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Original Research Article

Effects of different ratios of soluble to insoluble dietary fiber on growth performance and intestinal health of piglets

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ABSTRACT

This study investigated the impact of different ratios of soluble to insoluble dietary fiber (SDF:IDF) formulations by sugar beet pulp (SBP) supplementation on piglet growth performance, nutrient digestibility, immune function, intestinal morphology, intestinal microbiota and intestinal health. A total of 60 crossbred piglets (Duroc × [Landrace × Yorkshire]) at 40 d old with body weight of 10.0 ± 0.3 kg were randomly assigned to 5 treatments with 6 replicates per treatment and 2 piglets per replicate in a 21-d trial. The dietary treatments included a corn-soybean meal diet (0% SBP supplementation; CON), and diets supplemented with 2%, 4%, 6%, and 8% SBP, representing different SDF:IDF ratios at 10.16%, 13.53%, 16.79%, 19.86%, and 24.81%, respectively. The results indicated that the 8% SBP treatment had a negative effect on feed-to-gain ratio (linear, $P = 0.009$) compared with the CON treatment ($P = 0.021$). The apparent total tract digestibility (ATTD) of crude protein was lower in treatments supplemented with SBP ($P = 0.002$) and showed a linear decrease ($P = 0.001$), while the ATTD of IDF showed a linear increase ($P = 0.037$) in four SBP treatments compared to the CON treatment. The 4% SBP treatment increased serum concentrations of triglyceride (quadratic, $P = 0.019$) and K (linear, $P < 0.0037$), and decreased alanine transaminase concentration (quadratic, $P = 0.015$) compared with the CON treatment. The concentrations of Cit, Cys, Ile, Leu, Orn, Arg, taurine, urea, 1-methylhistidine, α-aminoadipic acid, α-aminobutyric acid and cystathionine in the 4% SBP treatment were highest among all treatments ($P < 0.05$). The serum concentrations of interleukin-6, interleukin-8, interleukin-10, transforming growth factor-β, and tumor necrosis factor-α in the 6% SBP treatment were higher than those in the CON treatment ($P < 0.05$), which also increased mucin-2 and G protein-coupled receptor 41 mRNA expression ($P < 0.05$) in colonic mucosa compared with the CON treatment and improved the intestinal barrier function. Diets containing more than 19.86% SDF:IDF could impair the intestinal health in piglets when SBP was used as the SDF source. Supplementing nursery piglet diets with 16.79% to 19.86% SDF:IDF is recommended for improving intestinal barrier function, increasing short-chain fatty acids concentrations, and improving intestinal microbiota composition.

1. Introduction

Piglets are frequently subjected to multifarious nutritional, psychological and environmental stresses during the weaning period, which can result in an imbalance of intestinal microflora, increasing incidence of diarrhea, and impaired growth performance (Gabler et al., 2019; Moeser et al., 2017). Previous studies

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have shown that dietary fiber (DF) supplementation can prevent post-weaning diarrhea and improve growth performance in weaned piglets (Yan et al., 2017; Zhao et al., 2018). Therefore, it is crucial to add appropriate amounts of dietary fiber in pig diets to regulate digestive physiological functions (Lee et al., 2022; Saddam et al., 2021). Various fibrous sources, including distillers dried grains with solubles such as soybean hulls, wheat bran, and sugar beet pulp (SBP), are commonly added to pig diets depending on the local availability of low-cost, fiber-rich ingredients (Li et al., 2021a). The dietary fiber cannot be digested in the small intestine by monogastric animals but can be fermented in the hindgut of these animals (Chiba, 2013). Numerous studies have shown that DF can enhance the abundance of Bacteroidetes and *Bifidobacterium*, which are generally considered beneficial for intestinal health (Li et al., 2021b; Thomson et al., 2012).

Dietary fiber is divided into soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) according to the solubility of the fiber. Sugar beet pulp is a pectin-rich SDF source which is a by-product from sugar beet production, and is highly fermentable and is used to reduce post-weaning diarrhea by promoting a beneficial shift in microbiota colonization (Huang et al., 2022; Li et al., 2019a). Sugar beet pulp can be fermented to produce short-chain fatty acids (SCFA), which can improve the intestinal microbiological environment, regulating the intestinal health of pigs (Díaz et al., 2020; Zhao et al., 2019a). A key mechanism of DF is hindgut fermentation and alterations in intestinal microflora, with SCFA production playing an indispensable role (Slavin, 2013).

Li et al. (2019b) found higher average piglet body weights and litter weights at weaning with an SDF:IDF of 25.73% in the gestation diet. Many studies have explored the effects of SBP on growth performance, nutrient digestibility, and fecal microflora in pigs (Shang et al., 2021a,b; Yan et al., 2017). The optimal ratio of SDF to IDF in fiber plays an important role in overall diet utilization and formulation of diets (Li et al., 2019b). However, few studies have investigated the optimal ratio of SDF to IDF for growth performance and intestinal health in piglets through supplementation with SBP. We hypothesized that different ratios of SDF to IDF formulated by SBP supplementation would exert different effects on intestinal health of piglets by improving gut microbiota composition and their metabolites. Therefore, in this study, sugar beet pulp was used to adjust the ratio of SDF to IDF in piglet diets to investigate the effect of SDF:IDF on growth performance, nutrient digestibility, immune function, intestinal morphology, intestinal microbiota and intestinal health of piglets.

2. Materials and methods

2.1. Animal ethics statement

All animal procedures were approved by the Committee of Animal Care at Hunan Agricultural University (approval number: CACAHU 2021-01106, Changsha, China). The experiment complied with the ARRIVE guidelines.

2.2. Animals and experimental design

A total of 60 crossbred piglets (Duroc × [Landrace × Yorkshire]) at 40 d old with average body weight of 10.0 ± 0.3 kg were randomly assigned to 5 distinct treatments with 6 replicates per treatment and 2 piglets per replicate (2 m × 1.2 m), and with the same number of males and females in each treatment. The 5 dietary treatments were as follows: (1) corn–soybean meal basal diet (CON); (2) basal diet supplemented with 2% sugar beet pulp (2% SBP); (3) basal diet supplemented with 4% sugar beet pulp (4% SBP); (4) basal diet supplemented with 6% sugar beet pulp (6% SBP); (5) basal diet supplemented with 8% sugar beet pulp (8% SBP). The 5 treatments represented different SDF:IDF of 10.16%, 13.53%, 16.79%, 19.86%, and 24.81%, respectively. Sugar beet pulp was purchased from COFCO International (Beijing, China). The animal experimental period lasted for 21 d.

2.3. Chemical analysis of diets and feces compositions

All diets were formulated to meet the NRC (2012) nutrient requirements (Table 1). Briefly, values of digestible energy, net energy, standardized ileal digestibility amino acids and available phosphorus were calculated using data provided by the China Feed Database (2019). Diets and fecal samples were weighed for determining dry matter (DM) (method 930.15; AOAC, 2007), crude protein (CP) (method 976.05; AOAC, 2007), ether extract (EE) (method 920.39; AOAC, 2007), ash (method 942.15; AOAC, 2007), SDF and IDF (method 991.43; AOAC, 2007). Gross energy (GE) was determined using a Parr 6400 Automatic Oxygen Bomb Calorimeter (Parr Instrument Co., Moline, IL, USA). Ca (GB/T 6436-2018), total phosphorus (GB/T 6437-2018) and Cr (GB/T 13088-2006) were determined according to the National Standard of the People's Republic of China.

2.4. Sampling collection and processing

Samples were collected on the last three days of the experiment period. All fecal samples from each replicate were mixed, dried at 65 °C, and pulverized for the analysis of nutrient digestibility. At the end of the trial, blood samples were collected by anterior vena cava puncture, centrifuged at 1500 × *g* for 10 min at room temperature, and then immediately stored at −80 °C for serum biochemical parameters analysis.

One piglet from each replicate was selected for slaughter after 12 h fasting at d 22. Tissue samples of approximately 2 cm jejunum and ileum were collected, and fixed in 4% paraformaldehyde for determining intestinal morphology. The mucosa of the ileum and colon were collected and stored at −80 °C. Furthermore, the ileal and colonic digesta of piglets were collected for SCFA and microbiota analysis.

2.5. Growth performance and diarrhea rate

The piglets from each replicate were weighed on an empty stomach at the beginning and end of the experimental period and the feed consumption of the piglets was recorded as well to determine average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G) according to the following formula:

ADFI = total feed intake/(test days × test number);

ADG = total weight gain/(number of test days × number of tests);

F/G = ADFI/ADG.

The diarrhea rate of each piglet was visually monitored each morning during the trial period and evaluated according to the method of Huang et al. (2022).

2.6. Apparent total tract digestibility of nutrients

Piglets in each dietary treatments were fed their respective diets plus dichromium trioxide (Cr_2O_3) in a 0.3% proportion as an exogenous marker. Approximately 150 g of fresh feces were collected and mixed with 10% hydrochloric acid and stored

Table 1
Ingredients and nutrient levels of experimental diets (%, air-dried basis).

Item	Treatments[1]				
	CON	2%SBP	4%SBP	6%SBP	8%SBP
Ingredients					
Corn	55.40	53.00	50.50	48.10	45.60
Soybean meal	6.80	6.80	6.80	6.70	6.70
Expanded soybean	6.00	6.00	6.00	6.00	6.00
Wheat flour	6.00	6.00	6.00	6.00	6.00
Whey powder	5.00	5.00	5.00	5.00	5.00
Fermented soybean meal	5.00	5.00	5.00	5.00	5.00
Enzymatic soybean[2]	5.00	5.00	5.00	5.00	5.00
Soybean oil	1.50	1.90	2.40	2.90	3.40
Sodium diacetate	1.00	1.00	1.00	1.00	1.00
Calcium hydrogen phosphate	0.40	0.40	0.40	0.40	0.40
Cane sugar	2.00	2.00	2.00	2.00	2.00
Chocolate powder	2.00	2.00	2.00	2.00	2.00
L-Lysine (98%)	0.60	0.60	0.60	0.60	0.60
NaCl	0.40	0.40	0.40	0.40	0.40
Calcium propionate	0.10	0.10	0.10	0.10	0.10
Premix[3]	2.50	2.50	2.50	2.50	2.50
Cr_2O_3	0.30	0.30	0.30	0.30	0.30
Sugar beet pulp	–	2.00	4.00	6.00	8.00
Total	100.00	100.00	100.00	100.00	100.00
Calculated nutrient levels					
Digestible energy, kcal/kg	3590.0	3600.0	3620.0	3620.0	3620.0
Net energy, kcal/kg	2520.0	2520.0	2520.0	2520.0	2520.0
SID lysine	1.23	1.23	1.23	1.23	1.23
SID methionine	0.36	0.36	0.36	0.35	0.35
SID methionine + cystine	0.60	0.60	0.60	0.59	0.59
SID threonine	0.67	0.67	0.67	0.66	0.66
Available phosphorus	0.41	0.40	0.41	0.41	0.40
Analyzed nutrient levels, %					
Crude protein	17.11	16.98	17.23	17.36	17.03
SDF	1.30	1.80	2.30	2.80	3.30
IDF	12.80	13.30	13.70	14.10	13.30
SDF:IDF ratio	10.16	13.53	16.79	19.86	24.81
Ether extract	5.23	5.54	5.95	6.15	6.26
Total Ca	0.42	0.44	0.42	0.43	0.48
Total phosphorus	0.54	0.52	0.54	0.55	0.52

