique, relative gene expression was expressed as a ratio of the target gene to the control gene.

2.9. 16S RNA sequencing

Bacterial DNA was isolated from ileal, cecal, and colonic digesta samples following the instructions provided by the manufacturer using a Stool DNA Kit (Omega Bio-tek, Norcross, GA, USA). The DNA content was determined using a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), and the DNA integrity was confirmed using 1% agarose gel electrophoresis. The 16S rRNA gene V3–V4 region was amplified by primers F338 (5′-ACTCCTACGGG AGGCAGCAG-3′) and R806 (5′-GGAC-TACHVGGGTWTCTAAT-3′). The AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) was used to purify the amplified DNA. The amplified DNA was then quantified using a QuantiFluor TM-ST fluorometer (Promega, USA). Equimolar pools of purified amplicons were assembled for paired-end sequencing (2 × 300) on the Illumina MiSeq platform. On the raw sequences, QIIME demultiplexed and applied quality-filtering (version 1.17). The remaining high-quality sequences were organized into operational taxonomic units (OTU) at 97% similarity by UPARSE after chimeric sequences were found and removed by UCHIME. Using the Silva (SSU128) 16S rRNA database, RDP Classifier (http://rdp.cme.msu.edu/) taxonomically assigned each 16S rRNA gene sequence with a 70% confidence interval.

2.10. SCFA concentrations

The SCFA concentrations of ileal, cecal and colonic digesta samples were determined as described by LH Zhang et al. (2019). Firstly, 0.5 g of digesta samples were dissolved in 8 mL of distilled water, mixed thoroughly, and centrifuged at 3000 × *g* for 5 min. The obtained supernatants were diluted 50 times using water, and then filtered through a 0.22-μm membrane. Each sample was detected by an Ion Chromatography system (DIONEX ICS-3000, Thermo Fisher, Waltham, MA, USA). SCFA were separated by an AS11 analytical column (250 × 4 mm) and an AG11 guard column under the gradient conditions (0 to 5 min, 0.8 to 1.5 mmol/L; 5 to 10 min, 1.5 to 2.5 mmol/L, 10 to 15 min, 2.5 mmol/L), and the flow rate was 1.0 mL/min. The gradient was carried out by potassium hydroxide. The SCFA concentrations are shown as mg/kg of digesta samples.

2.11. Statistical analysis

The SAS MIXED procedure was used to analyze growth performance and nutrient digestibility by using a pen as the experimental unit. Polynomial contrasts were utilized to calculate the linear and quadratic effects by various ratios of BSF replacing SBM in diets. Using a nonlinear regression (NLIN) model, ADG = a × (ratio of BSF replacing SBM)^2 + b × (ratio of BSF replacing SBM) + c, the ideal ratio of BSF replacing SBM in diets to ADG of piglets was evaluated. The χ^2 contingency test was used to analyze diarrhea rate data. The SAS GLM procedures were used to do a one-way Analysis of Variance on other data. For data processing, the individual pig was considered as an experimental unit. $P < 0.05$ was regarded as statistically significant, while a significant trend was defined as $0.05 \leq P < 0.10$.

3. Results

3.1. Growth performance

The effects of BSF replacing SBM on growth performance of weaned piglets are shown in Table 4. During all phases, BSF replacing SBM in piglet diets quadratically affected ADG and ADFI ($P < 0.05$). In comparison with other treatments, piglets from the BSF25 group showed the highest ADG and ADFI ($P < 0.05$). Compared to BSF0, ADG of piglets from the BSF75 and BSF100 groups decreased from d 15 to 28 and d 1 to 28 ($P < 0.05$). However, the rate of diarrhea in piglets from all treatments did not show any differences (Fig. 1). As shown in Fig. 2, the ratio of BSF replacing SBM in diets is plotted against the quadratic model of ADG of pigs for the entire period. ADG = −0.008175 (ratio of BSF replacing SBM)^2 + 0.3234 (ratio of BSF replacing SBM) + 346.8976 was the equation for the quadratic curve, and R^2 was 0.58. The ratio of BSF replacing SBM in diets equaling 19.96% marked the apex of ADG.

3.2. Nutrient digestibility

As shown in Table 5, the digestibility of OM, DM, GE and EE increased quadratically as the ratio of BSF replacing SBM increased in diets on d 14 ($P < 0.05$). The piglets from the BSF25 group showed enhanced digestibility of OM and DM compared to the BSF0, whereas the digestibility of EE reduced in the BSF100 group ($P < 0.05$).

3.3. Relative organ weight

As shown in Table 6, on d 28, piglets from the BSF100 group showed a significant increase in relative weight of kidney compared to piglets from the BSF0 and BSF25 groups ($P < 0.05$). However, no differences in the relative weight of heart, liver and spleen were observed.

3.4. Digestive enzyme activities

On d 28, there were no significant differences in pancreatic amylase, chymotrypsin, and trypsin activities of piglets from different treatments (Table 7). However, compared with BSF0 and BSF100, the activity of jejunal sucrase significantly increased in the BSF25 group ($P < 0.05$).

3.5. Intestinal morphology

In Table 8 and Fig. 3, effects of BSF replacing SBM in diets on intestinal morphology of weaned pigs on d 28 are shown. The piglets from the BSF25 group had a significantly higher villus height (VH) to crypt depth (CD) ratio in the duodenum compared to BSF0 ($P < 0.05$).

Table 4
Effects of black soldier fly (BSF) replacing soybean meal (SBM) on growth performance of weaned piglets.

Item	Diets[1]					SEM	*P*-value		
	BSF0	BSF25	BSF50	BSF75	BSF100		Treatment	Linear	Quadratic
Day 1–14									
ADG[2], g	274[c]	319[a]	303[b]	282[c]	281[c]	3.55	0.02	0.23	<0.01
ADFI[3], g	488[c]	572[a]	542[b]	507[c]	511[c]	7.86	<0.01	0.41	<0.01
FCR[4]	1.78	1.80	1.79	1.80	1.82	0.02	0.76	0.28	0.51
Day 15–28									
ADG, g	385[b]	441[a]	395[b]	338[c]	337[c]	8.29	<0.01	<0.01	<0.01
ADFI, g	713[b]	809[a]	741[b]	665[c]	661[c]	12.20	<0.01	<0.01	<0.01
FCR	1.86	1.83	1.88	1.97	1.96	0.04	0.08	<0.01	0.42
Day 1–28									
ADG, g	329[c]	383[a]	343[b]	311[d]	307[d]	4.05	<0.01	<0.01	<0.01
ADFI, g	598[c]	689[a]	640[b]	583[c]	581[c]	8.53	<0.01	<0.01	<0.01
FCR	1.82	1.80	1.87	1.88	1.89	0.04	0.24	0.04	0.82

SEM = standard error of the mean.
[a-d] Mean values within a row with different letters differ at $P < 0.05$.
[1] BSF0, control diet; BSF25, BSF replacing 25% SBM in diet; BSF50, BSF replacing 50% SBM in diet; BSF75, BSF replacing 75% SBM in diet; BSF100, BSF replacing 100% SBM in diet ($n = 6$).
[2] Average daily gain (ADG) = (body weight gain of the pen/piglets' number)/days.
[3] Average daily feed intake (ADFI) = (feed intake of the pen/piglets' number)/days.
[4] Feed conversion ratio (FCR) = feed intake of the pen/body weight gain of the pen.

