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Original Research Article

Differential responses of weaned piglets to supplemental porcine or chicken plasma in diets without inclusion of antibiotics and zinc oxide

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ABSTRACT

This study was conducted to investigate the effects of spray-dried porcine plasma protein (SDPP) or spray-dried chicken plasma protein (SDCP) supplementation in diets without the inclusion of antibiotics and zinc oxide (ZnO) on growth performance, fecal score, and fecal microbiota in early-weaned piglets. A total of 192 healthy weaning piglets (Duroc × Landrace × Yorkshire, 21 d old) were blocked by BW (6.53 ± 0.60 kg) and randomly assigned to 4 dietary treatments: negative control (NC, basal diet), positive control (PC), basal diet + ZnO at 2 g/kg and antibiotics at 0.8 g/kg), SDPP (containing 5% SDPP), and SDCP (containing 5% SDCP). The experiment lasted 14 d. The SDPP group had higher ($P < 0.05$) final BW, average daily gain and average daily feed intake than the NC and SDCP groups. The percentage of piglets with fecal scores at 2 or ≥2 was higher ($P < 0.05$) in the NC and SDCP groups than in the PC group. A decreased ($P < 0.05$) bacterial alpha diversity and Bacteroidetes abundance, but increased ($P < 0.05$) Firmicutes abundance were observed in the PC and SDPP groups when compared to the NC group. The relative abundance of *Lactobacillus* was higher ($P < 0.05$) in the SDPP than in the SDCP group, and that of *Streptococcus* was higher ($P < 0.01$) in the PC and SDPP groups than in the NC group. The PC group also had higher ($P < 0.01$) *Faecalibacterium* abundance than the NC and SDCP groups. Additionally, the SDCP group had higher ($P < 0.05$) serum urea nitrogen than those fed other diets, and lower ($P < 0.10$) short-chain fatty acids to branched-chain fatty acids ratio than the PC and SDPP groups. Overall, SDPP was a promising animal protein for piglets in increasing feed intake, modifying gut microbiota profile, reducing gut protein fermentation and alleviating diarrhea frequency, thus promoting growth performance, under the conditions with limited in-feed utilization of antibiotics and ZnO.

1. Introduction

Weaning is an essential process for piglets from sucking to eating dry feed, during which piglets normally suffer from dramatic changes in diets and environment (Montagne et al., 2007). Because of these changes, newly weaned piglets will firstly go through a starvation period with too low feed intake (Le Dividich and Sève, 2000) and then a compensation period when they start to

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consume much more feed (Torrallardona, 2010), which further results in the alteration of gut integrity, gut function and gut microbial community (Gresse et al., 2017; Campbell et al., 2013). Hence, during the weaning transition period, piglets have increased diarrhea incidence but decreased growth performance, which will ultimately cause animal welfare issues and significant economic losses (Campbell et al., 2013; Madec and Josse, 1983).

In the livestock industry, antibiotic growth promoters (AGP) have been used as an effective tool for decreasing diarrhea and improving growth in weaning piglets for decades (Nofrarías et al., 2006). Meanwhile, a therapeutic dosage of zinc oxide (ZnO), from 2,000 to 4,000 mg/kg in piglets' complete feed, can decline the colonization and population of pathogens in the intestine, and result in decreased post-weaning diarrhea and increased growth performance (Shelton et al., 2011; Cho et al., 2015). Hence, AGP and pharmacological levels of ZnO are widely adopted in weaning piglets' diets to reduce post weaning diarrhea and promote growth performance. However, antimicrobial resistance is increasingly becoming a global threat for human health, which prompts legislation to ban the use of AGP in feeds. Notably, EU countries have already banned the use of high dosage ZnO because of the potential environmental consequences (European Communities, 2003), and other countries also plan to restrict the use of high dosage ZnO in nursery piglets' diets. Therefore, it is of great importance to find an environmentally friendly strategy to solve the post-weaning problems in piglets.

Animal spray-dried plasma protein (SDP), such as spray-dried porcine plasma protein (SDPP) and spray-dried chicken plasma protein (SDCP) are complex mixtures, containing fibrinogen, immunoglobulin and albumin (Jiang et al., 2000). Having abundant IgG and good palatability and digestibility (Pierce et al., 2005; Ermer et al., 1994; Dijk et al., 2001; Zhang et al., 2015), SDP has been used in piglets' diet and shown to be capable of promoting intestinal development, resisting the challenge of bacterial pathogens, changing the volatile fatty acids (VFA) profile, reducing diarrhea incidence and improving piglets' growth around the weaning transition period (Zhang et al., 2015; Gao et al., 2011; Tran et al., 2018; Che et al., 2020; Peace et al., 2011). However, it is still obscure whether dietary SDPP or SDCP has comparable effects to ZnO plus antibiotics in post-weaning piglets, especially when growing attention has been paid to improve the environmental conditions. In the present study, we hypothesized that dietary supplementation with SDP has similar effects with AGP plus high dosage of ZnO in maintaining microbial community, producing VFA, alleviating diarrhea and promoting growth performance of early-weaned piglets.

2. Materials and methods

The experimental protocol involved in the present study was approved by the Animal Care and Use Committee of Sichuan Agricultural University (Ya'an, China).

The SDPP and SDCP were provided by Sonac (China) Biology Co., Ltd., and the nutrient compositions of SDCP and SDPP used in this study are presented in Table 1.

2.1. Experimental animals and design

A total of 192 healthy piglets (half female and half castrated males) with similar genetic background (Duroc × Landrace × Yorkshire) were blocked by initial BW (6.53 ± 0.60 kg, 21 d) and randomly assigned to 4 treatments with 12 replicate pens in each treatment and 4 piglets (half female) in each pen (1.5 m × 1.2 m). The 4 dietary treatments were: 1) negative control (NC, basal diet); 2) positive control (PC), NC supplemented with ZnO at 2 g/kg (Xingjia Biological, Changsha city, China), 4% Flavomycin premix at 0.3 g/kg, and 15% chlortetracycline premix at 0.5 g/kg; 3) SDPP (containing 5% SDPP); 4) SDCP (containing 5% SDCP). The experiment lasted 14 d.