SBP = sugar beet pulp; SID = standardized ileal digestibility; SDF = soluble dietary fiber; IDF = insoluble dietary fiber.
[1] CON, 2% SBP, 4% SBP, 6% SBP and 8% SBP are diets in which the ratios of SDF to IDF are 10.16%, 13.53%, 16.79%, 19.86%, and 24.81%, respectively.
[2] The commercial name was HP300, which was purchased from Hamlet Protein, Denmark.
[3] Premix provided the following per kilogram of diet: vitamin A, 14,000 IU; vitamin D, 2900 IU; vitamin B_2, 4.0 mg; vitamin B_3, 35 mg; vitamin B_5, 40 mg; vitamin B_7, 0.3 mg; vitamin B_{11}, 2.0 mg; vitamin B_{12}, 0.04 mg; Cu, 110 mg; Fe, 220 mg; Zn, 90 mg; Mn, 60 mg; I, 0.6 mg.

at −20 °C on the last three days of the experiment period. After sample collection, the fecal samples were thawed and pooled together, dried in a forced-draft oven at 65 °C for 72 h, then passed through a 40-mesh screen for analysis of nutrient digestibility. The chromium concentration in diet and feces were measured photometrically for calculating the ATTD with the following formula (Li et al., 2023):

$$\text{ATTD}\,(\%) = \left(1 - \frac{Cr_2O_3\ \text{in diet}\,(\%)}{Cr_2O_3\ \text{in feces}\,(\%)} \times \frac{\text{Feces nutrient}\,(\%)}{\text{Diet nutrient}\,(\%)}\right) \times 100$$

2.7. Serum parameters

Serum samples were thawed at 4 °C and mixed well prior to analysis. Serum biochemical parameters including total protein (TP), albumin (ALB), glucose (GLU), alanine transaminase (ALT), creatinine (CRE), blood urea nitrogen (BUN), triglyceride (TG), total cholesterol (TC), Ca, K, and Na of the piglets were determined using an Automatic Biochemical Autoanalyzer (Beckman CX4, Beckman Coulter Inc., Brea, CA, United States) and commercial kits (Shanghai Kehua Bio-Engineering Co., Ltd, Shanghai, China). The serum amino acid concentrations were determined by High-Performance Liquid Chromatography (Agilent 1290, Agilent Technologies, USA).

The serum concentrations of diamine oxidase (DAO, MM-043802), D-lactate (D-LA, MM-3373202), interleukin-1β (IL-1β, MM-042201), interleukin-6 (IL-6, MM-041801), interleukin-8 (IL-8, MM-041701), interleukin-10 (IL-10, MM-042502), interleukin-12 (IL-12, MM-7777601), interferon-gamma (IFN-γ, MM-041201), tumor necrosis factor-α (TNF-α, MM-038301) and transforming growth factor-β (TGF-β, MM-038201) were measured by enzyme linked immunosorbent assay kits following the manufacturer's instructions (Jiangsu Meimian Industrial Co., Ltd, Yancheng, China).

2.8. Intestinal morphology

Jejunum and ileum samples were fixed in 4% paraformaldehyde solution for 24 h, embedded in paraffin and sectioned and stained with hematoxylin and eosin (H&E), and the tissue sections were mounted on the machine using a panoramic section scanner. Five intact, well-oriented samples with obvious villous height and crypt depth were measured to calculate the villus height to crypt depth ratio (VH/CD), the goblet cell number per villus and epithelial length using Image-Pro Plus 6.0 analysis software (version 6.0, Rockville, MD, USA).

2.9. Short chain fatty acids in the ileal and colonic digesta

The concentrations of SCFA such as acetate, propionate, butyrate, isobutyrate, valerate and isovalerate in the ileal and colonic

digesta were analyzed by Gas Chromatography (GC) (8890, Agilent, USA) as described by Xiao et al. (2017).

2.10. Colonic digesta nitrogen metabolites

The ammonia nitrogen concentration of colonic digesta was detected using an indophenol colorimetric method based on the method of Almeida et al. (2017). Briefly, the colon content (1 g) was weighed, vortex shaken with pure water and extracted overnight in a refrigerator at 4 °C, and the supernatant was extracted with phenol and sodium hypochlorite reagents. Colonic ammonia nitrogen concentration was measured by UV spectrophotometer at a wavelength of 550 nm.

The standard stock solution was prepared by using indole and skatole standards adding methanol. The colon content (1 g) was weighed with chromatographically pure methanol and vortex shaken well. The supernatant was centrifuged at 12,000 × *g*, and then analyzed by Reversed-Phase High Performance Liquid Chromatography (Agilent 1290, USA) to detect indole and skatole contents. Chromatographic conditions: the temperature of the column was 35 °C, the ratio of mobile phase methanol and water was 60:40 (vol:vol), at a flow rate of 1.0 mL/min. The detector was a fluorescence detector, with excitation wavelength of 270 nm, and emission wavelength was 350 nm.

The biogenic amine content of colon digesta was determined by the method of Yang et al. (2014). The colon content (1 g) was weighed and perchloric acid solution was added to it at −20 °C and allowed to sit overnight. It was then centrifuged at low temperature, the supernatant collected, and the above operation was repeated again. The supernatant was then derivatized and the derivatized solution was analyzed to detect biogenic amines by High Performance Liquid Chromatography (Agilent 1200, Germany).

2.11. Colonic digesta fiber degrading enzymes

The colonic digesta levels of pectinase (MM-7778402), xylanase (MM-7778402), mannanase (MM-7778402) and cellulase (MM-7778402) were detected using commercial ELISA kits following the manufacturer's instructions (Jiangsu Meimian Industrial Co., Ltd, Yancheng, China).

2.12. RNA extraction and quantitative real-time polymerase chain reaction

Total RNA in the jejunal and ileal mucosa were extracted and purified using Trizol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's guidelines. The amount and purity of the RNA were examined using a Nanodrop One (NanoDrop, Thermo Fisher Scientific, USA). The integrity of the RNA was measured with 1% agarose gel electrophoresis using a Chemiluminescence Imaging Analyzer (ChemiDoc, Bio-Rad, USA). The procedures for reverse transcription and real-time PCR were described in our previous study (Jiang et al., 2021). The reverse transcription kit (*Evo M-MLV* RT Kit with gDNA Clean for qPCR, Cat. no. AG11705) for cDNA synthesis was purchased from Accurate Biotechnology (Hunan) Co., Ltd, ChangSha, China. RT-PCR was performed using the SYBR Green Premix *Pro Taq* HS qPCR kit (Cat. No. AG11701, Accurate Biotechnology (Hunan) Co., Ltd ChangSha, China) by a real-time PCR instrument (LightCycler480 II system, Roche, Basel, Switzerland). The primers for the RT-PCR were designed with the Primer-Blast tool based on NCBI Gene Bank. Beta-actin was used for housekeeping genes to normalize the expression levels of target gene using the $2^{-\Delta\Delta Ct}$ method. All primer sequences are shown in Table 2.

2.13. DNA extraction, PCR amplification and microbiota sequencing

Colonic digesta microbial genomic DNA was extracted using MN-NucleoSpin Kit (Qiagen Ltd., Germany) based on the manufacturer's protocol, and the concentration and quality of the colonic digesta DNA were examined with the Nanodrop One instrument (NanoDrop, Thermo Fisher Scientific, USA) and the 1% agarose gels for electrophoresis with a Chemiluminescence Imaging Analyzer (ChemiDoc, Bio-Rad, USA), respectively. The universal forward primer 338F (5′-GGACTACHVGGGGTWTCTAAT-3′) and universal reverse primer 806R (5′-GGACTACHVGGGGTWTCTA-3′) were used to amply the V3–V4 regions of 16S rRNA gene. The PCR amplification was conducted using the conditions as described in previous studies (Lan et al., 2023).

2.14. Bioinformatics analysis

The amplified products were extracted, purified, and quantified, and then sequenced using an Illumina HiSeq 2500 platform (Illumina Inc., San Diego, USA). Operational taxonomic units (OTU) were generated by clustering at 97% sequence similarity using USEARCH (version 7.0). Bioinformatics analysis was mainly performed through QIIME (version 1.7.0) and R packages (version 3.2.0) as in our previous study (Li et al., 2021c). Spearman's correlation analysis was used to perform the correlations between concentration of SCFA, metabolites and microbiota in accordance with the previous method (Lan et al., 2023).

2.15. Statistical analysis

Data were presented as the mean and standard error of the mean (SEM). Significant differences among different treatments were evaluated by one-way analysis of variance (ANOVA) with a Duncan's multiple comparison test using SPSS 23.0 software (SPSS Inc., Chicago, IL, USA). The linear and quadratic effects of SDF:IDF were then analyzed using regression analysis in SPSS 23. The $\chi 2$ contingency was used to calculate the diarrhea rate. Linear discriminant analysis (LDA) effect size (LEfSe) was performed to screen differential bacteria (LDA > 4). Principal component analysis (PCA) based on Bray–Curtis dissimilarity was used to compare the microbial community. Significant values were declared if $P < 0.05$, and $0.05 \leq P < 0.10$ was considered to be a tendency.

3. Results

3.1. Growth performance and diarrhea rate

Different ratios of SDF to IDF significantly influenced F/G in piglets ($P = 0.021$), with a linear increase (linear, $P = 0.009$) with SDF:IDF observed, with the 8% SBP-supplemented diet significantly increasing F/G compared to the CON treatment ($P < 0.05$). The 6% SBP treatment exhibited lower diarrhea rate compared to the CON treatment. However, no significant differences ($P > 0.05$) were observed among treatments on ADFI and ADG of piglets (Table 3).