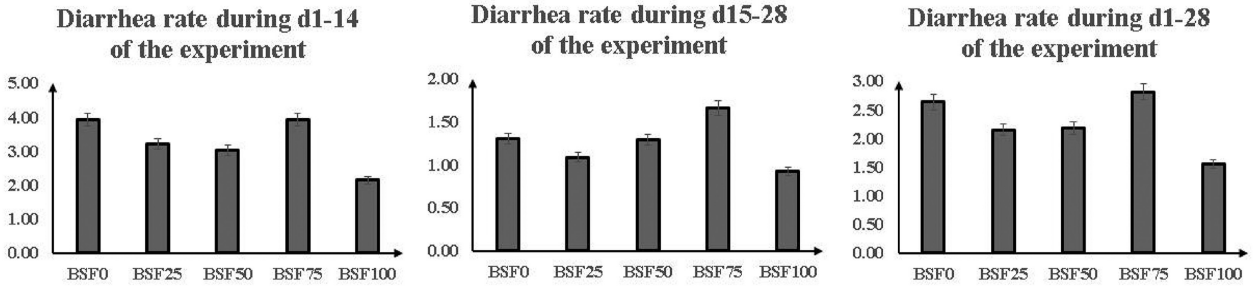

Fig. 1. Effects of BSF replacing SBM on diarrhea rates of weaned piglets. The results are presented as the mean ± SEM, $n = 6$. BSF0, control diet; BSF25, BSF replacing 25% SBM in diet; BSF50, BSF replacing 50% SBM in diet; BSF75, BSF replacing 75% SBM in diet; BSF100, BSF replacing 100% SBM in diet. BSF = black soldier fly; SBM = soybean meal.

3.6. Ileum immunoglobulin

As shown in Table 9, the levels of IgM and IgG in the ileum of piglets from the BSF25 group were higher compared with BSF0 and BSF100 on d 28 ($P < 0.05$).

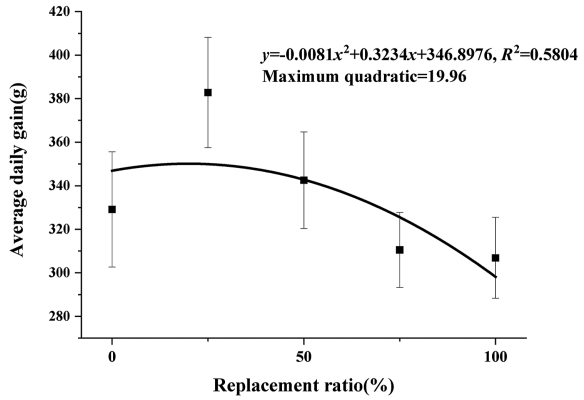

Fig. 2. Quadratic model of ADG in all stages of wean pigs plotted against the ratio of BSF replacing SBM in diets. The quadratic curve equation was: ADG = −0.008175 × (Ratio of BSF replacing SBM)^2 + 0.3234 × (Ratio of BSF replacing SBM) + 346.8976, and the R^2 was equal to 0.5804. When the ratio of BSF replacing SBM in diet was equal to 19.96%, the ADG (350.13 g/d) was at its highest point. ADG = average daily gain; BSF = black soldier fly; SBM = soybean meal.

3.7. Serum parameters

The effects of BSF substitution for SBM in diets on serum appetite hormones of weaned piglets are shown in Table 10. On d 28, compared with BSF0, concentrations of serum NPY and ghrelin in piglets from the BSF25 and BSF100 groups significantly increased and leptin level decreased in piglets from the BSF25 group ($P < 0.05$). Furthermore, there were no significant differences in DAO, D-lactate or endotoxin levels among piglets from the BSF0, BSF25 and BSF100 groups.

3.8. AMCase activity and AMCase mRNA expression

As shown in Fig. 4, no significant differences in mRNA expression of *AMCase* and AMCase activities in the stomach, jejunum and ileum were observed.

3.9. Tight junction proteins mRNA expression

In Fig. 5, no significant differences in mRNA expression of occludin, claudin-1 and *ZO-1* in the ileum were observed.

3.10. Intestinal microbiota

16S rRNA gene sequencing of chyme samples was conducted to investigate effects of BSF on piglet intestinal microbiota. The Venn diagram showed that 157 OTU were isolated from the ileal chyme of

Table 5
Effects of black soldier fly (BSF) replacing soybean meal (SBM) on nutrient digestibility of weaned piglets (%).

Item	Diets[1]					SEM	*P*-value		
	BSF0	BSF25	BSF50	BSF75	BSF100		Treatment	Linear	Quadratic
Day 14									
DM	78.30^{bc}	82.68^{a}	82.17^{a}	80.67^{ab}	76.54^{c}	0.82	<0.01	0.04	0.01
OM	83.30^{bc}	85.73^{a}	85.71^{a}	84.91^{ab}	82.07^{c}	0.59	<0.01	0.10	<0.01
CP	75.23	77.24	75.67	74.32	75.48	1.16	0.60	0.55	0.76
GE	77.86^{ab}	80.84^{a}	80.48^{a}	79.42^{a}	76.02^{b}	0.79	<0.01	0.08	<0.01
EE	57.21^{a}	60.28^{a}	59.83^{a}	58.00^{a}	51.04^{b}	1.40	<0.01	<0.01	<0.01
Day 28									
DM	81.96	82.34	81.82	80.86	79.61	1.02	0.45	0.09	0.39
OM	84.94	85.83	85.43	85.11	83.67	0.84	0.55	0.28	0.2
CP	72.63	79.22	77.06	74.55	76.36	1.53	0.12	0.61	0.14
GE	77.9	81.03	80.82	80.65	78.81	1.23	0.40	0.74	0.07
EE	61.04	65.18	64.1	60.32	59.71	2.16	0.40	0.32	0.18

SEM = standard error of the mean; DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; GE = gross energy.
[a-c] Mean values within a row with different letters differ at $P < 0.05$.
[1] BSF0, control diet; BSF25, BSF replacing 25% SBM in diet; BSF50, BSF replacing50% SBM in diet; BSF75, BSF replacing 75% SBM in diet; BSF100, BSF replacing 100% SBM in diet ($n = 6$).

Table 6
Effects of black soldier fly (BSF) replacing soybean meal (SBM) on relative organ weight of weaned piglets (%).

Item	Diets[1]			SEM	*P*-value
	BSF0	BSF25	BSF100		
Heart	0.49	0.49	0.47	0.01	0.65
Liver	3.09	3.01	3.45	0.12	0.08
Spleen	0.20	0.21	0.24	0.03	0.67
Kidney	0.46^{b}	0.49^{b}	0.66^{a}	0.03	<0.01

SEM = standard error of the mean.
[a, b] Mean values within a row with different letters differ at $P < 0.05$.
[1] BSF0, control diet; BSF25, BSF replacing 25% SBM in diet; BSF100, BSF replacing 100% SBM in diet ($n = 4$).

Table 7
Effects of black soldier fly (BSF) replacing soybean meal (SBM) on digestive enzyme activities of weaned piglets.

Item	Diets[1]			SEM	*P*-value
	BSF0	BSF25	BSF100		
Pancreas					
Amylase, U/g	31.78	36.66	32.24	2.60	0.40
Chymotrypsin, IU/g	29.69	33.58	30.92	2.13	0.47
Trypsin, U/g	10.31	13.53	11.80	0.76	0.07
Jejunum					
Sucrase, μmol/g	7.71^{b}	10.43^{a}	6.75^{b}	0.68	0.02
Maltase, U/mg	12.23	11.54	11.76	1.43	0.94
Lactase, U/mg	1.84	2.20	2.30	0.47	0.77

SEM = standard error of the mean.
[a, b] Mean values within a row with different letters differ at $P < 0.05$.
[1] BSF0, control diet; BSF25, BSF replacing 25% SBM in diet; BSF100, BSF replacing 100% SBM in diet ($n = 4$).

pigs from the BSF0, BSF25 and BSF100 groups, with 67 OTU being common and 316 being unique (Fig. 6A). There were no differences in bacterial alpha-diversity indices among the three treatments (Table 11). According to the PCoA based on Bray Curtis ($P = 0.063$, $R = 0.22$), no discernible differences at the OTU level in ileal microbiota of piglets from all treatments were found (Fig. 6B). Subsequently, effects of BSF on microbial composition in the ileum of piglets were further investigated (Fig. 6C–E). At the phylum level (Fig. 6F), piglets given BSF25 diets had a higher abundance of Cyanobacteria ($P < 0.05$) than piglets from other groups. At the family level (Fig. 6F), pigs fed BSF25 diets showed higher Oscillospiraceae abundance ($P < 0.05$), while pigs fed BSF100 diets had higher Pasteurellaceae, Saccharimonadaceae, Bacillaceae, and Staphylococcaceae abundance ($P < 0.05$). At the genus level (Fig. 6F), pigs fed BSF25 diets showed higher *Subdoligranulum* abundance ($P < 0.05$) compared to other groups, whereas pigs fed BSF100 diets had higher *Actinobacillus*, *Nosocomiicoccus*, *Tepidimicrobium*, *Pseudogracilibacillus*, *Cerasibacillus*, *Aerococcus* and *Staphylococcus* abundance ($P < 0.05$).