Table 1
Characterization of plasma protein powder (%, as-fed basis).

Item	SDPP	SDCP
IgG[1]	15.40	11.10
Gel strength[1], g/cm	310.00	87.00
Solubility[1]	78.60	71.30
Relative pepsine digestibility[1]	100.00	100.00
Dry matter[1]	91.20	91.40
Gross energy[2], Mcal/kg	4.84	4.85
Protein[1]	79.70	71.80
Fat[2] (acid hydrolysis)	2.40	5.30
Ash[1]	7.70	11.50
Calcium[2]	0.24	0.22
Phosphorus[2]	0.17	0.62
Amino acids[2]		
Asp	7.19	6.40
Thr	4.54	3.74
Ser	4.60	4.19
Glu	10.90	9.71
Gly	2.79	2.61
Ala	3.84	3.35
Cys	2.14	1.91
Val	4.51	3.77
Met	1.08	1.70
Ile	2.59	2.55
Leu	6.97	5.57
Tyr	3.82	3.14
Phe	4.08	3.27
Lys	6.18	4.63
His	2.32	1.86
Arg	4.53	4.45
Pro	4.55	3.48

SDPP = spray-dried porcine plasma protein; SDCP = spray-dried chicken plasma protein; IgG = immunoglobulin G.
[1] The determination of IgG, gel strength, solubility, relative pepsine digestibility, dry matter, protein, fat and ash were done in Darling Ingredients central laboratory (Son, The Netherlands).
[2] The determination of gross energy, Ca, P, amino acids were done in Animal Nutrition Institute (Sichuan Agricultural University, Chengdu, China).

2.2. Experimental diets

The experimental diets were formulated according to swine nutrition requirements as recommended by National Research Council (2012) (NRC, 2012). All the diets were iso-proteinic, isoenergetic and balanced for amino acid requirements. The ingredients and composition of the experimental diets are shown in Table 2. The spray-dried plasma in SDPP and SDCP groups replaced fish meal in the basal diet of the NC and PC groups. The diets were pelleted with the temperature no more than 65 °C in a commercial feed mill.

2.3. Housing and management

The experiment was conducted at a research farm (Pujiang county, Chengdu city, China). During the experiment, piglets were housed in the same room with 24-h light, and initial temperature controlled at 30 °C for the first 2 d then gradually being decreased to 28 °C and maintained. The relative humidity was controlled from 60% to 80%. Each pen was equipped with a stainless-steel trough and nipple drinker, and the piglets had ad libitum access to feed and water throughout the experimental period. All the piglets were fed 6 times daily during the 1st week, and 4 times daily during the 2nd week.

Table 2
Ingredients and nutrient composition of the experimental diets (air-dry basis).

Item	NC	PC	SDPP	SDCP
Ingredients, g/kg				
Corn (8.2% crude protein)	449.04	446.86	444.15	433.61
Whey powder (80% lactose)	202.60	200.00	200.00	200.00
Heat-treated soybean	50.00	50.00	50.00	50.00
Soybean meal, dehulled (46% crude protein)	211.00	211.50	222.20	230.50
Fish meal (63.28% crude protein)	63.40	63.40	–	–
Porcine plasma protein powder [1]	–	–	50.00	–
Chicken plasma protein powder [1]	–	–	–	50.00
Soybean oil	–	–	4.70	5.60
L-Lys·HCl (78.8%)	4.19	4.18	2.69	4.04
DL-Met	1.86	1.86	1.44	1.13
L-Thr	1.28	1.29	0.28	0.40
L-Trp	0.42	0.42	–	–
L-Val	1.08	1.08	0.1	0.48
Dicalcium phosphate	1.98	2.09	8.75	8.33
Limestone	5.89	5.88	8.44	8.67
Sodium chloride	3.50	3.50	3.50	3.50
Premix [2]	3.75	3.75	3.75	3.75
ZnO [3]	–	2.00	–	–
AGP [4]	–	0.80	–	–
Total	1,000	1,000	1,000	1,000
Calculated composition, %				
NE, Mcal/kg	2.499	2.499	2.499	2.499
Crude protein	20.60	20.60	20.60	20.60
SID AA				
Lys	1.35	1.35	1.35	1.35
Met	0.49	0.49	0.44	0.41
Met + Cys	0.74	0.74	0.74	0.74
Thr	0.79	0.79	0.79	0.79
Trp	0.25	0.25	0.25	0.25
Val	0.86	0.86	0.86	0.86
Ca	0.80	0.80	0.80	0.80
STTD-P	0.43	0.43	0.43	0.43

NC = negative control; PC = positive control; SDPP = spray-dried porcine plasma protein; SDCP = spray-dried chicken plasma protein; AGP = antibiotic growth promoters; SID = standardized ileal digestibility; STTD-P, standard total-tract digestibility of phosphorus.
[1] The porcine plasma protein powder and chicken plasma protein powder were provided by Sonac (China) Biology Co., Ltd.
[2] The premix provided the following per kilogram of the diet: copper, 6 mg; iron, 100 mg; manganese, 4 mg; zinc, 100 mg; iodine, 0.14 mg; selenium, 0.30 mg; vitamin A, 11,375 IU; vitamin D_3, 3,500 IU; vitamin E, 28 mg; vitamin K, 3.5 mg; vitamin B_1, 3.5 mg; vitamin B_2, 8.75 mg; niacin, 35 mg; pantothenic, 17.5 mg; vitamin B_6, 4.2 mg; vitamin B_{12}, 42 μg; biotin, 175 μg; folic acid, 1.75 mg. Meanwhile, it provided the following per tonne of the diet: choline chloride (50%), 1,000 g; oregano oil (Ropadiar, Ropapharm, Netherlands), 200 g; ethoxyquin (with purity 33%, Zhongdan, China), 300 g; anti-mould additive (Ruinom Antimold, Chongqing Huaruilong, China), 500 g; flavour (FMA, Dadhank, China), 500 g.
[3] Zinc oxide (purity 99.7%) by Xinjia Biological (Changsha, China).
[4] Provided the following per kilogram of diet: 300 mg of 4% Flavomycin premix and 500 mg of 15% chlortetracycline premix.