3.2. Apparent nutritional digestibility

The effects of the ratio of SDF to IDF on nutrient digestibility are showed in Table 4. Different ratios of SDF to IDF significantly influenced the ATTD of CP and total phosphorus ($P = 0.002$ and 0.002, respectively); the ATTD of CP and total phosphorus in the 4% SBP treatment were significantly lower than those in the CON treatment ($P < 0.05$). The ATTD of CP showed a linear decrease ($P = 0.001$), the ATTD of total phosphorus displayed a quadratic decrease ($P = 0.005$), and the ATTD of IDF and ash increased linearly ($P = 0.037$ and 0.033, respectively) with increasing SDF:IDF.

Table 2
Primer sequences used for quantitative reverse transcription-PCR.

Genes	Primer	Sequences (5′ to 3′)	Size, bp	Accession number
MUC1	Forward	GTGCCGACGAAAGAACTG	187	XM_021089728.1
	Reverse	TGCCAGGTTCGAGTAAGAG		
MUC2	Forward	CTGTGTGGGGCCTGACAA	65	XM_021082584.1
	Reverse	AGTGCTTGCAGTCGAACTCA		
GPR41	Forward	TGGAGACCTTACGTGTTG	75	NM_001315601.1
	Reverse	CGAGGATGAGAAGTAGTAGAT		
GPR43	Forward	CGTGTTCATCGTTCAGTA	76	NM_001278758.1
	Reverse	GAAGTTCTCATAGCAGGTA		
GPR40	Forward	TGCTCTGACCTCCTGCTGG	235	NM_001278783.1
	Reverse	CACACACCCCCCAGGAATAG		
β-Actin	Forward	CTGCGGCATCCACGAAACT	147	XM_003124280.5
	Reverse	AGGGCCGTGATCTCCTTCTG		

MUC1 = mucin-1; *MUC2* = mucin-2; *GPR41* = G protein-coupled receptors 41; *GPR43* = G protein-coupled receptors 43; *GPR40* = G protein-coupled receptors 40.

Table 3
Effects of the ratio of SDF to IDF on growth performance and diarrhea rate of piglets.

Item	Treatments[1]					SEM	*P*-value		
	CON	2%SBP	4%SBP	6%SBP	8%SBP		ANOVA	Linear	Quadratic
IBW, kg	9.95	10.05	10.14	9.91	10.01	0.252	0.910	0.970	0.612
FBW, kg	19.50	19.18	19.94	19.67	19.60	0.704	0.869	0.666	0.778
ADG, g/d	454.97	434.76	466.87	464.40	456.93	28.730	0.816	0.605	0.905
ADFI, g/d	845.39	818.66	873.30	871.34	908.87	46.924	0.403	0.096	0.566
F/G	1.86[b]	1.90[b]	1.88[b]	1.88[b]	1.99[a]	0.043	0.021	0.009	0.102
Diarrhea rate, %	11.43	14.28	12.92	8.57	12.86				

SDF = soluble dietary fiber; IDF = insoluble dietary fiber; SBP = sugar beet pulp; IBW = initial body weight; FBW = final body weight; ADG = average daily gain; ADFI = average daily feed intake; F/G = feed-to-gain ratio.
[a,b] Means in the same row with different superscripts are significantly different ($P < 0.05$) by one-way ANOVA. Data are presented as mean and SEM ($n = 6$).
[1] CON, 2% SBP, 4% SBP, 6% SBP and 8% SBP are diets in which the ratios of SDF to IDF are 10.16%, 13.53%, 16.79%, 19.86%, and 24.81%, respectively.

3.3. Serum biochemical parameters

As shown in Table 5, different ratios of SDF to IDF significantly influenced the serum concentrations of TG ($P = 0.043$), ALT ($P = 0.007$) and K ($P = 0.037$) in piglets. The concentrations of TG and ALT showed quadratic increases ($P = 0.019$ and 0.015, respectively). Different ratios of SDF to IDF affected the BUN concentration (quadratic, $P = 0.005$). The concentrations of TG and K in 4% SBP treatment were significantly higher ($P < 0.05$), while the ALT concentration was lower compared to that of the CON treatment ($P < 0.05$).

3.4. Serum amino acids and derivatives

The dietary 4% SBP supplementation significantly increased serum concentrations of Cit, Cys, Ile, Orn and Arg compared with the CON treatment ($P < 0.05$). The Cit, Cys, Ile and Arg concentrations all showed quadratic increases with increasing SDF:IDF ratio from 10.16% to 16.79% ($P < 0.001$, $P = 0.012$, 0.019 and 0.037, respectively). Different ratios of SDF to IDF in diets had significant effects on the serum concentrations of taurine ($P = 0.008$), urea ($P = 0.044$), 1-methylhistidine ($P = 0.004$), α-aminoadipic acid ($P = 0.042$), α-aminobutyric acid ($P = 0.016$) and cystathionine ($P = 0.008$), with the highest concentrations of these derivatives found in the 4% SBP treatment. All concentrations were significantly higher in the 4% SBP treatment than in the CON treatment except for urea ($P < 0.05$), and 1-methylhistidine which showed linear ($P = 0.038$) and quadratic ($P = 0.010$) increases respectively (Table 6).

3.5. Serum inflammatory cytokines

Different ratios of SDF to IDF significantly affected the concentrations of inflammatory cytokines such as INF-γ (linear, $P = 0.005$; quadratic, $P = 0.001$), IL-1β (linear, $P = 0.001$; quadratic, $P = 0.001$), IL-6 (linear, $P = 0.001$), IL-8 (linear, $P = 0.008$; quadratic, $P < 0.001$),

Table 4
Effects of the ratio of SDF to IDF on apparent total tract digestibility of dietary nutrients of piglets' diet (%).

Item	Treatments[1]					SEM	*P*-value		
	CON	2%SBP	4%SBP	6%SBP	8%SBP		ANOVA	Linear	Quadratic
DM	49.78	50.22	42.90	46.06	49.28	6.917	0.802	0.741	0.389
GE	50.46	56.44	49.80	52.65	55.11	6.121	0.778	0.691	0.881
CP	62.63[a]	56.30[a]	45.98[b]	53.67[ab]	45.64[b]	4.295	0.002	0.001	0.210
EE	53.39	64.07	59.32	63.36	65.61	5.865	0.260	0.083	0.607
IDF	40.48	46.84	46.74	48.95	51.95	5.070	0.270	0.037	0.745
SDF	48.48	55.20	59.63	55.92	57.33	5.000	0.264	0.112	0.168
Ash	42.39	53.43	52.87	54.84	59.63	7.106	0.211	0.033	0.600
Ca	58.92	64.97	58.79	65.87	63.56	5.696	0.602	0.433	0.821
Total phosphorus	71.78[a]	46.07[c]	56.60[bc]	61.67[ab]	60.73[ab]	5.476	0.002	0.601	0.005

SDF = soluble dietary fiber; IDF = insoluble dietary fiber; DM = dry matter; GE = gross energy; CP = crude protein; EE = ether extract.
[a-c] Means in the same row with different superscripts are significantly different ($P < 0.05$) by one-way ANOVA. Data are presented as mean and SEM ($n = 6$).
[1] CON, 2% SBP, 4% SBP, 6% SBP and 8% SBP are diets in which the ratios of SDF to IDF are 10.16%, 13.53%, 16.79%, 19.86%, and 24.81%, respectively.

Table 5
Effects of the ratio of SDF to IDF on serum biochemical indexes of piglets (mmol/L).

Item	Treatments[1]					SEM	*P*-value		
	CON	2%SBP	4%SBP	6%SBP	8%SBP		ANOVA	Linear	Quadratic
TP, g/L	49.00	50.67	46.00	49.67	50.17	3.769	0.753	0.876	0.553
ALB, g/L	25.50	26.17	24.50	27.50	26.50	2.600	0.827	0.571	0.848
GLU	5.17	6.13	5.00	5.63	5.65	0.516	0.231	0.689	0.923
CRE	59.00	66.67	58.17	64.50	64.17	5.570	0.485	0.518	0.938
TC	2.06	2.43	1.98	2.09	2.26	0.190	0.156	0.851	0.756
TG	0.40^{b}	0.50^{ab}	0.63^{a}	0.45^{b}	0.46^{b}	0.071	0.043	0.642	0.019
ALT, U/L	47.00^{a}	50.67^{a}	32.83^{b}	46.67^{a}	51.83^{a}	5.034	0.007	0.619	0.015
NH_3, μmol/L	1.68	1.94	2.31	1.53	1.68	0.280	0.072	0.507	0.074
BUN	1.09	1.69	1.96	1.55	0.78	0.414	0.059	0.416	0.005
Ca	2.25	2.25	2.49	2.25	2.36	0.104	0.114	0.324	0.340
K	5.17^{b}	5.83^{ab}	6.17^{a}	5.50^{ab}	6.00^{a}	0.327	0.037	0.080	0.135
Na	140.33	140.00	151.17	140.00	150.67	6.387	0.177	0.160	0.984
DAO, U/mL	25.89	24.67	24.23	24.54	24.27	1.171	0.618	0.211	0.402
D-LA, μmol/mL	519.24	510.41	510.63	542.61	540.31	29.151	0.676	0.265	0.566

IDF = insoluble dietary fiber; SDF = soluble dietary fiber; TP = total protein; ALB = albumin; GLU = glucose; CRE = creatinine; TC = total cholesterol; TG = triglyceride; ALT = alanine transaminase; NH_3 = ammonia; BUN = blood urea nitrogen; DAO = diamine oxidase; D-LA = D-lactate.
[a,b] Means in the same row with different superscripts are significantly different ($P < 0.05$) by one-way ANOVA. Data are presented as mean and SEM ($n = 6$).
[1] CON, 2% SBP, 4% SBP, 6% SBP and 8% SBP are diets in which the ratios of SDF to IDF are 10.16%, 13.53%, 16.79%, 19.86%, and 24.81%, respectively.

Table 6
Effects of the ratio of SDF to IDF on serum amino acids and derivatives of piglets (ng/mL).