The Venn diagram revealed that cecal microbiota contained 729, 647 and 771 OTU from pigs fed BSF0, BSF25, and BSF100 diets, of which 274 OTU were unique and 465 OTU were shared (Fig. 7A). There were no significant differences among three treatments in bacterial alpha-diversity indices (Table 11). Significant variations in microbiota of piglets from three treatments were observed from the PCoA at OTU level (Fig. 7B) ($P = 0.012$, $R = 0.44$). At the phylum level, in comparison to piglets from other groups, piglets fed BSF25 diets exhibited a higher ($P < 0.05$) abundance of Campilobacterota, and piglets fed BSF100 diets showed ($P < 0.05$) higher abundance of Patescibacteria (Fig. 7C and F). At the family level, pigs given BSF100 diets showed higher ($P < 0.05$) abundance of Oscillospiraceae, Erysipelotrichaceae, Anaerovoracaceae, Saccharimonadaceae, Acholeplasmataceae, Bacillaceae and Staphylococcaceae compared to other groups, while pigs given BSF25 diets showed greater ($P < 0.05$) abundance of Veillonellaceae and Campylobacteracea

Table 8
Effects of black soldier fly (BSF) replacing soybean meal (SBM) on intestinal morphology of weaned piglets.

Item	Diets[1]			SEM	*P*-value
	BSF0	BSF25	BSF100		
Duodenum					
Villus height, μm	347.78	412.14	346.70	21.99	0.13
Crypt depth, μm	345.71	283.53	331.98	27.95	0.32
Villus height to crypt depth ratio	1.01^{b}	1.46^{a}	1.08^{b}	0.07	<0.01
Jejunum					
Villus height, μm	355.29	417.12	354.54	15.22	0.05
Crypt depth, μm	242.23	239.32	300.05	14.79	0.05
Villus height to crypt depth ratio	1.50^{ab}	1.77^{a}	1.18^{b}	0.12	0.03
Ileum					
Villus height, μm	233.59	276.56	235.11	32.03	0.59
Crypt depth, μm	269.98	276.34	290.09	12.99	0.57
Villus height to crypt depth ratio	0.89	1.00	0.81	0.13	0.60

SEM = standard error of the mean.
[a, b] Mean values within a row with different letters differ at $P < 0.05$.
[1] BSF0, control diet; BSF25, BSF replacing 25% SBM in diet; BSF100, BSF replacing 100% SBM in diet ($n = 4$).

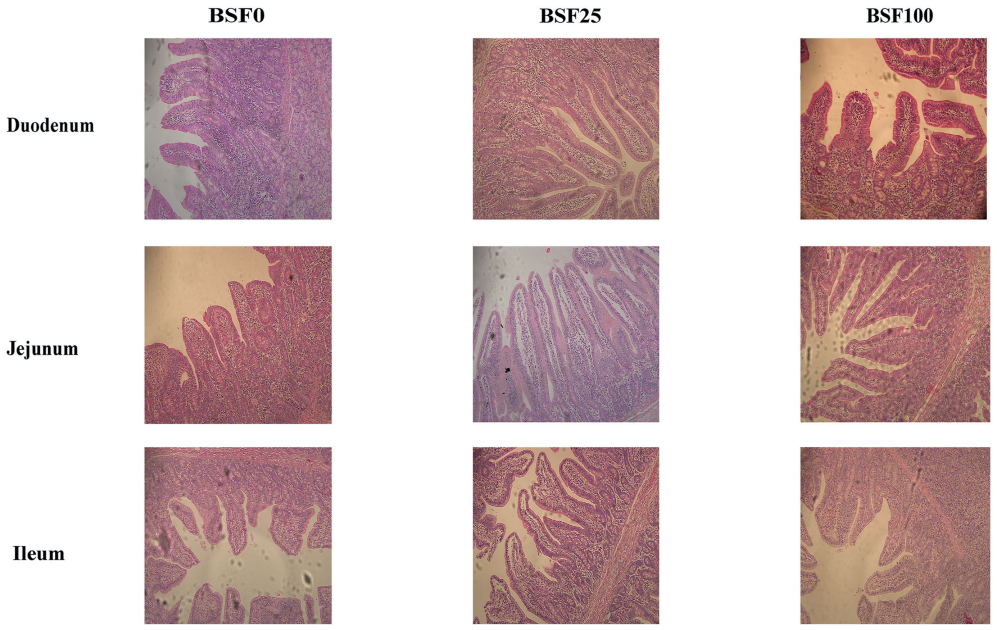

Fig. 3. The photomicrograph of small intestinal segments of piglets from BSF0, BSF25 and BSF100 groups. All specimens were examined under a light microscope (Nikon Eclipse Ci, Tokyo, Japan) at 400× magnification. $n = 4$. BSF0, control diet; BSF25, BSF replacing 25% SBM in diet; BSF100, BSF replacing 100% SBM in diet. BSF = black soldier fly; SBM = soybean meal.

Table 9
Effects of black soldier fly (BSF) replacing soybean meal (SBM) on ileal immune functions of weaned piglets (μg/mg).

Item	Diets[1]			SEM	*P*-value
	BSF0	BSF25	BSF100		
Immunoglobulin A	11.31	15.43	10.35	1.16	0.05
Immunoglobulin G	6.10[b]	8.73[a]	5.41[b]	0.74	0.04
Immunoglobulin M	6.15[b]	8.53[a]	5.14[b]	0.55	0.01

SEM = standard error of the mean.
[a, b]Mean values within a row with different letters differ at $P < 0.05$.
[1] BSF0, control diet; BSF25, BSF replacing 25% SBM in diet; BSF100, BSF replacing 100% SBM in diet ($n = 4$).

Table 10
Effects of black soldier fly (BSF) replacing soybean meal (SBM) on serum parameters of weaned piglets.

Item	Diets[1]			SEM	*P*- value
	BSF0	BSF25	BSF100		
Growth hormone, ng/mL	1.61	1.85	1.22	0.25	0.24
Leptin, ng/mL	1.62[a]	1.46[b]	1.54[ab]	0.04	0.04
Neuropeptide Y, pg/mL	29.54[b]	34.59[a]	33.19[a]	0.90	<0.01
5-Hydroxytryptamine, pg/mL	319.91	357.12	353.51	9.16	0.07
Ghrelin, pg/mL	97.40[b]	116.67[a]	111.70[a]	1.8	<0.01
Peptide YY, pmol/mL	2.49	2.43	2.54	0.04	0.41
Cholecystokinin, pg/mL	25.95	25.19	28.67	1.22	0.16
Glucagon-like peptide-1, pmol/L	1.85	1.82	1.90	0.07	0.34
Glucagon-like peptide-2, pg/mL	450.15	492.05	466.9	13.03	0.24
Endotoxin, EU/L	64.60	62.15	66.51	1.24	0.10
Diamine oxidase, U/L	3.70	3.85	3.96	0.09	0.18

SEM = standard error of the mean; EU = endotoxin units.
[a, b] Mean values within a row with different letters differ at $P < 0.05$.
[1] BSF0, control diet; BSF25, BSF replacing 25% SBM in diet; BSF100, BSF replacing 100% SBM in diet ($n = 4$).