2.4. Growth performance

All the piglets were weighed individually at the beginning (d 0) and end (d 14) of the experiment after removing the feed for 12 h, and the feed consumption per pen was recorded daily throughout the experiment. On the basis of the data above, average BW, average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F) were calculated.

2.5. Fecal scoring

The fecal scoring was visually observed each morning and afternoon by an observer blinded to the treatments according to Sun et al. (2008) and modified with industry practice. Fresh excreta were ranked based on the following scale: 0 = solid; 1 = semi-solid; 2 = semi-liquid; 3 = liquid. Piglets were defined as having diarrhea when feces score was 3. Then, the percentage of piglets receiving different scores was calculated as follow:

$$\text{Percentage of piglets} = \frac{\text{No. of piglets scored at different scores}}{\text{No. of observed piglets}} \times 100\%$$

2.6. Sample collection

In the afternoon of the 14th day, fecal samples were collected by frequent grab sampling of the male piglets in each pen (n = 12, 12 piglets/treatment). The fecal samples were immediately put in 2-mL sterile tubes and stored at −80 °C until analysis.

Blood samples (n = 8, 8 piglets/treatment) were collected from the vena cava anterior on the morning of the 15th day after overnight fasting and immediately transferred into vacuum tubes. Serum was isolated by centrifugation at 3,500 × g for 15 min, and stored at −20 °C until further analysis.

2.7. Sample analysis

2.7.1. Serum urea nitrogen

Serum urea nitrogen (SUN) was measured using a specific commercial assay kit (C013-1; Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

2.7.2. Fecal microbial diversity

The fecal samples were sent to Novogene Bioinformatics Technology Co., Ltd for microbial diversity analysis by 16S rDNA gene

sequencing. Total genome DNA from fecal samples was extracted using QIAamp DNA Stool Mini Kits (Qiagen Inc., Hilden, Germany) according to the manufacturer's instructions. The concentration and purity of DNA was monitored on 1% (wt/vol) agarose gels electrophoresis. Then, the DNA was diluted to 1 ng/μL using sterile water. PCR amplifications were conducted to amplify the variable regions V3 − V4 of bacterial 16S rRNA using primer 515F (5′-GTGCCAGCMGCCGCGGTAA - 3′) and 806R (5 ′ - GGACTACHVGGGTWTCTAAT-3′). The PCR reaction was set up in a 30-μL reaction volume which included 2-μL sterile water, 10-μL DNA template, 3-μL of each primer and 15-μL 2 × Phusion Master Mix (New England Biolabs, United States). The PCR cycle conditions used were as follows: initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, elongation at 72 °C for 30 s and finally at 72 °C for 5 min. PCR products were detected by electrophoresis on 2% agarose gel containing SYBR green. All amplicons with main strip between 400 and 450 bp were purified with Qiagen Gel Extraction Kit (Qiagen Inc., Hilden, Germany). A sequencing library was generated using TruSeq DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, USA) following the manufacturer's recommendations and index codes were added. Qubit 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system were used to check the quality of the sequencing library. Finally, pyrosequencing for 16S rDNA was operated on an Illumina HiSeq2500 platform and 250 bp paired-end reads were generated. Raw reads were selected and assembled using FLASH (V1.2.7) and QIIME (V1.7.0). UCHIME algorithm (UCHIME Algorithm) was used to obtain the effective tags. Sequence analyses were performed using Uparse software (Uparse v7.0.1001). The same operational taxonomic units (OTU) were with at least 97% similarity. The representative sequence for each OTU was screened, and the GreenGene Database was used based on RDP classifier (Version 2.2) algorithm to annotate taxonomic information. The OTU abundance information was normalized using a standard of sequence number corresponding to the sample with the least sequences. Then QIIME (Version 1.7.0) software was used for the analysis of microbial alpha diversity and beta diversity. Furthermore, Tax4Fun was used for microbial community functional profile predictions.

2.7.3. Fecal volatile fatty acids concentration

After determination of fecal microbial diversity, the remaining feces were used to measure the concentration of VFA using gas chromatography in the lab of Animal Nutrition Institute, Sichuan Agricultural University. About 0.7 g of each sample was weighed and diluted with 1.5 mL distilled water in a centrifuge tube. All samples were stood for 30 min and then centrifuged at 20,000 × *g* for 15 min. One milliliter supernatant was transferred to a new tube and mixed with 0.2 mL of 25% metaphosphoric acid and 23.3 μL of 210 mmol/L crotonic acid. Being mixed homogeneously and incubated at 4 °C for 30 min, the tube was centrifuged at 20,000 × *g* for 10 min, and 0.3-mL supernatant was transferred to another tube. Next, 0.9-mL methanol was added, mixed, and then centrifuged at 10,000 × *g* for 5 min. Supernatant was filtrated through 0.22-μm membrane and collected into 1.5-mL tubes. The VFA (acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate) were quantified using a gas chromatographic system (VARIAN CP- 3800, America).