Item	Treatments[1]					SEM	*P*-value		
	CON	2%SBP	4%SBP	6%SBP	8%SBP		ANOVA	Linear	Quadratic
Asp	1.30^{ab}	0.94^{b}	1.75^{a}	1.42^{ab}	1.06^{b}	0.267	0.045	0.991	0.116
Thr	2.66	1.80	3.31	2.32	2.04	0.540	0.079	0.550	0.360
Ser	6.13	5.42	6.76	5.55	5.54	0.919	0.562	0.613	0.637
Glu	18.28^{ab}	15.05^{b}	25.65^{a}	23.69^{a}	17.94^{ab}	3.596	0.038	0.331	0.076
Sar	0.54	0.65	0.89	0.69	0.53	0.152	0.148	0.938	0.022
Gly	43.62	38.69	45.86	39.02	40.18	7.100	0.814	0.683	0.923
Ala	24.35	20.17	28.07	28.38	25.12	4.355	0.348	0.327	0.622
Cit	3.57^{bc}	3.83^{bc}	7.06^{a}	5.01^{ab}	2.27^{c}	1.061	0.002	0.554	<0.001
Val	5.82^{ab}	4.56^{b}	8.46^{a}	7.73^{a}	4.94^{b}	1.241	0.015	0.620	0.028
Cys	1.06^{b}	1.10^{b}	2.16^{a}	1.39^{b}	1.13^{b}	0.338	0.017	0.555	0.012
Met	1.98	1.86	2.48	2.15	1.69	0.398	0.350	0.740	0.133
Ile	4.73^{b}	4.05^{b}	7.95^{a}	5.79^{b}	4.94^{b}	0.971	0.005	0.329	0.019
Leu	7.99^{b}	6.86^{b}	11.77^{a}	9.42^{ab}	6.94^{b}	1.463	0.013	0.888	0.016
Tyr	4.40	3.79	5.35	4.70	3.84	0.864	0.370	0.914	0.251
Phe	4.07	3.96	5.27	4.66	3.58	0.832	0.308	0.877	0.093
Orn	2.94^{b}	2.47^{b}	5.02^{a}	3.43^{b}	3.45^{b}	0.644	0.008	0.180	0.078
Lys	15.11	12.50	21.19	17.22	13.91	3.240	0.100	0.750	0.114
His	1.30	1.35	2.27	1.69	1.17	0.459	0.151	0.924	0.040
Arg	8.79^{b}	7.33^{b}	14.02^{a}	9.25^{b}	7.58^{b}	2.043	0.021	0.915	0.037
Hypro	6.92	4.80	6.98	7.18	6.84	1.216	0.293	0.420	0.628
Pro	10.28	9.06	14.01	11.66	10.30	2.037	0.177	0.566	0.171
Taurine	2.17^{b}	1.63^{b}	3.23^{a}	2.41^{ab}	1.99^{b}	0.406	0.008	0.637	0.053
Urea	21.58^{ab}	27.03^{ab}	37.93^{a}	26.01^{ab}	12.56^{b}	7.693	0.044	0.279	0.006
1-Methylhistidine	0.81^{c}	0.81^{c}	2.11^{a}	1.65^{ab}	1.25^{bc}	0.353	0.004	0.038	0.011
α-Aminoadipic acid	1.45^{b}	1.04^{b}	3.49^{a}	1.24^{b}	1.04^{b}	0.865	0.042	0.749	0.073
α-Aminobutyric acid	0.40^{b}	0.32^{b}	0.61^{a}	0.41^{b}	0.31^{b}	0.088	0.016	0.652	0.029
Cystathionine	0.49^{b}	0.40^{b}	0.83^{a}	0.53^{b}	0.51^{b}	0.111	0.008	0.461	0.054
β-alanine	1.47	1.14	1.83	1.61	1.48	0.301	0.263	0.480	0.531

SDF = soluble dietary fiber; IDF = insoluble dietary fiber; SBP = sugar beet pulp.
[a-c] Means in the same row with different superscripts are significantly different ($P < 0.05$) by one-way ANOVA. Data are presented as mean and SEM ($n = 6$).
[1] CON, 2% SBP, 4% SBP, 6% SBP and 8% SBP are diets in which the ratios of SDF to IDF are 10.16%, 13.53%, 16.79%, 19.86%, and 24.81%, respectively.

IL-10 (quadratic, $P = 0.001$), IL-12 (linear, $P = 0.006$), TGF-β (linear, $P = 0.004$) and TNF-α (quadratic, $P = 0.001$) in piglets' serum; the concentrations of IL-6, IL-8, IL-10, TGF-β, and TNF-α in the 6% SBP treatment were significantly higher than those in the CON treatment ($P < 0.05$) (Fig. 1).

3.6. Jejunal and ileal morphology

As shown in Table 7 and Fig. 2, no significant differences were observed in villus height (VH), crypt depth (CD), VH/CD ratio, goblet cell number per villus and epithelial length in the jejunum and ileum ($P > 0.05$). The small intestinal villi of the piglets exhibited varying degrees of wear as dietary SDF:IDF increased, with the 6% and 8% SBP treatments showing irregularly shaped villi and significant wear compared to the CON treatment.

3.7. Short chain fatty acids concentrations in the ileal and colonic digesta

Table 8 presents the concentrations of six total SCFA in the ileal and colonic digesta of piglets. In the ileum, different ratios of SDF to IDF significantly affected the concentration of butyrate ($P = 0.015$);

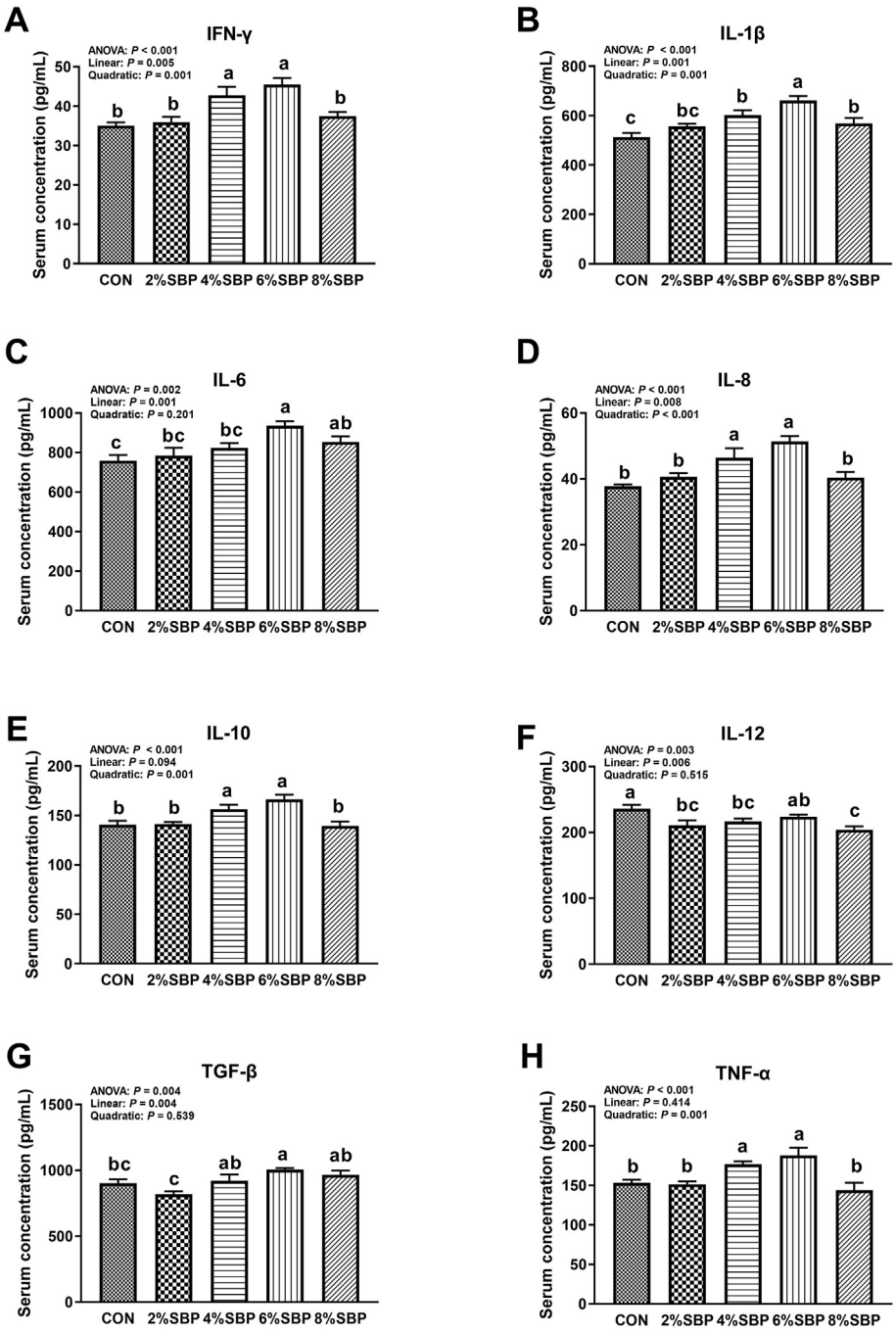

Fig. 1. Effects of the ratio of SDF to IDF on serum concentrations of cytokines of piglets. (A) Interferon-gamma (IFN-γ). (B) Interleukin-1β (IL-1β). (C) Interleukin-6 (IL-6). (D) Interleukin-8 (IL-8). (E) Interleukin-10 (IL-10). (F) Interleukin-12 (IL-12). (G) Transforming growth factor-β (TGF-β). (H) Tumor necrosis factor-α (TNF-α). CON, 2% SBP, 4% SBP, 6% SBP and 8% SBP are diets in which the ratios of SDF to IDF are 10.16%, 13.53%, 16.79%, 19.86%, and 24.81%, respectively. [a-c] Values with different lowercase letters are significantly different ($P < 0.05$). Data are presented as mean and SEM ($n = 6$). SDF = soluble dietary fiber; IDF = insoluble dietary fiber; SBP = sugar beet pulp.

the ileal digesta concentration of butyrate significantly increased in the 2% SBP treatment compared to those fed the CON diet ($P < 0.05$), and butyrate concentration tended to increase linearly ($P = 0.061$) and quadratically ($P = 0.091$) with the ratio of SDF to IDF increasing from 10.16% to 13.53%. In colonic digesta, dietary 2% SBP supplementation tended to increase isobutyrate ($P = 0.077$) and isovalerate ($P = 0.063$) concentrations, and also showed a linear increase in isobutyrate ($P = 0.011$) and isovalerate ($P = 0.007$) concentrations compared with the CON treatment.

3.8. The concentrations of microbial nitrogen metabolites in colonic digesta

Compared with the CON treatment, the dietary 2% SBP supplementation showed significantly higher spermine, and spermidine concentrations ($P < 0.05$). Spermine showed a linear increase ($P = 0.009$), while spermidine followed a quadratic ($P = 0.011$) increase. Dietary 6% SBP supplementation significantly increased the concentrations of biogenic amines and cadaverine compared to

Table 7
Effects of the ratio of SDF to IDF on intestinal morphology of piglets.