(Fig. 7D and F). At the genus level, pigs fed BSF25 diets demonstrated a higher ($P < 0.05$) abundance of *Subdoligranulum, Megasphaera*, *Catenibacterium*, *Slackia, Fournierella*, *Olsenella* and *Campylobacter* than those from the BSF0 and BSF100 groups. In addition, pigs fed BSF100 diets demonstrated a higher ($P < 0.05$) abundance of *Cerasibacillus*, *Pseudogracilibacillus*, *Candidatus_Saccharimonas*, *Anaeroplasma*, *Mogibacterium* and *Staphylococcus* than those fed the other two diets (Fig. 7E and F).

In the colon, 770, 729 and 815 OTU were found in pigs given BSF0, BSF25, and BSF100 diets, of which 210 OTU were unique and 560 OTU were shared (Fig. 8A). The Ace and Chao index of colonic samples of piglets from the BSF25 group were significantly lower than BSF0 and BSF100 (Table 11). Significant variations in the microbiota of piglets from various treatments were observed from the PCoA at OTU level (Fig. 8B) ($P = 0.026$, $R = 0.30$). At the phylum level, the abundance of Patescibacteria increased ($P < 0.05$) in piglets from the BSF100 group in comparison to other groups (Fig. 8C and F). At the family level, pigs given BSF25 diets demonstrated increased ($P < 0.05$) Ruminococcaceae, Muribaculaceae, Butyricicoccaceae, Acidaminococcaceae, Atopobiaceae and Veillonellaceae abundance compared to other groups, and piglets fed BSF100 diets showed higher ($P < 0.05$) abundance of Streptococcaceae, Rikenellaceae, Saccharimonadaceae, Micrococcaceae, Acholeplasmataceae, Corynebacteriaceae, Bacillaceae and Staphylococcaceae (Fig. 8D and F). At the genus level, the level of *Subdoligranulum*, *Blautia*, *Phascolarctobacterium*, *Faecalibacterium*, *Olsenella*, *Megasphaera*, *Eubacterium_eligens_group*, *Oribacterium*, *Negativibacillus*, *Fournierella*, *Butyricicoccus*, *Allisonella*, *Enterorhabdus*, *Lachnoclostridium*, *Desulfovibrio* and *Eubacterium_ventriosum_group* was higher in piglets fed BSF25 diets than those in the BSF0 and BSF100 groups ($P < 0.05$). In addition, pigs fed BSF100 diets had greater ($P < 0.05$) abundance of *Streptococcus*, *Rikenellaceae_RC9_gut_group*, *Candidatus_Saccharimonas*, *Pseudogracilibacillus*, *Rothia*, *Anaeroplasma*, *Corynebacterium*, *Mogibacterium, Staphylococcus* and *Quinella* than those fed the other two diets (Fig. 8D and F).

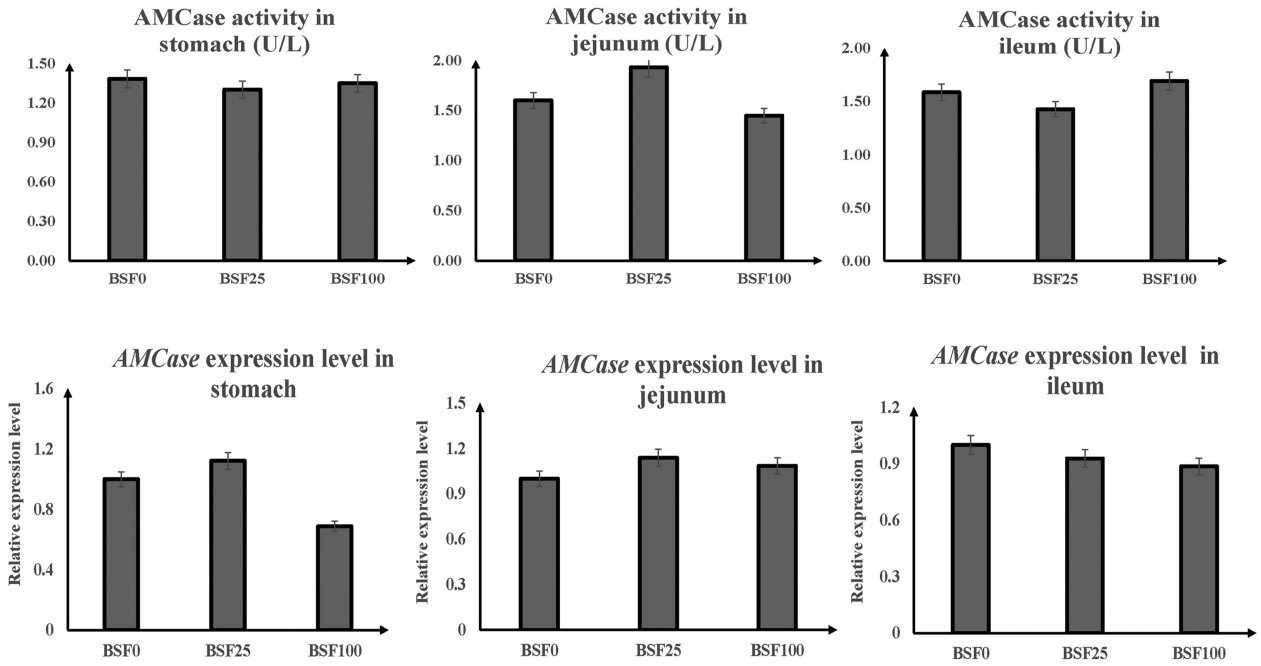

Fig. 4. Effects of BSF replacing SBM on AMCase activity and expression level of *AMCase* in stomach, jejunum and ileum tissues of piglets. The results are presented as the mean ± SEM, $n = 4$. BSF0, control diet; BSF25, BSF replacing 25% SBM in diet; BSF100, BSF replacing 100% SBM in diet. BSF = black soldier fly; SBM = soybean meal; AMCase = acidic mammalian chitinase.

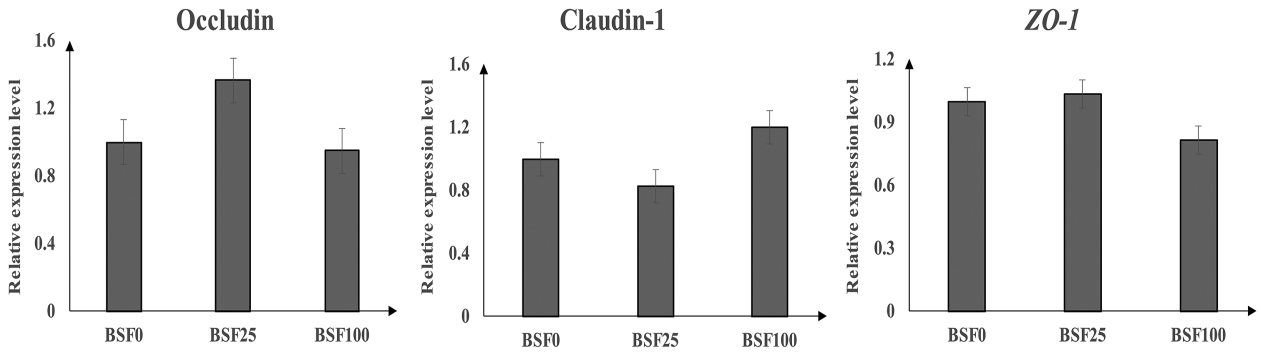

Fig. 5. The expression level of tight junction proteins of piglets from BSF0, BSF25, and BSF100 groups. (A) Occludin, (B) claudin-1, (C) *ZO-1*. The results are presented as the mean ± SEM, $n = 4$. BSF0, control diet; BSF25, BSF replacing 25% SBM in diet; BSF100, BSF replacing 100% SBM in diet. BSF = black soldier fly; SBM = soybean meal; *ZO-1* = zonula occludens-1.