3. Statistical analysis

All the growth performance, fecal microbial diversity, relative abundance at phylum and genus level in feces, and fecal VFA data were analyzed using the PROC MIXED procedure of SAS (SAS 9.4, Inst. Inc., Cary, NC) with a randomized complete block design. Block was a random effect. The PROC GLIMMIX with gamma transformation was used to analyze the data when the residuals did not fit the normal distribution. Statistical differences among treatments were determined by Tukey's multiple-range test. The chi-squared test was used to statistically analyze the percentage of piglets with different fecal scores. Results were expressed as least-squares means with pooled standard error. $P < 0.05$ was declared to be statistically significant. For predicted function of fecal microbial community, bootstrap Mann–Whitney U test was used to test differences in gene distribution, with cutoffs of $P < 0.01$, false discovery rate <0.1, mean counts >10.

4. Results

4.1. Growth performance and fecal score

As shown in Table 3, dietary treatment had a significant ($P < 0.05$) effect on the final BW, ADG and ADFI during this experiment. Piglets fed SDPP diet had higher ($P = 0.002$) ADFI than those fed the NC and SDCP diet. As a result, the ADG and BW at d 14 was significantly higher ($P = 0.006$, $P = 0.016$) in the SDPP than in the NC and SDCP groups. In addition, the feed conversion ratio tended ($P = 0.101$) to be affected by dietary treatment, with the PC and SDPP piglets showing numerically higher G:F than the NC and SDCP piglets. Fecal scoring results (Table 4) showed that the PC diet decreased the percentage of piglets scored at 2, and ≥2 from d 0 to 7 ($P = 0.006$, $P = 0.007$), d 8 to 14 ($P = 0.015$, $P = 0.052$), and the overall period ($P = 0.048$, $P = 0.038$) when compared with the NC diet. Furthermore, the percentage of piglets scored at 2, and ≥2 from d 0 to 7 ($P = 0.006$, $P = 0.007$), and that scored ≥2 from d 0 to 14 ($P = 0.038$) was lower in the PC than in the SDCP group. Meanwhile, the SDPP group showed a lower ($P = 0.006$, $P = 0.007$) percentage of piglets scored at 2, and ≥2 from d 0 to 7 than the SDCP group, but no difference ($P > 0.05$) was observed between the PC and SDPP groups in each stage.

4.2. Microbial diversity in feces

The rarefaction curves shown in Fig. 1 indicated that the depth of sampling was adequate to assess the microbial communities. As shown in Table 5, at the 97% similarity level, the OTU numbers and Chao 1 index were decreased ($P = 0.011$, $P = 0.029$) in the PC and SDPP groups when compared with those in the NC group. Meanwhile, the SDPP group had a lower ($P = 0.021$) abundance-based coverage estimator (ACE) value than the NC group and lower ($P = 0.011$) Shannon index than the SDCP group.

The Venn diagram described the common and unique OTU among the 4 groups (Fig. 2). The NC, PC, SDPP and SDCP groups,

Table 3
Effects of dietary supplementation with SDPP and SDCP on growth performance in weaning piglets.

Item	Treatments				Pooled SEM	*P*-value
	NC	PC	SDPP	SDCP		
Body weight, kg						
Initial (d 0)	6.53	6.53	6.54	6.53	0.440	1.000
Final (d 14)	8.34^{b}	8.63^{ab}	8.83^{a}	8.36^{b}	0.627	0.016
Day 0 to 14						
No. of pigs	48	48	48	48		
ADG, g	129^{b}	150^{ab}	164^{a}	131^{b}	14.4	0.006
ADFI, g	208^{b}	225^{ab}	238^{a}	212^{b}	15.4	0.002
G:F	0.62	0.66	0.68	0.61	0.025	0.101

NC = negative control; PC = positive control; SDPP = spray-dried porcine plasma protein; SDCP = spray-dried chicken plasma protein.
[a,b] Means in a row with different letter show significant differences ($P < 0.05$).

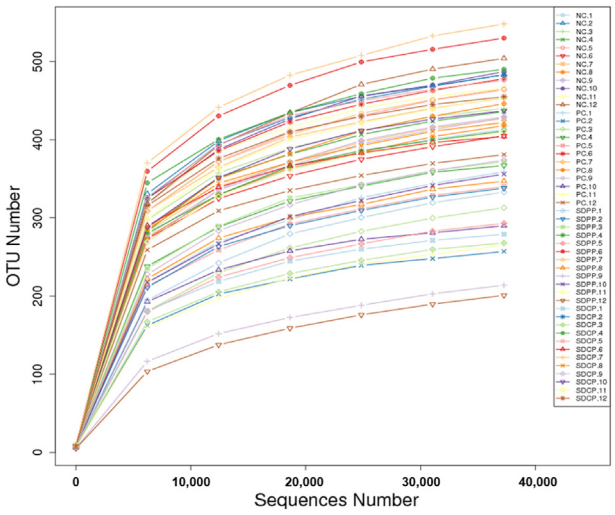

Fig. 1. Rarefaction curves. OUT = operational taxonomic unit; NC = negative control; PC = positive control; SDPP = spray-dried porcine plasma protein; SDCP = spray-dried chicken plasma protein.

Table 4
Effects of dietary supplementation with SDPP and SDCP on fecal scoring distribution (% of total number) in weaning piglets.

Item	Treatments				*P*-value
	NC	PC	SDPP	SDCP	
Day 0 to 7					
Score = 2	21.13^{ab}	15.48^{c}	19.05^{bc}	26.19^{a}	0.006
Score = 3	1.79	1.19	0.30	0.89	0.289
Score ≥2	22.92^{ab}	16.67^{c}	19.35^{bc}	27.08^{a}	0.007
Day 8 to 14					
Score = 2	65.18^{a}	55.95^{b}	58.93^{ab}	53.57^{b}	0.015
Score = 3	1.49	1.79	3.27	4.17	0.104
Score ≥2	66.67	57.74	62.20	57.74	0.052
Day 0 to 14					
Score = 2	43.15^{a}	35.71^{b}	38.99^{ab}	39.88^{ab}	0.048
Score = 3	1.64	1.49	1.79	2.53	0.500
Score ≥2	44.79^{a}	37.20^{b}	40.77^{ab}	42.41^{a}	0.038

NC = negative control; PC = positive control; SDPP = spray-dried porcine plasma protein; SDCP = spray-dried chicken plasma protein.
^{a,b} Means in a row with different letter show significant differences ($P < 0.05$).