Item	Treatments[1]					SEM	*P*-value		
	CON	2%SBP	4%SBP	6%SBP	8%SBP		ANOVA	Linear	Quadratic
Jejunum									
Villus height, mm	0.37	0.36	0.41	0.28	0.32	0.062	0.283	0.189	0.653
Crypt depth, mm	0.21	0.26	0.21	0.15	0.15	0.047	0.164	0.050	0.456
VH/CD	2.07	1.54	2.00	2.08	2.47	0.486	0.463	0.226	0.265
Goblet cell number per villus	8.40	13.30	4.63	9.20	13.73	4.539	0.270	0.524	0.308
Epithelial length, mm	0.79	0.74	0.85	0.59	0.65	0.118	0.225	0.112	0.595
Ileum									
Villus height, mm	0.32	0.26	0.33	0.32	0.34	0.037	0.302	0.256	0.445
Crypt depth, mm	0.14	0.16	0.14	0.15	0.18	0.036	0.804	0.448	0.605
VH/CD	2.53	1.97	2.36	2.25	1.97	0.453	0.678	0.420	0.955
Goblet cell number per villus	10.53	13.63	8.90	9.10	10.03	3.647	0.703	0.504	0.951
Epithelial length, mm	0.65	0.56	0.67	0.65	0.71	0.067	0.275	0.169	0.333

SDF = soluble dietary fiber; IDF = insoluble dietary fiber; SBP = sugar beet pulp; VH/CD = villus height to crypt depth ratio.
Data are presented as mean and SEM ($n = 6$).
[1] CON, 2% SBP, 4% SBP, 6% SBP and 8% SBP are diets in which the ratios of SDF to IDF are 10.16%, 13.53%, 16.79%, 19.86%, and 24.81%, respectively.

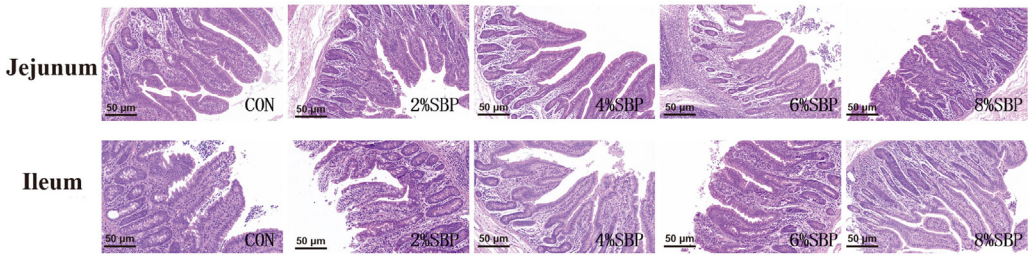

Fig. 2. Effects of the ratio of SDF to IDF on intestinal morphology of jejunum and ileum (H&E staining; scale bar = 50 μm). CON, 2% SBP, 4% SBP, 6% SBP and 8% SBP are diets in which the ratios of SDF to IDF are 10.16%, 13.53%, 16.79%, 19.86%, and 24.81%, respectively. SDF = soluble dietary fiber; IDF = insoluble dietary fiber; SBP = sugar beet pulp.

Table 8
Effects of the ratio of SDF to IDF on the concentration of SCFA in ileal and colonic digesta of piglets (μg/g).

Item	Treatments[1]					SEM	*P*-value		
	CON	2%SBP	4%SBP	6%SBP	8%SBP		ANOVA	Linear	Quadratic
Ileal digesta									
Total SCFA	812.39	1107.77	940.69	781.46	720.09	190.541	0.293	0.242	0.174
Acetate	651.72	893.84	766.51	576.88	561.34	167.000	0.262	0.195	0.203
Propionate	64.82	58.37	67.60	88.49	75.75	21.774	0.689	0.296	0.987
Isobutyrate	11.55	15.54	21.30	16.01	28.72	9.790	0.470	0.124	0.807
Butyrate	58.52^{b}	102.41^{a}	49.00^{b}	70.61^{ab}	32.78^{b}	19.004	0.015	0.061	0.091
Isovalerate	18.50	26.67	27.22	21.37	14.12	12.866	0.827	0.629	0.284
Valerate	7.29	10.95	9.07	8.11	7.39	1.850	0.284	0.531	0.121
Colonic digesta									
Total SCFA	7800.62	7835.78	7214.72	7560.36	8214.22	713.208	0.711	0.732	0.254
Acetate	3931.95	4094.55	3915.14	3693.80	4257.56	268.492	0.320	0.680	0.295
Propionate	1751.18	1778.47	1568.50	1943.02	1968.20	211.814	0.347	0.218	0.310
Isobutyrate	168.61	173.24	171.19	121.85	94.82	32.555	0.077	0.011	0.211
Butyrate	1225.41	1089.21	1025.65	1208.14	1438.80	233.428	0.467	0.306	0.125
Isovalerate	271.28	284.11	252.31	183.19	125.83	59.195	0.063	0.007	0.267
Valerate	452.21	416.21	281.93	410.36	329.01	109.643	0.526	0.313	0.559

SDF = soluble dietary fiber; IDF = insoluble dietary fiber; SCFA = short-chain fatty acids; SBP = sugar beet pulp.
[a,b] Means in the same row with different superscripts are significantly different ($P < 0.05$) by one-way ANOVA. Data are presented as mean and SEM ($n = 6$).
[1] CON, 2% SBP, 4% SBP, 6% SBP and 8% SBP are diets in which the ratios of SDF to IDF are 10.16%, 13.53%, 16.79%, 19.86%, and 24.81%, respectively.

the CON treatment ($P < 0.05$); biogenic amines showed a quadratic ($P = 0.011$) increase, while cadaverine showed a linear increase ($P = 0.003$) with increasing SDF:IDF. The dietary 8% SBP supplementation also significantly increased the concentration of tyramine, and tyramine concentration displayed linear ($P < 0.001$) and quadratic ($P = 0.005$) increases. In addition, the NH_3–N concentration significantly decreased in a linear manner ($P = 0.002$). No significant differences in indole and skatole were observed among the treatments of piglets ($P > 0.05$) (Table 9).

3.9. The concentrations of fiber degrading enzymes in colonic digesta

The dietary 8% SBP supplementation significantly increased the concentrations of cellulase and pectinase compared to the CON treatment ($P < 0.05$); cellulase and pectinase displayed linear increases ($P = 0.001$ and 0.026, respectively). As SDF:IDF increased, the concentration of xylanase showed a linear increase ($P = 0.005$). The concentration of mannanase in 8% SBP supplementation

Table 9
Effects of the ratio of SDF to IDF on microbial nitrogen metabolite concentrations in colonic digesta of piglets (μg/g).

Item	Treatments[1]					SEM	*P*-value		
	CON	2%SBP	4%SBP	6%SBP	8%SBP		ANOVA	Linear	Quadratic
Indole	11.33	17.58	14.16	16.73	21.98	4.180	0.158	0.038	0.722
Skatole	12.51	20.07	22.53	23.44	27.42	5.451	0.117	0.012	0.551
NH_3–N, mg/dL	13.93^{a}	14.55^{a}	12.08^{ab}	10.49^{ab}	7.57^{b}	2.182	0.026	0.002	0.292
Biogenic amines	153.85^{b}	234.16^{ab}	187.10^{b}	321.50^{a}	154.90^{b}	43.140	0.003	0.363	0.011
Monoamine									
Tyramine	1.16^{b}	1.11^{b}	1.51^{b}	3.33^{b}	9.02^{a}	1.595	<0.001	<0.001	0.005
Tryptamine	7.32	9.39	8.94	6.76	7.90	1.216	0.200	0.595	0.274
Phenethylamine	44.99^{ab}	63.92^{a}	50.85^{ab}	4.26^{c}	44.78^{b}	8.867	<0.001	0.003	0.811
Diamine									
Cadaverine	3.48^{b}	4.44^{b}	7.44^{b}	206.69^{a}	22.81^{b}	32.851	<0.001	0.003	0.057
Spermine	2.46^{b}	4.74^{a}	1.90^{b}	2.33^{b}	0.85^{b}	0.889	0.004	0.009	0.083
Spermidine	69.87^{b}	119.27^{a}	86.73^{b}	76.57^{b}	71.28^{b}	12.042	0.002	0.151	0.011
1,7-Heptadiamine	25.19	31.47	28.81	23.26	20.19	6.368	0.436	0.213	0.212

SDF = soluble dietary fiber; IDF = insoluble dietary fiber; SBP = sugar beet pulp.
[a,b] Means in the same row with different superscripts are significantly different ($P < 0.05$) by one-way ANOVA. Data are presented as mean and SEM ($n = 6$).
[1] CON, 2% SBP, 4% SBP, 6% SBP and 8% SBP are diets in which the ratios of SDF to IDF are 10.16%, 13.53%, 16.79%, 19.86%, and 24.81%, respectively.

Table 10
Effects of the ratio of SDF to IDF on fiber degrading enzymes levels of colonic digesta of piglets.

Item	Treatments[1]					SEM	*P*-value		
	CON	2%SBP	4%SBP	6%SBP	8%SBP		ANOVA	Linear	Quadratic
Cellulase, pg/mL	8098.52^{b}	10,615.48^{b}	10,645.47^{b}	10,953.66^{ab}	13,868.25^{a}	1424.172	0.010	0.001	0.782
Xylanase, U/L	141.22	164.58	178.22	174.50	219.00	24.161	0.051	0.005	0.700
Pectinase, ng/mL	25.27^{b}	36.11^{a}	31.75^{ab}	30.52^{ab}	39.32^{a}	4.239	0.029	0.026	0.934
Mannanase, pg/mL	3592.30^{b}	3190.74^{b}	3242.53^{b}	3568.80^{b}	6176.58^{a}	852.516	0.008	0.007	0.010

SDF = soluble dietary fiber; IDF = insoluble dietary fiber; SBP = sugar beet pulp.
[a,b] Means in the same row with different superscripts are significantly different ($P < 0.05$) by one-way ANOVA. Data are presented as mean and SEM ($n = 6$).
[1] CON, 2% SBP, 4% SBP, 6% SBP and 8% SBP are diets in which the ratios of SDF to IDF are 10.16%, 13.53%, 16.79%, 19.86%, and 24.81%, respectively.

significantly increased compared to that of the CON treatment ($P < 0.05$), exhibiting linear ($P = 0.007$) and quadratic ($P = 0.010$) increases (Table 10).