3.11. SCFA concentrations

Table 12 showed effects of BSF replacing SBM in diets on piglet intestinal SCFA concentrations. In the colon, pigs fed BSF25 diets had higher ($P < 0.05$) butyrate concentration compared to those from the BSF0 and BSF100 groups. Compared to BSF25, piglets fed BSF100 diets had reduced ($P < 0.05$) lactate concentrations.

4. Discussion

Recently, BSF was thought to be a valuable substitute for SBM in piglet diets. The BSF used in the current study was full fat BSF. The CP level of the BSF in this study was 36.69%, the CP content of full fat BSF has been reported to range from 27.5% to 43.9%, which was slightly lower than that of (43.51%) conventional SBM (Spranghers et al., 2017; Onsongo et al., 2018; Tyshko et al., 2021). The EE level of the BSF in this study was 31.34%, the EE level of full fat BSF has been reported to range from 29.4% to 51.5%, which was higher than that of (1.59%) conventional SBM (Shumo et al., 2019; Rawski et al., 2020; de Souza Vilela et al., 2021). The chitin content of the BSF in this study was 3.95%; the chitin level of BSF has been reported to range from 3.87% to 7.21% (Lu et al., 2022). The differences in chitin content might be related to the development stages of black soldier fly (Wang et al., 2020). The molecular structure of chitin, which is regarded as an indigestible fiber, is similar to cellulose, and the active ingredient of chitin is chitosan (Finke, 2007). Additionally, the BSF in the current study was rich in amino acids, and most reported BSF has also been demonstrated to be abundant in amino acids (Lu et al., 2022). Overall, BSF has great potential to replace SBM as a sustainable protein source.

The findings of this study demonstrated that ADG and ADFI of piglets changed quadratically with the increase of the ratio of BSF replacing SBM in diets. Compared with BSF0, inclusion of BSF at 25% replacing SBM significantly increased ADG of piglets, and this result was consistent with previous results, wherein BSF in small amounts could improve piglet performance (Hartinger et al., 2022). The

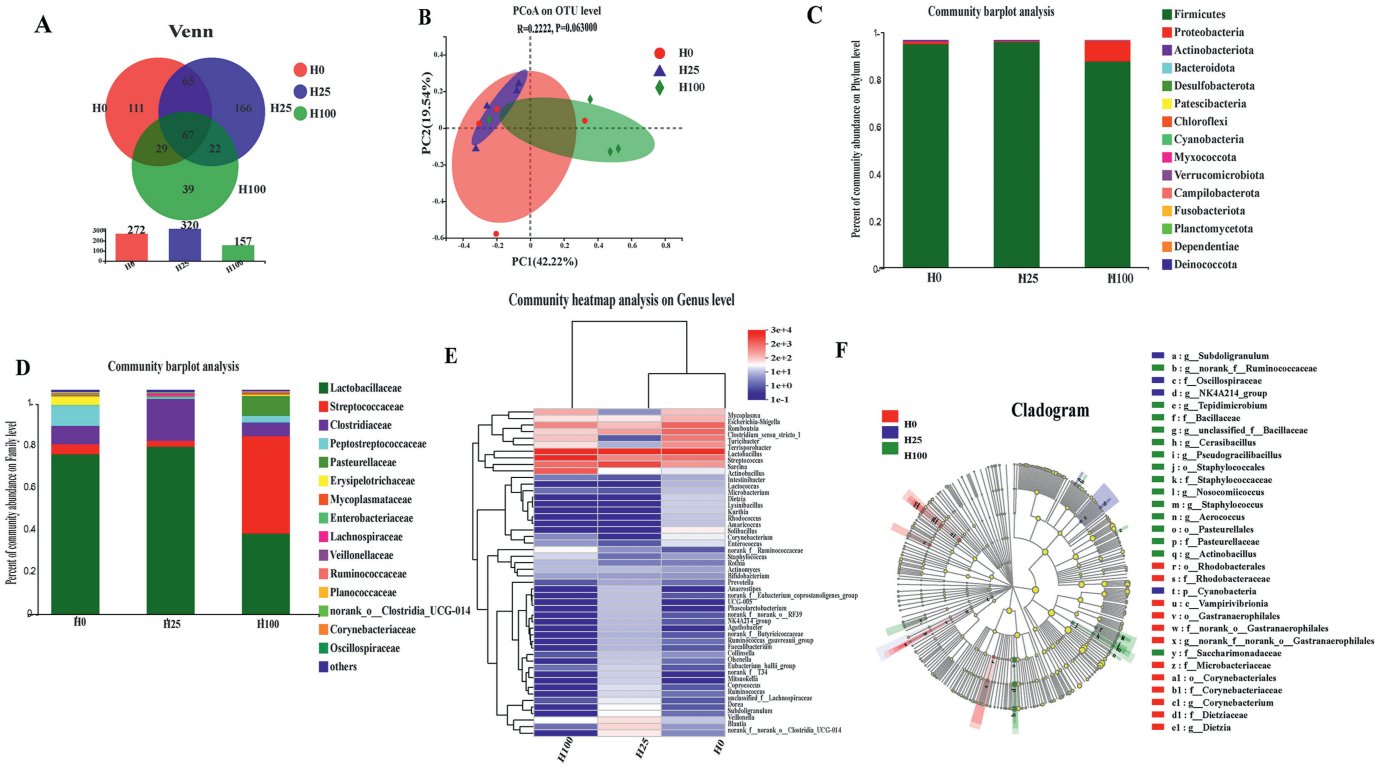

Fig. 6. Effects of BSF replacing soybean meal on microbial composition of ileal chyme. (A) Venn diagram. (B) PCoA based on Bray–Curtis distance calculated from OTU abundance matrix ($R = 0.22$, $P = 0.06$). (C, D) Barplot analysis of microbial community compositions at phylum and family levels. (E) Heatmap analysis of microbial community compositions at genus levels. (F) The discriminant analysis of LEfSe multi-level species difference from phylum to genus level. $n = 4$. H0, control diet; H25, BSF replacing 25% SBM in diet; H100, BSF replacing 100% SBM in diet. BSF = black soldier fly; SBM = soybean meal; PCoA = principal coordinate analysis.

improved ADG of piglets might be due to the increase of ADFI, and insect meal has been thought to increase diet palatability (Jin et al., 2016). According to Biasato et al. (2019), ADFI of weaned piglets increased when partly defatted BSF at 10% was added to diets replacing SBM from d 24 to d 61. However, Spranghers et al. (2018) reported that adding full fat and defatted BSF to piglet diets had no impact on piglet performance. When BSF was utilized to replace 25% or 50% of the animal protein in piglet diets, Crosbie et al. (2021) found no significant changes in ADG at any stages. Hakenasen et al. (2021) reported that piglets receiving diets where BSF replaced SBM tended to develop slightly slower than those receiving a control diet. In the current study, we also found that the addition of BSF at 50% replacing SBM had no significant effects on piglet ADG, but the inclusion of BSF at 75% and 100% replacing SBM had a negative impact on piglet ADG. The differences in effects of BSF on growth performance of piglets may be related to the life stage of insects, rearing substrate of insects, dietary inclusion levels and fat extraction techniques (Crosbie et al., 2020). It is worth noting that chitin is a nitrogen-containing fiber, and high levels of chitin might possess anti-nutritional properties. This may explain why pigs fed diets rich in BSF fare less well in terms of growth performance (Bach and Babayan, 1982).

Table 11
Effects of black soldier fly (BSF) replacing soybean meal (SBM) on alpha diversity indices of ileum, cecum and colon chyme microbial community of weaned piglets.