Table 5
Effects of dietary supplementation with SDPP and SDCP on the average richness and diversity of feces bacteria community at the 3% dissimilarity level.

Item	Treatments				Pooled SEM	*P*-value
	NC	PC	SDPP	SDCP		
OTU numbers	485^{a}	398^{b}	393^{b}	456^{ab}	26.8	0.011
ACE	491^{a}	412^{ab}	406^{b}	462^{ab}	25.6	0.021
Chao1	489^{a}	410^{b}	406^{b}	464^{ab}	26.5	0.029
Shannon	5.75^{ab}	5.20^{ab}	5.07^{b}	5.97^{a}	0.246	0.011

NC = negative control; PC = positive control; SDPP = spray-dried porcine plasma protein; SDCP = spray-dried chicken plasma protein; OUT = operational taxonomic unites; ACE = abundance-based coverage estimator.
^{a,b} Means in a row with different letter show significant differences ($P < 0.05$).

respectively, had 24, 15, 19 and 40 unique OTU. Furthermore, the PCoA plot among groups (Fig. 2) demonstrated that fecal bacterial communities of the PC and SDPP groups had similar distribution, which was distinct from the NC and SDCP groups.

4.3. Microbial composition in feces

At the phylum level, the relative abundances of the top 4 phyla were significantly affected ($P < 0.05$) by dietary treatments (Table 6). Compared with the NC group, the PC group had increased ($P = 0.030$) relative abundance of Firmicutes but decreased ($P = 0.005$, $P = 0.004$) relative abundances of Bacteroidetes and Tenericutes. In comparison to the NC group, both SDPP and SDCP groups had higher ($P = 0.030$) relative abundances of Firmicutes but similar Tenericutes abundance. No difference was observed between the NC and SDCP groups with regard to the relative abundances of Bacteroidetes and Spirochaetes. However, Bacteroidetes and Spirochaetes appeared to be more abundant ($P = 0.005$, $P = 0.001$) in NC than in SDPP pigs. Notably, the relative abundances of the top 4 phyla were not significantly different between the PC and SDPP groups. In contrast, increased ($P = 0.004$) relative abundance of Tenericutes was observed in SDCP pigs when compared with that in the PC group.

As shown in Table 7, three of the top ten genera presented appeared to be influenced by dietary treatments. The relative abundance of *Lactobacillus* was significantly higher ($P = 0.017$) in the SDPP than in the SDCP group. Meanwhile, the relative abundance of *Streptococcus* increased ($P = 0.003$) in the PC and SDPP groups when compared with the NC group. It also appeared that an increased abundance of *Faecalibacterium* ($P = 0.004$) existed in the PC group when compared with the NC and SDCP group.

4.4. Functional profiling of the bacterial communities

As shown in Fig. 3, there was distinct predicted microbial function among the 4 groups. Results suggested that bacteria in the NC group probably functioned in bacterial motility and protein repair. The microbial community in the PC group had a wide function, which mainly related to the function of cell process. Moreover, microbial function in the SDPP group seemed to be associated with quorum sensing, and transporter effects. Furthermore, amino acid metabolism related function was predicted to be striking in microbial communities of SDCP pigs.

4.5. Concentration of urea nitrogen in serum and VFA in feces

As shown in Table 8, piglets in the SDCP group had higher ($P < 0.001$) SUN than those in the other groups. Consequently, despite dietary treatment failing to affect ($P > 0.05$) the concentration of different kinds of volatile fatty acids in feces, the fecal isovalerate concentration tended to be higher ($P = 0.104$) in the SDCP group than in the PC group. Moreover, the ratio of short chain fatty acids (SCFA) to branch chain fatty acids (BCFA) ratio tended to be higher ($P = 0.058$) in both the PC and SDPP groups than that in the NC and SDCP groups.

5. Discussion

Animal SDP, produced from blood collected in abattoirs, contains fibrinogen, immunoglobulin and albumin (Jiang et al., 2000). Spray-dried porcine plasma protein, one of the major SDP used in animal feed, was reported to have a good palatability, and can improve the feed ingestion in weaning piglets to shorten the transition period from weaning to initial eating (Torrallardona, 2010). Our results showed that piglets fed the SDPP diet had higher feed intake than those fed the NC or SDCP diet, but no distinct difference was observed between SDPP and PC (ZnO plus AGP) piglets. As a result, the increased feed intake in the SDPP group led to a greater final BW and ADG than those in the NC and SDCP groups. Meanwhile, the beneficial effects of SDP have been shown to be associated with immunoglobulin concentration (Gatnau, 1990) which is distinct among plasma protein sources (Zhang et al., 2015). The content of IgG in SDPP used in this trial was 1.38 times (15.4% vs. 11.1%) higher than that