3.10. Relative mRNA expression involved in G protein-coupled receptors (GPR) and intestinal barrier function in the ileal and colonic mucosa

The relative mRNA expression level of *GPR 40* in the 8% SBP treatment significantly higher than the CON treatment in ileal mucosa (linear, $P < 0.001$; quadratic, $P = 0.001$). The relative mRNA expression levels of *GPR 41* (linear, $P < 0.001$) and mucin-2 (*MUC 2*) (linear, $P < 0.001$) were significantly higher in 6% SBP treatment compared to the CON treatment in colonic mucosa. Moreover, the relative mRNA expression level of *GPR 40* supplemented with 8% SBP was superior to the CON treatment in colonic mucosa (linear, $P = 0.004$; quadratic, $P = 0.015$), the mRNA expression level of *GPR 43* with supplemented 8% SBP was extremely significant (up-regulated by many orders of magnitude) compared to that of the CON treatment in colonic mucosa (linear, $P < 0.001$; quadratic, $P < 0.001$) (Fig. 3A–E).

3.11. Colonic microbiota

The OTU of the colonic content of the piglets showed that there were 1426, 1704, 2160, 1322 and 1288 OTU from the CON, 2% SBP, 4% SBP, 6% SBP and 8% SBP treatments, respectively, totaling 7900 OTU, with 689 common OTU among treatments (Fig. 4A). As shown in Table 11 and Table 12, there was no difference in α-diversity, such as Chao1, Simpson and Shannon indexes, and in the top 10 bacterial abundances at family level among all treatments with different ratios of SDF to IDF ($P > 0.05$). PCA revealed that there was no clear separation of the microbial community among treatments, indicating no shift in gut microbial communities induced by different ratio of SDF to IDF formulated by SBP supplementation (Fig. 4B).

The relative abundances of the top 20 bacteria at the phylum level (Fig. 4C) and the top 30 bacteria at the genus level (Fig. 4D) were extracted and analyzed. The dominant two phyla of the colon in five treatments were Firmicutes and Bacteroidetes. At the genus level, *Prevotella_9*, *Megasphaera*, *Lactobacillus* and *Clostridium_sensu_stricto_1* were predominant in the colonic content of the piglets. The LEfSe analysis was used to identify the significantly different bacteria from family to species levels between treatments (Fig. 4E). At the family level, Alteromonadaceae was greatly enriched in the 8% SBP treatment. The genera *Escherichia_shigella*, *Eubacterium_ruminantium_group* and *Subdoligranulum* were enriched in the CON treatment, an increased richness of *Paracoccus* in the 4% SBP treatment, *Lawsonia* in the 6% SBP treatment as well as *Marinobacter* in the 8% SBP treatment were observed in the colon of piglets. At the species level, *Marinobacter*_unclassified was enriched in the CON treatment, *Paracoccus_homiensis* and *Akkermansia_muciniphila* were enriched in the 4% SBP treatment, *Lawsonia_intracellularis* was enriched in the 6% SBP treatment and *Marinobacter*_unclassified was enriched in the 8% SBP treatment.

3.12. Correlation analysis between gut microbiota and SCFA and metabolites of piglets

The Spearman correlation showed that genera including *Veillonellaceae_unclassified*, *Prevotella_7*, *Olsenella*, *Megasphaera*,

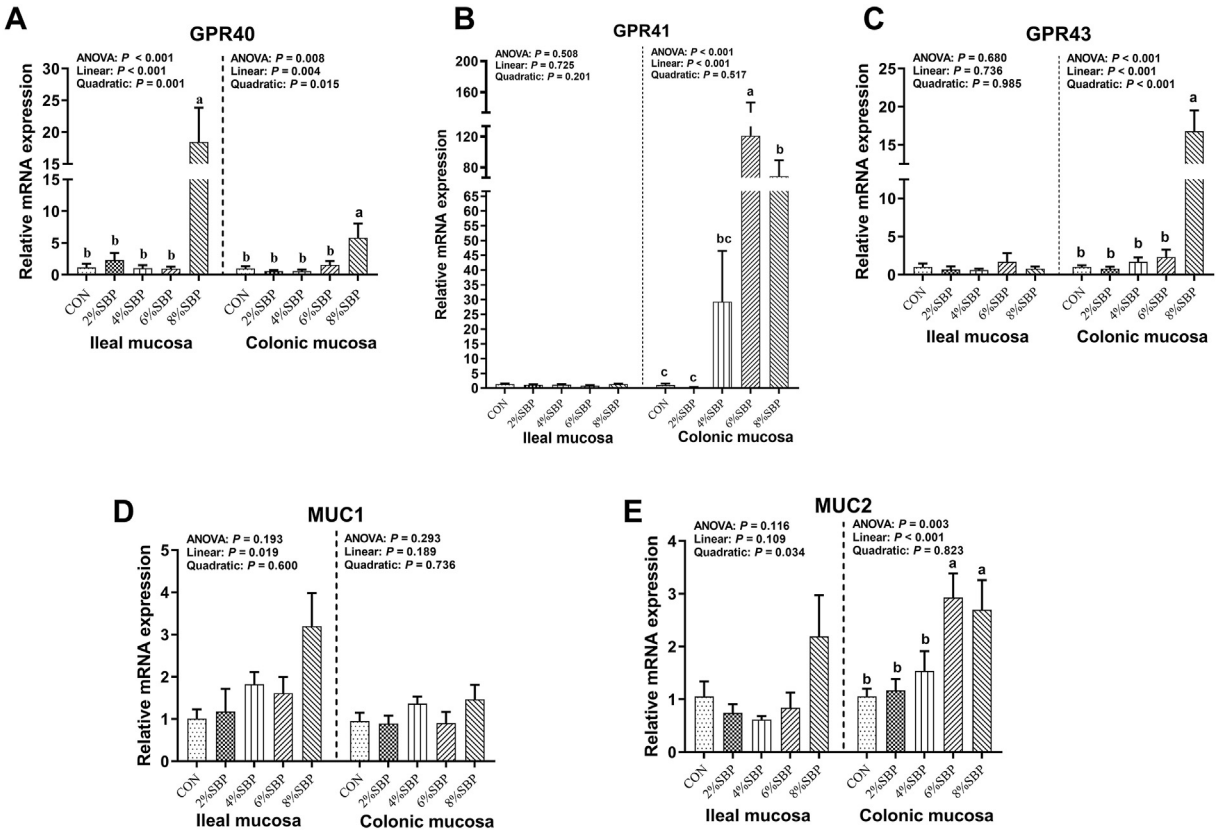

Fig. 3. Effects of the ratio of SDF to IDF on the gene mRNA expression involved in G protein-coupled receptors and intestinal barrier function of ileal mucosa and colonic mucosa in piglets. (A) G protein-coupled receptors 40 (*GPR40*). (B) G protein-coupled receptors 41 (*GPR41*). (C) G protein-coupled receptors 43 (*GPR43*). (D) Mucin-1 (*MUC1*). (E) Mucin-2 (*MUC2*). CON, 2% SBP, 4% SBP, 6% SBP and 8% SBP are diets in which the ratios of SDF to IDF are 10.16%, 13.53%, 16.79%, 19.86%, and 24.81%, respectively. $^{a-c}$ Values with different lowercase letters are significantly different ($P < 0.05$). Data are presented as mean and SEM ($n = 6$). SDF = soluble dietary fiber; IDF = insoluble dietary fiber; SBP = sugar beet pulp.

Mitsuokella, *Lactobacillus*, *Dialister*, and *Streptococcus* demonstrated a positive correlation with the concentration of valerate in the colon ($P < 0.05$). Moreover, concentrations of indole and skatole were positively related to *Prevotella*, Prevotellaceae_*NK3B31_group*, and *Phascolarctobacterium* ($P < 0.05$) (Fig. 5).

4. Discussion

This study investigated the effects of different ratios of soluble to insoluble dietary fiber on growth performance and intestinal health of piglets. Many previous studies have shown that high fiber levels in piglet diets impair digestion and absorption, thus affecting growth performance (Schedle et al., 2008; Shang et al., 2020). However, more and more studies are showing the beneficial effects of appropriate fiber levels in piglets (Badaras et al., 2022; Shang et al., 2021a; Zhao et al., 2018). Shang et al. (2021b) reported that the ratio of SDF to IDF (5% SBP and 7.5% wheat bran) was approximately 20.80% during lactation, which may be optimal SDF:IDF for improving piglet growth performance. An inclusion of high amount of SBP would affect the ratio of SDF:IDF, affecting utilization of dietary nutrients by piglets and growth performance. Wang et al. (2016) reported that the ATTD of CP and GE reduced linearly when SBP inclusion increased from 12% up to 24% in piglet diets, resulting in a linear decrease in ADFI and ADG. In this study, the growth performance of piglets was not affected when the SDF:IDF ratio ranged from 16.79% to 19.86%, while 24.81% SDF:IDF increased F/G linearly and decreased the ATTD of CP linearly, which was similar to results of Wang et al. (2016). Our recent study showed that SBP exhibited rapid fermentation rates producing acetate, which can provide energy for the intestinal tract and promote the proliferation of beneficial microbiota, thus reducing the diarrhea rate in piglets (Feng et al., 2023). Piglets supplemented with optimal levels of SBP produced feces with a lower dry-matter content, and reduced nutritional diarrhea (Gill et al., 2000), which may largely explain our results.

Serum biochemical parameters and amino acids were monitored to reflect the organism health and dietary metabolism (Wang et al., 2009). Dietary 4% SBP (16.79% SDF:IDF) enhanced serum TG and K concentrations compared with the CON treatment (10.16% SDF:IDF); our results were in consistent with previous studies, in which diets containing 12.5% SBP increased TG concentration in pigs (Weber and Kerr, 2012). Our recent study also revealed that a 3% SBP diet (17.04% SDF:IDF) elevated serum TG and K concentrations greater than the 7.16% SDF:IDF diet (Feng et al., 2023). Triglyceride is one of the most important indexes of lipid metabolism; our results show that different ratios of SDF:IDF affected lipid metabolism and the digestion and absorption of serum minerals in piglets. Reportedly, ALT is a liver-specific enzyme and ALT concentrations increase when the liver is damaged or a related injury occurs; thus, low ALT levels generally indicate better health status (Lu et al., 2019). In the present study, we observed a decrease in serum ALT with the 16.79% SDF:IDF treatment, indicating that dietary 4% SBP (16.79% SDF:IDF) was more suitable for inclusion to ensure normal kidney and liver function.