Item	Diets[1]			SEM	*P*-value
	BSF0	BSF25	BSF100		
Ileum					
Sobs	129.50	132.25	82.50	23.92	0.32
Shannon	1.58	1.59	1.81	0.25	0.77
Simpson	0.36	0.31	0.28	0.08	0.80
Ace	210.18	206.73	154.88	40.94	0.59
Chao	197.88	191.76	122.47	36.63	0.34
Cecum					
Sobs	465.75	371.25	482.75	44.32	0.24
Shannon	3.27	3.16	3.66	0.40	0.67
Simpson	0.16	0.13	0.08	0.05	0.58
Ace	568.85	448.82	572.68	51.11	0.23
Chao	588.89	455.82	573.76	56.81	0.27
Colon					
Sobs	539.25	470.25	526.25	17.60	0.07
Shannon	3.83	3.81	3.79	0.30	0.99
Simpson	0.10	0.07	0.07	0.03	0.71
Ace	620.20[a]	537.64[b]	638.46[a]	17.89	0.02
Chao	635.18[a]	545.95[b]	651.23[a]	22.79	0.03

SEM = standard error of the mean.
[a, b] Mean values within a row with different letters differ at $P < 0.05$.
[1] BSF0, control diet; BSF25, BSF replacing 25% SBM in diet; BSF100, BSF replacing 100% SBM in diet ($n = 4$).

The appetite and feed intake of piglets are generally regulated by a variety of neural signals (Sartin et al., 2011). In this study, compared with BSF0, the level of NPY and ghrelin significantly increased, and the level of leptin significantly decreased in the serum of piglets from the BSF25 group. NPY is synthesized by the arcuate nucleus of the hypothalamus, and is proved to be an appetite promoting neurotransmitter (Polkowska et al., 2006; Bahar and Sweeney, 2008). Barb et al. (2006) indicated that injection of NPY into the lateral ventricle of the brain improved feed intake of pigs. Vizcarra et al. (2007) found that after active immunization with ghrelin, feed intake of pigs significantly decreased. Increasing levels of the two factors above may contribute to an increase in feed intake. Leptin reduces feed intake by inhibiting the appetite-stimulating process and activating the hypothalamic

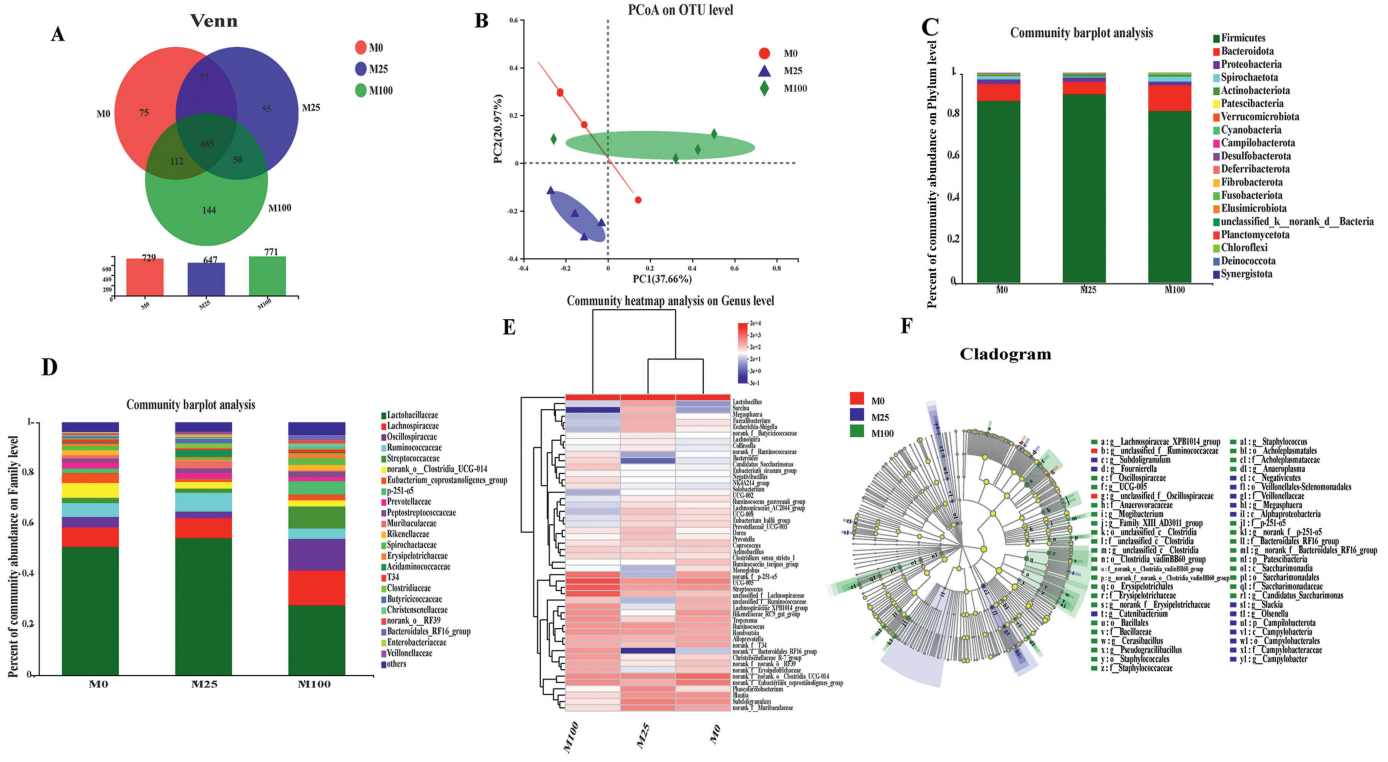

Fig. 7. Effects of BSF replacing soybean meal on microbial composition of cecal chyme. (A) Venn diagram. (B) PCoA based on Bray–Curtis distance calculated from OTU abundance matrix ($R = 0.44$, $P = 0.01$). (C, D) Barplot analysis of microbial community compositions at phylum and family levels. (E) Heatmap analysis of microbial community compositions at genus levels. (F) The discriminant analysis of LEfSe multi-level species difference from phylum to genus level. $n = 4$. M0, control diet; M25, BSF replacing 25% SBM in diet; M100, BSF replacing 100% SBM in diet. BSF = black soldier fly; SBM = soybean meal; PCoA = principal coordinate analysis.