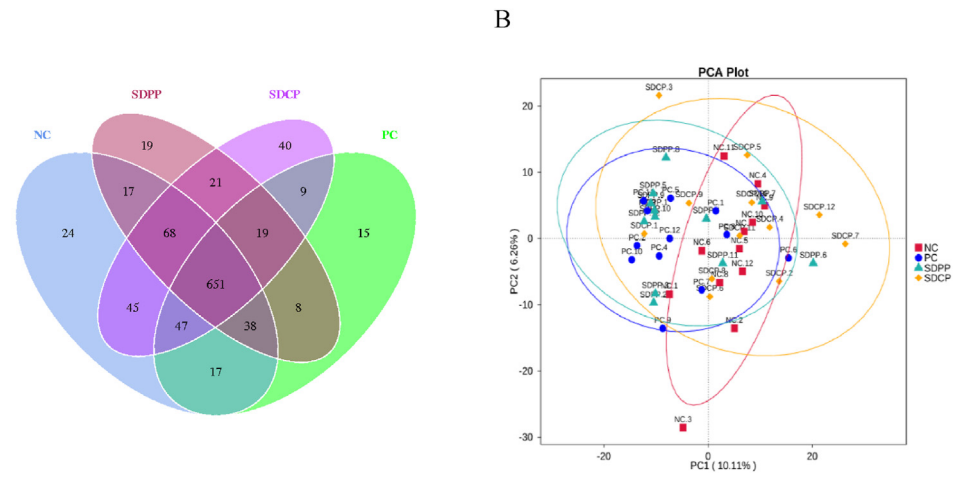

Fig. 2. The Venn diagram and principal component analysis (PCA) of bacterial communities in feces among groups. (A) Venn diagram among groups (B) PCA among groups. NC = negative control; PC = positive control; SDPP = spray-dried porcine plasma; SDCP = spray-dried chicken plasma.

in SDCP. The specific IgG in SDPP was suggested to have anti-pathogen effects in pigs (Torrallardona, 2010), resulting in fewer nutrients being used for the immune response, therefore improving BW gain (Zhang et al., 2015). In addition, the lower solubility, lower gel strength and lower IgG content in SDCP suggested that the proteins might be less well conserved. These observations might explain why the SDPP diet rather than the SDCP diet had similar beneficial effects to the PC diet in improving piglets' growth performance. Dietary supplementation with ZnO and AGP has been shown to be capable of suppressing the colonization of pathogenic bacteria in the intestine and decreasing post-weaning diarrhea (Nofrarías et al., 2006; Shelton et al., 2011; Cho et al., 2015). In this trial, there were more than 37% of piglets with fecal score ≥2 which might be ascribed to the high crude protein level (20%) and the high content of soybean meal that have been approved in the previous studies (Li et al., 1990; Wen et al., 2018; Zhang et al., 2020). Notably, the percentage of piglets with fecal score at 2, or ≥2 were lower in the PC than in the NC and SDCP groups, but was not different between the PC and SDPP groups, which was consistent with the results of growth performance. Moreover, the SDPP group also showed a much lower percentage of piglets with fecal score at 2 or ≥2 than the SDCP group, indicating the positive role of SDPP in protecting gut health.

The microbial communities colonized in the intestine are adapted to the environment and nutrients in the intestinal lumen (Kamada et al., 2013), reflecting the health condition and growth performance of the host (Palm et al., 2015). In the current study, decreased microbial diversity (OTU, ACE, Chao1) was observed when piglets were fed with the SDPP diet and the PC diet, which was contradictory to a previous study into intrauterine growth retardation (IUGR) piglets that showed that SDPP inclusion improved bacterial diversity (Che et al., 2020). This inconsistency might be related to the fact that normal and IUGR piglets have extremely different gut microbiome (D'Inca et al., 2010). Previous studies indicated that dietary supplementation with ZnO or AGP reduced the fecal microbial diversity in weaning piglets because of the antibacterial effects (Wang et al., 2012; Shen et al., 2014). Therefore, the similar effects of SDPP and PC diets on microbial

Table 6
Effects of dietary supplementation with SDPP and SDCP on average relative abundance of phylum (% of total sequences) in feces.

Phylum	Treatments				Pooled SEM	*P*-value
	NC	PC	SDPP	SDCP		
Firmicutes	66.62^{b}	79.52^{a}	78.55^{a}	75.64^{a}	3.428	0.030
Bacteroidetes	27.2^{a}	15.08^{b}	12.69^{b}	18.09^{ab}	3.308	0.005
Spirochaetes	1.52^{a}	0.84^{ab}	0.31^{b}	1.09^{a}	0.290	0.001
Tenericutes	0.89^{a}	0.09^{b}	0.56^{ab}	0.82^{a}	0.284	0.004
Proteobacteria	0.70	1.13	1.94	1.09	0.462	0.261

NC = negative control; PC = positive control; SDPP = spray-dried porcine plasma protein; SDCP = spray-dried chicken plasma protein.
^{a,b} Means in a row with different letter show significant differences ($P < 0.05$).

Table 7
Effects of dietary supplementation with SDPP and SDCP on average relative abundance of genus (% of total sequences) in feces.

Genus	Treatments				Pooled SEM	*P*-value
	NC	PC	SDPP	SDCP		
Lactobacillus	20.25^{ab}	22.40^{ab}	31.70^{a}	10.51^{b}	5.280	0.017
Subdoligranulum	2.24	5.46	4.16	5.59	1.982	0.092
Blautia	2.78	7.21	4.19	3.00	1.636	0.225
Faecalibacterium	2.05^{b}	10.94^{a}	5.05^{ab}	2.13^{b}	2.187	0.004
Unidentified_Ruminococcaceae	5.43	1.15	1.32	1.94	1.420	0.218
Campylobacter	1.70	2.52	1.09	0.63	1.011	0.486
Streptococcus	0.10^{b}	0.93^{a}	1.49^{a}	0.29^{ab}	0.475	0.003
Unidentified_Clostridiales	2.53	1.56	2.3	3.58	0.748	0.225
Holdemanella	1.08	2.69	2.01	1.46	0.519	0.112
Unidentified_Lachnospiraceae	0.65	0.84	0.44	1.58	0.321	0.061

NC = negative control; PC = positive control; SDPP = spray-dried porcine plasma protein; SDCP = spray-dried chicken plasma protein.
^{a,b} Means in a row with different letter show significant differences ($P < 0.05$).