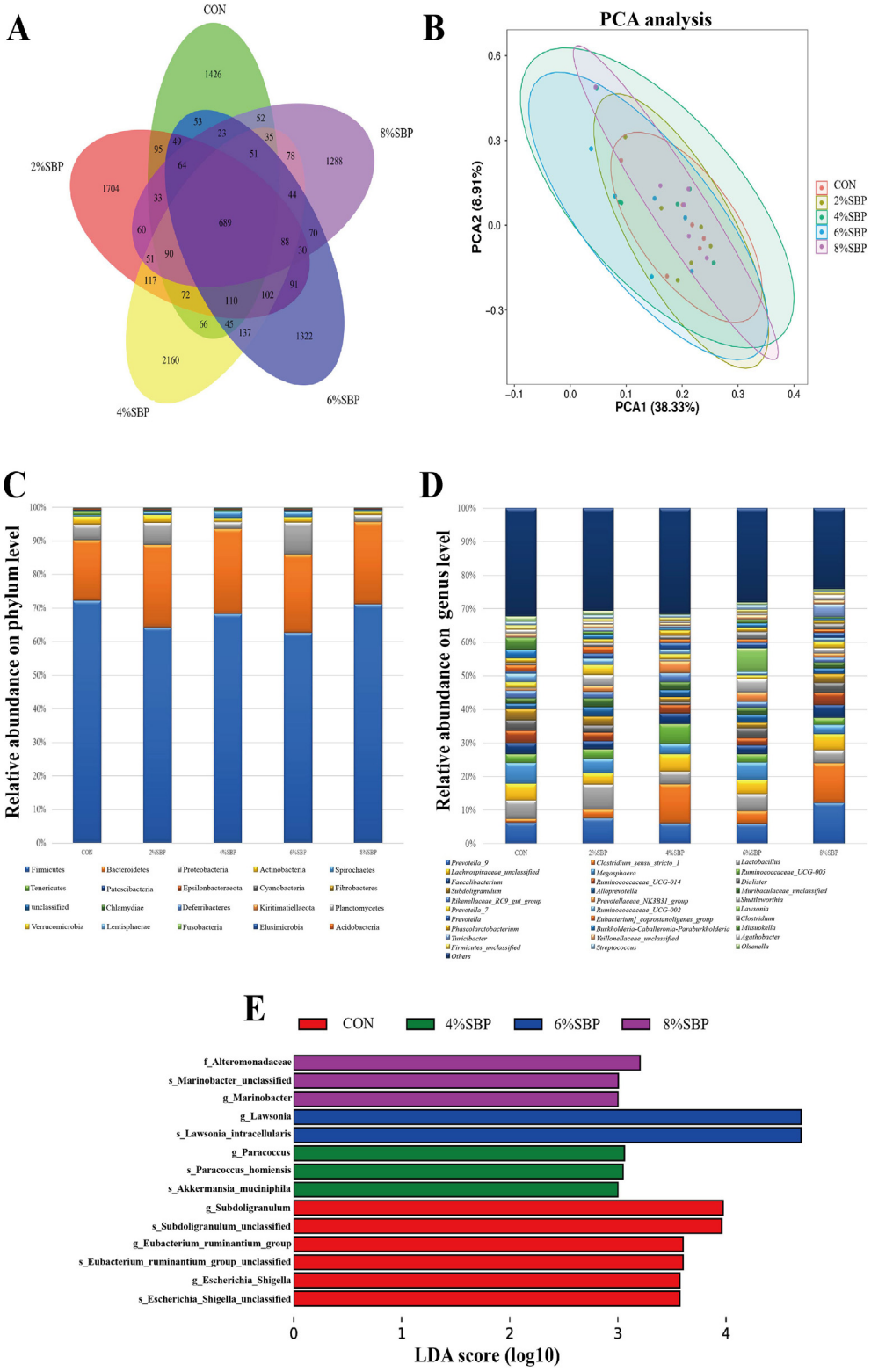

Fig. 4. Effects of the ratio of SDF to IDF on colonic microbial composition and structure. (A) Operational taxonomic units (OTU). (B) Principal component analysis (PCA) based on Bray–Curtis dissimilarity. (C, D) The differential bacteria at phylum level and genus level. (E) linear discriminant analysis effect size (LEfSe) analysis of colonic microbiota. CON, 2% SBP, 4% SBP, 6% SBP and 8% SBP are diets in which the ratios of SDF to IDF are 10.16%, 13.53%, 16.79%, 19.86%, and 24.81%, respectively. SDF = soluble dietary fiber; IDF = insoluble dietary fiber; SBP = sugar beet pulp.

Table 11
The α-diversity analysis results of microbiota in colon of piglets fed with different ratio of SDF to IDF.

Item	Treatments[1]					SEM	*P*-value		
	CON	2%SBP	4%SBP	6%SBP	8%SBP		ANOVA	Linear	Quadratic
Chao1 index	832.00	926.32	1034.33	797.64	746.15	203.597	0.646	0.515	0.249
Simpson index	0.98	0.99	0.95	0.95	0.94	0.044	0.738	0.194	1.000
Shannon index	7.59	7.82	7.61	7.08	6.97	0.836	0.817	0.300	0.658

SDF = soluble dietary fiber; IDF = insoluble dietary fiber; SBP = sugar beet pulp.
Data are presented as mean and SEM ($n = 6$).
[1] CON, 2% SBP, 4% SBP, 6% SBP and 8% SBP are diets in which the ratios of SDF to IDF are 10.16%, 13.53%, 16.79%, 19.86%, and 24.81%, respectively.

Table 12
Comparison of the top 10 most abundant microbiota at family level in colon of piglets fed with different ratio of SDF to IDF (%).

Family level	Treatments[1]					SEM	*P*-value		
	CON	2%SBP	4%SBP	6%SBP	8%SBP		ANOVA	Linear	Quadratic
Ruminococcaceae	23.57	20.37	22.46	15.40	19.10	5.160	0.558	0.239	0.737
Prevotellaceae	13.30	18.91	17.55	17.07	20.79	5.630	0.748	0.307	0.846
Lachnospiraceae	14.05	13.42	12.86	15.56	16.12	4.367	0.934	0.526	0.629
Veillonellaceae	14.44	9.15	5.46	11.79	8.08	4.965	0.443	0.372	0.326
Clostridiaceae_1	1.15	2.63	11.83	3.77	12.15	9.889	0.688	0.305	0.896
Lactobacillaceae	5.48	7.50	3.77	5.05	3.82	2.537	0.585	0.319	0.827
Erysipelotrichaceae	3.71	4.01	2.36	2.86	6.08	2.318	0.559	0.494	0.205
Muribaculaceae	1.69	2.71	2.47	1.99	1.78	0.964	0.790	0.810	0.297
Acidaminococcaceae	3.31	1.80	1.81	2.13	1.37	0.726	0.118	0.038	0.359
Rikenellaceae	2.17	1.98	2.75	1.81	1.50	0.826	0.643	0.423	0.383

SDF = soluble dietary fiber; IDF = insoluble dietary fiber; SBP = sugar beet pulp.
Data are presented as mean and SEM ($n = 6$).
[1] CON, 2% SBP, 4% SBP, 6% SBP and 8% SBP are diets in which the ratios of SDF to IDF are 10.16%, 13.53%, 16.79%, 19.86%, and 24.81%, respectively.

An interesting finding of the present study is that the serum concentrations of Lys and Met were not affected as the SDF:IDF ratio increased, indicating that the supply of these limiting amino acids were adequate, which may partially explain why ADG and ADFI were not affected by the SDF:IDF ratio. Induced serum concentrations of Asp, Thr, Glu, Cit, Cys, Ile, Leu, Orn, Arg and amino acid derivatives including 1-methylhistidine, α-aminoadipic acid, α-aminobutyric acid and cystathionine were observed in the 4% SBP treatment (16.79% SDF:IDF), which are mainly related to piglet immunity and intestinal oxidative metabolism (Rakhshandeh, 2011). Although Asp and Cys are non-essential amino acids, they also have an important effect on homeostasis and metabolism in piglets (Wang et al., 2021). Both Asp, Leu, Glu and taurine play essential roles in regulating the immune response and enhancing the immune function via the proliferation of lymphocytes and synthesis of γ-aminobutyrate (Wu, 2009). Arginine is a potent secretagogue for growth hormone, which may relate to growth performance. These amino acids were found to play a crucial role in maintaining the intestinal barrier and prevent the translocation of microorganisms from the intestinal into the systemic circulation (Li et al., 2007). The same results were also found in our recent study (Feng et al., 2023), possibly because appropriate ratios of SDF:IDF improved the digestion and metabolism of amino acids and improved intestinal immunity.

Fiber-degrading enzymes play crucial roles in the utilization of DF, reflecting fiber digestibility (Shang et al., 2020). Dietary manipulation is an effective strategy to promote the secretion of fiber-degrading enzymes. Sugar beet pulp is rich in pectin, which to some extent increases the expression of β-xylanase gene *xynB*, regulating the secretion of β-xylanase, thereby improving fiber digestibility and regulating gastrointestinal tract morphology, improving pig growth performance (Ivarsson et al., 2014). In this study, dietary SBP supplementation affected cellulase, pectinase and mannanase levels in the digestive tract of the piglets. Dietary 8% SBP supplementation linearly and quadratically increased the levels of fiber-degrading enzymes in piglets. A plausible explanation for these observations is that SBP contributes to the synthesis and secretion of fiber-degrading enzymes in the intestine of piglets, ultimately improving fiber degradation and digestion. Higher fiber-degrading enzymes activity was conducive to nutrient absorption, increasing the ATTD of IDF in piglets.

To determine whether growth performance, diarrhea rate and nutrient digestibility may be associated with changes in the intestinal health of the piglets, we further evaluated the effects of the ratio of SDF to IDF on intestinal immune function to explore the underlying mechanisms. Similar to the changes of serum cytokines concentration observed in our study, 4% and 6% SBP increased serum INF-γ, IL-1β, IL-8, IL-10, and TNF-α concentrations, but 8% SBP reduced the concentrations of the above in the serum, Chen et al. (2017) found that dietary pectic oligosaccharide supplementation enhanced the concentration of IFN-γ in ileal mucosa, thus improving piglet immunity against porcine rotavirus. IFN-γ is a major product of Th1 cells with broad-spectrum antiviral agent and immunomodulatory effects involving macrophages. Previous studies have found that IFN-γ was able to reduce TNF-α secretion through localized macrophage populations (Schroder et al., 2004). The consistent trend in IFN-γ and TNF-α in this study may be due to the fact that IFN-γ could increase cellular sensitivity to the proapoptotic effect of TNF-α by promoting the expression of TNF-α receptor on the surface of tumor cells (Barroso-Arévalo et al., 2021). The reason why 4% and 6% SBP increased serum INF-γ, IL-1β, IL-8, IL-10, and TNF-α concentrations, while 8% SBP reduced concentrations for this study was that the immune function of the piglets was still under development. Thus, supplementation with the appropriate amount of DF can stimulate the immunity of the piglets, while high DF can increase the nutrient demand of the pigs and reduce their immune response (Trachsel et al., 2019).