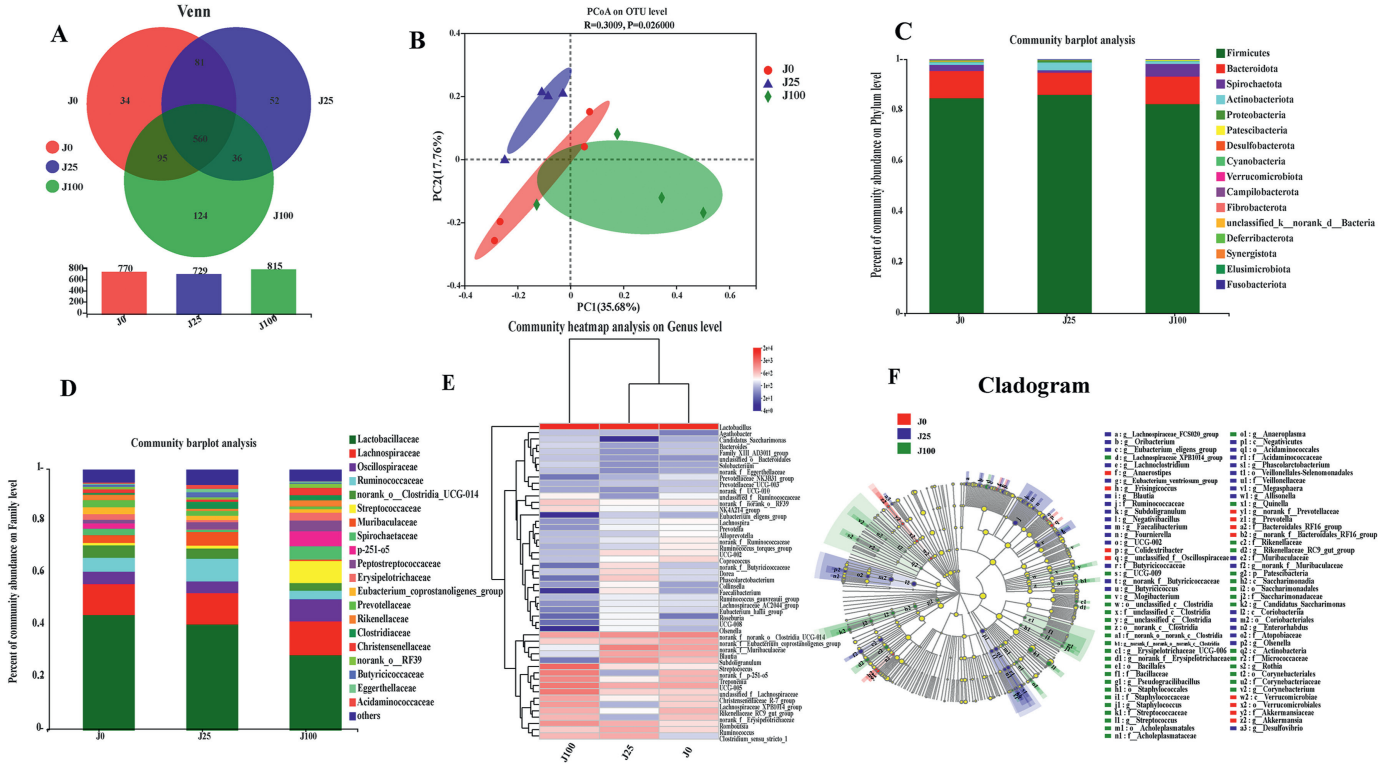

Fig. 8. Effects of BSF replacing soybean meal on microbial composition of colonic chyme. (A) Venn diagram. (B) PCoA based on Bray–Curtis distance calculated from OTU abundance matrix ($R = 0.30$, $P = 0.03$). (C, D) Barplot analysis of microbial community compositions at phylum and family levels. (E) Heatmap analysis of microbial community compositions at genus levels. (F) The discriminant analysis of LEfSe multi-level species difference from phylum to genus level. $n = 4$. J0, control diet; J25, BSF replacing 25% SBM in diet; J100, BSF replacing 100% SBM in diet. BSF = black soldier fly; SBM = soybean meal; PCoA = principal coordinate analysis.

Table 12
Effects of BSF replacing SBM on SCFA levels in ileum, cecum and colon chyme of weaned piglets (mg/kg).

Item	Diets[1]			SEM	*P*-value
	BSF0	BSF25	BSF100		
Ileum					
Lactic acid	2167.71	3844.17	2507.51	1499.79	0.72
Acetic acid	944.20	928.87	791.97	294.31	0.93
Propionic acid	9.40	19.26	11.23	4.40	0.31
Formic acid	227.29	417.52	218.57	114.93	0.44
Isobutyric acid	2.38	0.52	4.96	2.78	0.56
Butyric acid	15.98	22.52	5.01	9.91	0.49
Isovaleric acid	6.97	2.58	8.61	2.55	0.30
Valeric acid	13.03	0.33	0.67	6.26	0.33
Total SCFA	3386.94	5235.78	3548.53	1397.11	0.61
Cecum					
Lactic acid	522.96	546.75	262.67	98.06	0.15
Acetic acid	4585.75	5778.01	5212.39	665.52	0.30
Propionic acid	1749.01	2260.91	2395.40	159.32	0.06
Formic acid	109.69	122.58	55.92	19.03	0.10
Isobutyric acid	6.51	22.40	32.27	7.04	0.06
Butyric acid	819.81	956.01	702.86	130.09	0.44
Isovaleric acid	30.62	29.02	47.82	11.80	0.47
Valeric acid	64.29	164.83	82.97	34.62	0.09
Total SCFA	7642.55	9678.25	8591.40	3196.90	0.09
Colon					
Lactic acid	1417.22[a]	1370.28[a]	838.34[b]	108.98	0.02
Acetic acid	4508.67	5381.19	5155.21	473.98	0.45
Propionic acid	2250.64	2366.19	2234.80	324.52	0.95
Formic acid	74.32	109.32	88.07	16.74	0.39
Isobutyric acid	93.19	83.54	161.74	28.64	0.19
Butyric acid	573.71[b]	753.77[a]	501.95[b]	60.98	0.04
Isovaleric acid	68.01	163.70	92.41	25.58	0.09
Valeric acid	182.30	177.47	214.82	51.09	0.86
Total SCFA	9414.16	10,607.71	9488.24	687.67	0.44

BSF = black soldier fly; SBM = soybean meal; SCFA = short-chain fatty acids; SEM = standard error of the mean.
[a, b] Mean values within a row with different letters differ at $P < 0.05$.
[1] BSF0, control diet; BSF25, BSF replacing 25% SBM in diet; BSF100, BSF replacing 100% SBM in diet ($n = 4$).

appetite suppression process (Sartin et al., 2011). Ramsay et al. (2004) showed that leptin injection significantly reduced feed intake of piglets. Piglets from the BSF25 group had lower blood leptin levels and less of an inhibitory impact on their feed intake, which may account for increased feed consumption.

Compared to BSF0, DM and OM digestibility improved in piglets from the BSF25 and BSF50 groups, whereas EE digestibility decreased in piglets from the BSF100 group. Hartinger et al. (2022) reported that BSF increased DM and OM digestibility of broiler chickens. With regards to other insect species, Jin et al. (2016) found that adding dried mealworm to diets for weaned pigs resulted in a linear increase in DM digestibility. However, Spranghers et al. (2018) observed piglets fed BSF diets showed similar nutrient digestibility to those fed basal diets. Biasato et al. (2019) also reported that nutrient digestibility was not affected by partially defatted BSF meal inclusion in weaned piglet diets. Cullere et al. (2016) also found that 10% to 15% BSF in broiler diets did not affect nutrient digestibility, but decreased the digestibility of EE. The changes in nutrient digestibility may be influenced by insect species and life stages, feed inclusion levels, and fat extraction techniques, similar to the reason for changes in growth performance. The improved nutrient digestibility may be due to the chitin contained in the diet. A certain component of chitin can be digested in piglets to produce chito-oligosaccharides. The addition of chito-oligosaccharides to piglet diets has been reported to improve nutrient digestibility (Heim et al., 2014; He et al., 2009; Han et al., 2007). In the present study, a limited amount of BSF exhibited a favorable effect on nutrient digestibility; however, a larger amount of BSF had the opposite effect. According to Spranghers et al. (2018), protein digestibility was shown to be superior in pigs given 4% full-fat and defatted BSF diets than in the control group, whereas values for 8% full-fat BSF diets were lower than in the control group. A high content of chitin might explain the decreased nutrient digestibility of piglets supplemented with high levels of BSF. The chitin content of insects was negatively correlated with the digestibility of nutrients (Marono et al., 2015).

The integrity of the intestine is crucial to the digestion and utilization of nutrients in piglets (Biasato et al., 2019). Some important morphological features are regarded as markers of ideal intestinal function, such as VH and CD (Zhang et al., 2016). In the current study, BSF replacing SBM at 25% SBM enhanced VH to CD ratio in weaned piglets. Medium-chain fatty acid (MCFA) such as lauric acid found in BSF may explain the improvement in intestinal morphology. MCFA has been observed to improve intestinal structure by positively affecting crypt cell renewal. However, some studies reported that BSF in diets did not significantly affect intestinal morphology of piglets (Spranghers et al., 2018; Biasato et al., 2019). These differences might also be associated with the dosing and processing of functional compounds such as chitin and lauric acid contained in BSF. Moreover, the disaccharidase activity is related to the ability of piglets to digest and transport carbohydrate, and disaccharidase activity is considered to be able to evaluate the development of the intestine. The increase of disaccharidase activity could represent a rapid maturation of the intestine (Monaco et al., 2011). The present study showed that BSF at 25% to replace SBM increased maltase activity, suggesting that BSF at 25% to replace SBM could improve the ability of piglets to utilize carbohydrates and promote the maturation of the intestine compared to BSF0.