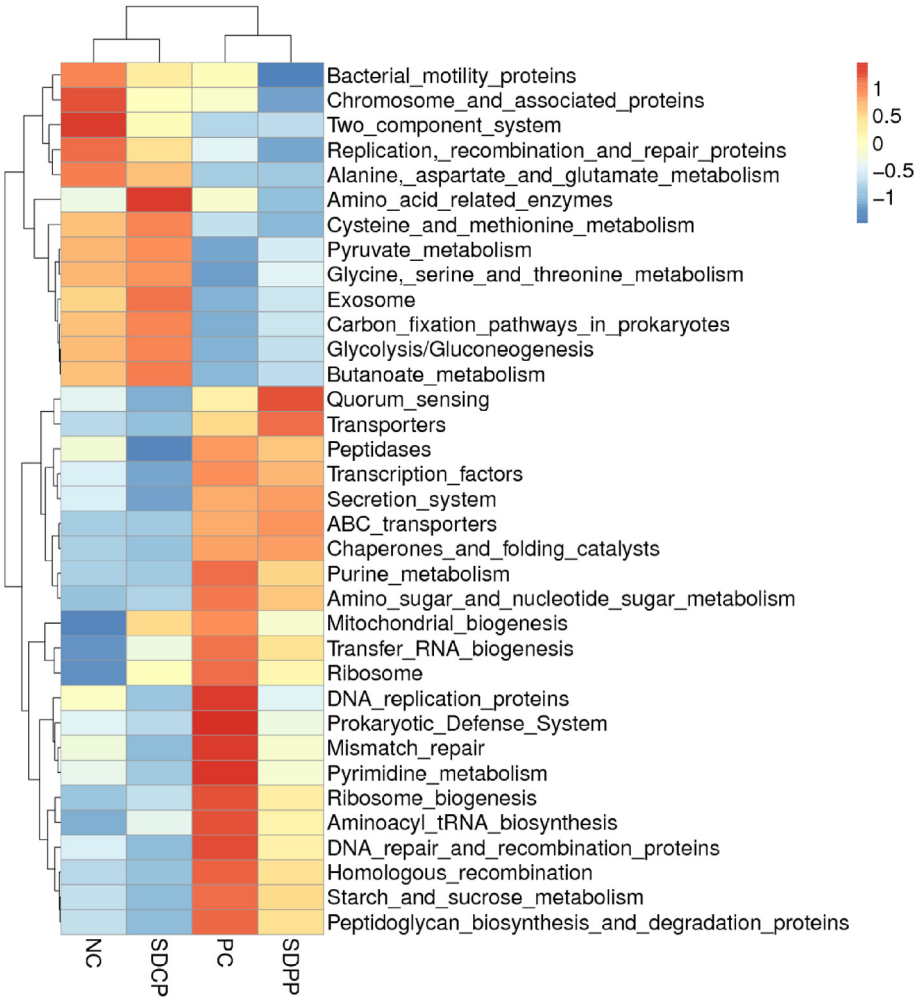

Fig. 3. Predicted function of fecal microbial community. The value of each functional gene was log10 transformed. All 4 levels of the KEGG pathways are shown in the heatmap. NC = negative control; PC = positive control; SDPP = spray-dried porcine plasma protein; SDCP = spray-dried chicken plasma protein; KEGG = Kyoto Encyclopedia of Genes and Genomes.

Table 8
Effects of dietary supplementation with SDPP and SDCP on SUN and VFA concentration in feces.

Item	Treatments				Pooled SEM	*P*-value
	NC	PC	SDPP	SDCP		
SUN, mmol/L	4.50[a]	5.54[a]	6.19[a]	8.67[b]	0.597	<0.001
VFA, μmol/g						
Acetate	50.40	47.16	47.78	45.70	4.276	0.889
Propionate	19.10	20.08	20.65	18.95	1.823	0.898
Butyrate	10.60	7.95	9.10	9.43	1.443	0.618
Valerate	2.08	1.65	2.11	2.40	0.459	0.618
Isobutyrate	2.20	1.52	1.72	2.30	0.314	0.198
Isovalerate	3.44	2.11	2.47	3.73	0.569	0.104
Total SCFA [1]	80.14	75.23	77.58	74.11	6.890	0.929
Total BCFA [2]	5.64	3.63	4.18	6.02	0.878	0.131
SCFA:BCFA ratio	17.08	28.37	28.86	15.93	4.393	0.058

NC = negative control; PC = positive control; SDPP = spray-dried porcine plasma protein; SDCP = spray-dried chicken plasma protein; SUN = serum urea nitrogen; VFA = volatile fatty acids; SCFA = short fatty acids; BCFA = branch chain fatty acid.
[a,b] Means in a row with different letter show significant differences ($P < 0.05$).
[1] Total SCFA included acid acetate, propionate and butyrate.
[2] Total BCFA included isobutyrate and isovalerate.

diversity suggested that SDPP might have similar antibacterial activity with ZnO plus AGP, which was consistent with the fecal scoring results in this trial.

In the current study, the 2 dominant phyla in piglets' feces were Firmicutes and Bacteroidetes, which was consistent with previous study (Che et al., 2020). In rodents, compared with normal mice, obese mice had increased relative abundance of Firmicutes, but decreased relative abundance of Bacteroidetes (Ley et al., 2005). In humans, accompanied with the process of weight loss, decreased Firmicutes abundance and increased Bacteroidetes abundance were discovered (Ley et al., 2006). The above observations suggest that higher host BW is always accompanied with higher Firmicutes abundance, and lower Bacteroidetes abundance. Therefore, the higher BWs in PC and SDPP pigs might partly be attributed to altered gut microbiome. Our results also suggested the cross-talk between intestinal microbiome and host performance. The phylum Spirochaetes contained 4 genera including important pathogenic species which can cause swine dysentery (Tsinganou and Gebbers, 2010). The lower abundance of Spirochaetes in the SDPP group gives evidence to the lower fecal score. In addition, Tenericutes appeared to be lower in the PC group than in the NC and SDCP groups, which was consistent with the previous study that antibiotics intervention decreased Tenericutes in the colon of piglets (Mu et al., 2017).