Some markers of mucosal barrier function, in particular mucin-2 (*MUC2*), increased in the colonic mucosa of piglets supplemented 6% SBP. Previous studies have reported that high fiber diet (with

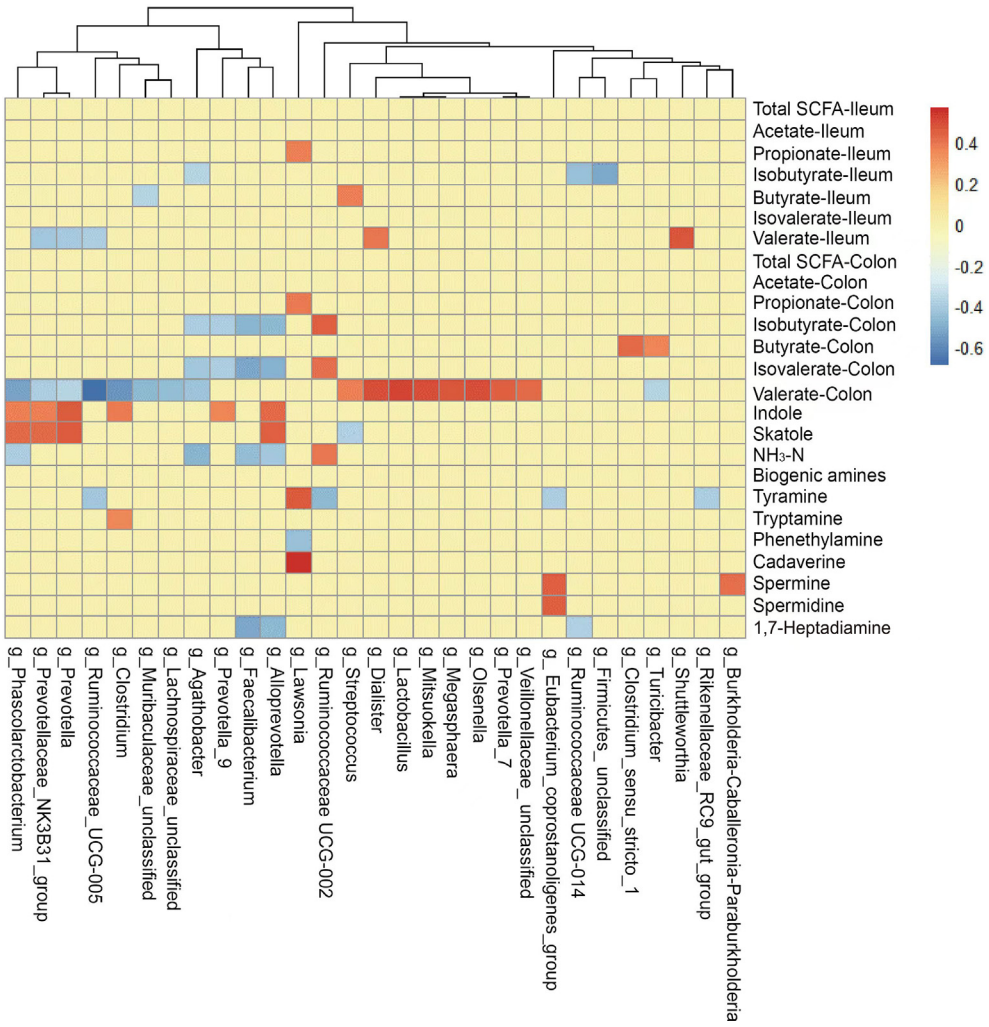

Fig. 5. Correlation between concentration of short-chain fatty acids (SCFA), metabolites and gut microbiota. Red presents a significant positive correlation; blue represents a significantly negative correlation. The intensity of the colors represents the degree of association, with increasing intensity representing higher absolute value for the correlation.

10% SBP) increased SCFA concentration in cecum and colon, increased goblet cell numbers and had a tendency to increase the relative mRNA expression of *MUC2*, promoting gut health (Wellington et al., 2020). This indicates that 19.86% SDF:IDF can stimulate immune response, consistent with the concentrations of inflammatory cytokines in serum.

Short-chain fatty acids, products of microbial fermentation, exhibited a wide range of functions from immunomodulation to metabolism (Wang et al., 2019). There were differences in SCFA concentrations in feces when piglets were fed with corn bran, SBP, wheat bran and soybean hulls, respectively. The concentration of acetate was lower in the SBP group indicating that dietary fiber sources affect the production of SCFA (Zhao et al., 2019b; Zhu et al., 2004). It is well known that gut microbiota degraded dietary fiber to produce SCFA, which bind to intestinal mucosal GPR to regulate host metabolism, thereby affecting nutrient utilization and deposition (Cani and Delzenne, 2007). SCFA-sensing GPRs include GPR41, and GPR43 which were presented in intestinal epithelial cells, adipocytes, and immune cells, GPR40 was found to be a receptor for medium- and long-chain fatty acids and an open reading frame pseudogene of GPR41 (Han et al., 2023). It was found that GPR41 was only coupled to Gi/o and activated in the affinity order of propionate > butyrate > acetate, whereas GPR43 was coupled to Gi/o and Gq- dual-coupled GPR activated in the affinity order butyrate = propionate > acetate. The function of GPR43 is mainly mediated by Gi/o and is only coupled to Gq in the intestine, promoting the secretion of glucagon like peptide-1 in L-cells (Koh et al., 2016). High soluble fiber diets elevated GPR40 levels in animals, which maybe the reason for the highest *GPR40* mRNA expression in 8% SBP treatment in ileal and colonic mucosa of piglets. Sodium butyrate effectively regulates function of T lymphocytes through motivating GPR43 to reduce the level of inflammatory factors like IL-6, IL-8 and TNF-α (Yang and Zhao, 2021), which is accordance with our results. *GPR43* mRNA expression in the colonic mucosa of the 8% SBP treatment was the highest and serum levels of IL-6, IL-8, TNF-α, IFN-γ and IL-1β lower than those of the 6% SBP treatment. GPR41 played a crucial role in increasing Treg cells and protecting immunity (Yang and Zhao, 2021). In the current study, the mRNA expression of *GPR41* and *MUC2* in colonic mucosa was the highest in 6% SBP treatment, the reasons for the this might be a result of 19.86% SDF:IDF promoted SCFA production and also improved the intestinal barrier function in piglets (Diao et al., 2020).

The important reason for the beneficial impacts of DF on intestinal is improved gut microbiota composition and their metabolites. Gut microbiota occupied a crucial role in host health; the interaction and connection between diet, gut microbe and host have been intensively studied (Li et al., 2021d; Wang et al., 2020). Therefore, we further investigated the effects of SBP on the intestinal functions of piglets to explore the underlying mechanisms.

Generally, Firmicutes and Bacteroidetes were the most dominant phyla in pigs (Ley et al., 2008). Our results showed that the dominant two phyla of colon among five treatments were Firmicutes and Bacteroidetes, which was similar to the results obtained by Xu et al. (2019), who reported that pigs fed SBP showed an increased abundance in phyla Firmicutes and increased the Chao1 index in colon of pigs. The increase of Proteobacteria in the 6% SBP treatment in our current study, a possible reason is that SBP is rich in soluble fiber components such as pectin, and excessive levels of pectin can negatively affect intestinal health. In our current study, dietary 4% SBP supplementation increased the number of microbial OTU in colon, which suggested that proper addition of SBP could improve microbial diversity and intestinal health in the piglets. However, further increasing the proportion of SBP did not improve microbial diversity and may even have the opposite result. Another finding of our study was that the abundances of *Clostridium_sensu_stricto_1* in the 4% SBP treatment was higher than the other treatments. *Clostridium_sensu_stricto_1* plays a significant role in amino acid utilization of diets in animals as amino acid-metabolizing bacteria (Neis et al., 2015), which may be an important factor for the highest concentrations of serum Cit, Cys, Ile and Arg in the 4% SBP treatment.

Intestinal microbial nitrogen metabolites that are not digested and absorbed in the foregut enter the hindgut and can be fermented by microbes to produce NH_3–N, biogenic amines, and indoles. NH_3 is a product of protein fermentation in the substrate; our results showed that the concentration of NH_3 decreased as the SDF:IDF ratio increased, which was similar to Bikker et al. (2006), who showed that the addition of DF to the diets was associated with an increase in fiber fermentation, a decrease in protein fermentation, and a decrease in NH_3 production. In the case of biogenic amines, we found that the concentration of cadaverine in colon contents was approximately dozens of times greater than that of the other treatments after 6% SBP supplementation. One reason for such a huge difference could be an increased lysine decarboxylation by gut microbes; another reason may be due to the decreased production of SCFA. The high concentrations of spermine and spermidine in 2% SBP supplementation may be related to decarboxylation of arginine (Yu et al., 2018). Taken together, the optimal SDF to IDF ratio could improve gut microbiota by altering gut microbial composition, as well as their metabolites.

Our research showed that different SDF:IDF formulating by SBP supplementation in diet affected piglet growth performance and intestinal health. However, there were some limitations in our study. Firstly, we cannot guarantee that the same SDF:IDF with other fiber sources will come to the same conclusion. Secondly, whether there are more suitable fiber sources than SBP in piglet diets to adjust the SDF:IDF ratio from effectiveness needs further evaluation. Further studies are needed to clearly determine the effect and mechanisms of dietary fiber on the growth performance and the intestinal health of piglets.

5. Conclusion

In summary, our results demonstrated that the ratios of SDF to IDF ranging from 13.53% to 24.81% improved the ATTD of IDF in piglets. It was beneficial for enhancing intestinal barrier function, increasing SCFA production, and improving intestinal microbiota composition when SDF:IDF was up to 19.86%. The ratio of SDF to IDF ranging from 16.79% to 19.86% is recommended for promoting intestinal health in the piglet diet with SBP supplementation.

Author contributions

Luya Feng: Conceptualization, Software, Data Curation, Writing-original draft preparation, Formal analysis and Visualization. **Luya Feng, Zhenfu Luo, Jing Wang, Kunfu Wu, Wenliang Wang, Zhimou Liu, Juping Wen, Zhenbin Wang:** Methodology, Investigation. **Bi'e Tan, Xiaokang Ma:** Supervision; Validation. **Bi'e Tan:** Funding acquisition. **Bi'e Tan, Xiaokang Ma, Jing Wang, Gregory J. Duns:** Writing-review & 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.

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