Improved piglet immune function may be indicated by higher concentrations of IgA, IgM and IgG. Compared to BSF0, BSF at 100% to replace SBM decreased IgA, IgM and IgG levels. The improvement in immune function might be associated with chitin and lauric acid. It has been reported that the chitin contained in BSF, in the form of chito-oligosaccharides, could improve immune responses mediated by the humoral system and the cells (Lee et al., 2008; Xing et al., 2017). Additionally, lauric acid can be used as a direct energy source for intestinal cells, and the increase in its content may contribute to the intestinal functional development of piglets treated with BSF (Bach and Babayan, 1982). However, the evidence shows that a certain threshold of chitin could promote immune responses; however, when the level of chitin is above this threshold there may be a negative effect on immune function (Yousef et al., 2012). Moreover, the content of chitin might also affect the immune function of BSF or its components (Xing et al., 2017).

It was considered that tight junction protein expression levels, such as those of ZO-1, occludin and claudin-1, may be used to effectively assess the function of the intestinal barrier (Søfteland et al., 2019). Additionally, the levels of DAO, D-lactate, and endotoxin were taken into account as indicators of gut mucosal barrier function (Zhang et al., 2016). No significant alterations were observed in tight junction protein expression levels or levels of serum endotoxin, DAO or D-lactate, indicating that BSF supplementation may not have any impacts on pig intestinal barrier function. It has been reported that feeding BSF to weaning piglets exposed to enterotoxigenic *Escherichia coli K88* increased the levels of tight junction proteins claudin-3 and occludin (Jin et al., 2021). The piglets in the present study did not face the disease challenge, which may explain the lack of significance.

Maintaining host health depends heavily on intestinal microbiota (Tang et al., 2019). In the current study, BSF was able to modulate the proliferation of certain bacteria. Compared with BSF0, the abundance of beneficial bacteria was higher in the BSF25 group.

The abundance of *Subdoligranulum* was higher in the ileum of piglets from the BSF25 group compared to BSF0. Van Hul et al. (2020) indicated that *Subdoligranulum* was positively associated with HDL level, and negatively correlated with IL-6 level. Compared to BSF0, the BSF25 group had a considerably higher abundance of Veillonellaceae in the cecum. As Fresno Rueda et al. (2021) described, Veillonellaceae improved the gut barrier function, immunological system, and the integrity of intestinal mucosa. In the colon, the abundance of Negativicutes and Coriobacteriales significantly increased in the BSF25 group compared to BSF0. Negativicutes may be crucial for maintaining intestinal homeostasis, He et al. (2016) discovered that heat stress drastically decreased Negativicutes abundance in the duck gut. Additionally, Pittayanon et al. (2020) found that patients with ulcerative colitis had considerably less Coriobacteriales in the intestine than healthy people. Previous studies demonstrated that BSF functional components, such as chitin and lauric acid, might promote the colonization of beneficial bacteria, while preventing the colonization of potentially harmful microorganisms (Liaqat and Eltem, 2018; Spranghers et al., 2017). MCFA can infiltrate the lipid membrane of pathogenic bacterial cell, thus resulting in the decrease of cytosolic pH (Skrivanova et al., 2006). However, a higher abundance of potentially harmful bacteria, including Pasteurellaceae and Staphylococcaceae, was found in the gut of piglets from the BSF100 group compared to BSF0 (Klinsoda et al., 2020; Li et al., 2022a). High levels of chitin may be associated with the increase in pathogenic bacteria. This may also contribute to the poor performance of piglets from the BSF100 group.

Furthermore, the replacement of SBM with BSF at ratio of 25% may promote the colonization of fiber-degrading bacteria in the gut of weaned piglets. Compared to BSF0, a higher abundance of *Subdoligranulum* in the cecum, and Ruminococcaceae, *Faecalibacterium*, *Butyricicoccus* in the colon were observed in piglets from the BSF25 group. *Subdoligranulum* is an important fiber-degrading bacterium that is essential for maintaining piglet intestinal health (Hill et al., 2022; Liu et al., 2021). Ruminococcaceae is an important cellulose and hemicellulose fermenting bacterium (Ley et al., 2008). *Faecalibacterium* is also considered a microbial biomarker of intestinal homeostasis (Onarman Umu et al., 2018). *Faecalibacterium* can degrade fiber to produce butyrate, and *Faecalibacterium* may improve intestinal health through regulating energy balance (Zhang et al., 2019; He et al., 2016). *Butyricicoccus* can also produce butyrate by degrading fiber, which can improve intestinal health of piglets by alleviating inflammatory responses and suppressing the growth of undesirable bacteria and pathogens (Pittayanon et al., 2020; Biddle et al., 2013; Li et al., 2021). The increased abundance of fiber-degrading bacteria might be related to the chitin contained in the exoskeleton of insects. Chito-oligosaccharides, a possible prebiotic, may have a significant impact on modulating gut flora (Borrelli et al., 2017). Additionally, previous studies have demonstrated that BSF in diets might enhance the abundance of bacteria that produce SCFA, and the increase may be related to the capacity of bacteria to degrade chitin (Borrelli et al., 2017; Biasato et al., 2020).

The content of butyrate in the colon was significantly higher in piglets from the BSF25 group compared to BSF0. The increased SCFA levels are considered to be beneficial to the health of piglet intestines. SCFA is crucial for improving intestine structure and function as well as regulating appetite hormones (Deehan et al., 2017; Blaak et al., 2020). Butyric acid not only provides colonic cells with energy but also promotes the development of the intestine by increasing the number of epithelial cells (Rérat et al., 1987). SCFA can also create an acidic environment in the hindgut, preventing harmful microorganisms from colonizing (Delzenne et al., 2020). Furthermore, we found that the abundance of SCFA-producing bacteria increased in the intestine of piglets from the BSF25 group, which might explain increased SCFA levels in piglets.

Before being domesticated by humans, pigs had the ability to gradually digest chitin in order to feed on insects and mushrooms in the wild (Kawasaki et al., 2021). According to previous reports, the *AMCase* gene was shown to be expressed in the stomach tissues of growing pigs and weaned piglets (Tabata et al., 2019). At the same time, the secretion of AMCase may increase with the development of gastric tissues (Tabata et al., 2017). In this study, no differences were observed in *AMCase* expression level and AMCase activities in gastric tissues of piglets from all groups. This may indicate that piglets have the ability to digest chitin without dietary chitin induction, and this ability is independent of dietary chitin content. However, the chitin content in diets of the BSF100 group might be too high for piglets to degrade and utilize, which may explain why piglets from the BSF100 group showed poor performance.

5. Conclusion

In the current study, BSF replacing 25% SBM in piglet diets (4.5% BSF in diet) could increase the level of appetite hormones, digestive enzyme activity, immune parameters, and modulate the structure of intestinal microbiota to improve nutrient digestibility and growth performance of piglets. Piglets might have the ability to degrade chitin to release the nutrients encapsulated by chitin and produce chito-oligosaccharides as prebiotics to improve health status. However, BSF replacing 100% SBM in piglet diets (18.3% BSF in diet) showed an adverse effect on piglet performance as piglets cannot secret enough chitin degrading enzyme and a limited amount of chitinase might explain the poor performance of piglets. In the future, adding exogenous chitinase may further improve the application effects of insect protein.

Author contributions

Sujie Liu, Yongxi Ma, Yonggai Duan, Jianjun Zang, Xiangshu Piao and **Defa Li:** Designed the experiment. **Sujie Liu, Xiaolin Zhang** and **Longxian Li:** Conducted the experiment. **Sujie Liu, Jian Wang** and **Tenghao Wang:** Collected and analyzed the experimental data. **Sujie Liu:** Wrote the manuscript. **Jian Wang, Xiangshu Piao, Yongxi Ma** and **Defa Li:** Revised the manuscript. All authors have read and approved the final manuscript.

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.

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