At the genus level, piglets fed with the SDPP diet rather than the PC diet had the highest fecal *Lactobacillus* and *Streptococcus* abundances, which agreed well with previous study (Tran et al., 2018).

As we know, *Lactobacillus* and *Streptococcus* are 2 major genera in pig feces, belonging to *Firmicutes*, with the function of producing lactate (Kajihara et al., 2017) that can inhibit putrefying bacteria growth (Yang et al., 2015), and show positive effects in piglets' intestine (Castillo et al., 2007). In the current study, the greater abundance of *Lactobacillus* and *Streptococcus* in the SDPP group was consistent with its better growth performance and lower fecal score, which reflected a distinct way in enhancing piglets' performance and declining diarrhea compared with antibiotics and zinc oxide. Furthermore, piglets fed the PC diet had higher *Faecalibacterium* abundance than those fed NC and SDCP diets, whereas, did not differ from piglets consuming the SDPP diet. *Faecalibacterium* is the most abundant member of the phylum Firmi*cutes* and can produce butyrate to reduce gut inflammation (Louis et al., 2014). The lower abundance of *Faecalibacterium* in the NC and SDCP groups may in part explain their higher fecal score.

Predictive functional analysis of bacterial communities showed that the main functions of the bacteria in 4 groups were distinct from each other. Bacterial motility is beneficial for the survival of bacteria (Halverson, 2005) and has been shown to contribute to the virulence of many enteric bacteria (Kim, 2013), particularly those infecting the mucosal surface (Flo and Aderem, 2005), with harmful effects on host health. Hence, the higher abundance of bacteria shown to be highly relevant to motility and protein repair in the NC group indicated a worse intestinal microbial environment, which accounted for the highest fecal score in this group. Previous study reported that the lower protein digestibility in the small intestine resulted in more undigested proteins passing into the large intestine, which would further be degraded by microbiota (Windey et al., 2012). In this study, the highlighted function of bacteria in SDCP pigs was predicted to be related to amino acid metabolism, which partly reflected the lower protein digestibility in the small intestine of these pigs. Quorum sensing is a density-dependent cell–cell signaling that drives a change in behavior when the bacterial population reaches a certain density (Abisado et al., 2018). In this trial, gut microbiota in SDPP pigs was predicted to be highly correlated with quorum sensing, presumably as a result of increased abundance of *Lactobacillus*, which produced peptides with antimicrobial activity (Das et al., 2017) and then decreased fecal score and promoted growth performance through a feedback mechanism. However, whether this phenomenon was linked to an increased abundance of *Lactobacillus* remains to be elucidated by further study.

On the other hand, we found that dietary supplementation with SDCP increased piglets' SUN compared with other groups. SUN is an index reflecting dietary protein utilization. The higher the SUN indicates the lower the protein utilization is (Zhang et al., 2015) in the SDCP group. Previous study reported that dietary supplementation with SDPP increased the VFA concentration in colonic digesta of postweaning piglets (Che et al., 2020), whereas we found no difference in the concentration of different kinds of VFA among groups. The inconsistency might be related to the different sampling position, considering that the VFA would be absorbed in the distal colon (Topping and Clifton, 2001), which finally led to a similar fecal VFA concentration among groups in this study. However, an increased SCFA-to-BCFA ratio was found in the PC and SDPP groups compared with that in the NC and SDCP groups. The higher SCFA-to-BCFA ratio was usually ascribed to the higher SCFA or the lower BCFA. It has been documented that BCFA are the fermentation products of protein with toxic effects, whereas SCFA are beneficial for the host (Cook and Sellin, 1998; Havenaar, 2011). In combination with the bacterial functional prediction and the higher SUN in the SDCP group, it would appear that the SDCP diet had lower protein digestibility and utilization than other groups, which might have restricted its positive effects on growth performance in weaned piglets.

6. Conclusion

Dietary supplementation of SDPP during the 14-d post-weaning period is helpful to alleviate weaning-associated stresses via increasing feed intake, *Lactobacillus* abundance and fecal SCFA-to-BCFA ratio, contributing to lower fecal score and better growth performance. Spray-dried porcine plasma protein showed a potential to protect the gut health of early-weaned piglets fed diets without supplemental ZnO and AGP, and provides a practical basis for the control of dietary protein levels and use of protein sources in weaning piglets to deal with the challenges resulting from the banned use of in-feed AGP and the limitation of ZnO usage.

Author contributions

Li Zhe, **Lunxiang Yang, Fangyuan Chen** and **Peng Wang** carried out the animal rearing trial. **Li Zhe**, **Yong Zhuo**, **Jiayong Tang**, **Xiaoling Zhang**, **Xuemei Jiang**, **Lingjie Huang** and **Ruinan Zhang** did the laboratory work and data statistical analysis. **Li Zhe**, **Sen Lin** and **Zhengfeng Fang** wrote the manuscript. **Yan Lin**, **Shengyu Xu**, **Lianqiang Che**, **Gang Tian**, **Bin Feng** and **De Wu** participated in the design of the experiment and the editing of the manuscript. **Lourens Heres** and **Zhengfeng Fang** conceived and supervised the experiment. All authors have read the manuscript and agree to publish the version of the manuscript.

Conflicts of interest

Lourens Heres is an employee of Sonac, which is one of financial supporters of the present study.

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