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Abstract

This study aimed to evaluate the effects of mono- and multi-strain LAB probiotics on the growth performance, nutrient digestibility, blood profiles, fecal noxious gas emission, intestinal microbiota and intestinal morphology of weaning pigs challenged with or without *Escherichia coli (E. coli)* and *Salmonella enterica (*SE*)*. In Exp. 1, a total of 60 crossbred weaning pigs were randomly allotted to one of five dietary treatments. The dietary treatments included: NC (negative control; basal diet with no supplement), PC (positive control; basal diet with 0.01% *Lactiplantibacillus plantarum* (LP) containing 1.0×10^8 CFU/g), K (basal diet with 0.1% *Pediococcus acidilactici* K (K) containing 1.0×10^9 CFU/g), WK1 (basal diet with 0.1% *Pediococcus pentosaceus* SMFM2016-WK1 (WK1) containing 1.0×10^9 CFU/g), K-WK1 (basal diet with 0.05% K + 0.05% WK1 containing 1.0×10^9 CFU/g). The average daily gain (ADG) was higher in the K group than in the WK1 group. Diarrhea score was lower in the K-WK1 group than in the NC group. At the genus level, *Roseburia* abundance in WK1 was higher than in the other treatment groups. At the species level, *Blautia wexlerae* abundance was lower in WK1 than in the other groups, whereas *Succinivibrio dextrinosolvens* abundance was higher in WK1. The serum pro-inflammatory cytokine levels in the PC and WK1 groups were as low as those in the NC group. Experiment 2 was conducted with two trials in a 2 × 5 factorial arrangement of treatments consisting of two levels of challenge (challenge and non-challenge) with *E. coli* and SE and five levels of probiotics same as Exp.1. Supplementation with LP and WK1 resulted in higher ADG and lower diarrhea scores than those in the other groups. Consequently, supplementation of WK1 showed a particularly positive effect on growth performance and diarrhea, villus height and intestinal microbiota in oral challenge experiment and feeding trial. Therefore, WK1 might be the most effective among the probiotics used in this experiment.

Keywords: oral challenge, probiotics, intestinal microbiota, weaning pigs, *Pediococcus pentosaceus*

Introduction

Colibacillosis and *Salmonellosis* are among the most detrimental diseases for the health problems of weaning piglets, resulting in post-weaning diarrhea (PWD), mortality, and reduced growth performance [1-3]. Pathogenic *Escherichia coli* (*E.coli*) and *Salmonella enterica* (SE) infections are the key causes of *Colibacillosis* and *Salmonellosis*, respectively [4, 5]. *E. coli* transmitted by the oral route can cause diseases such as hemorrhagic colitis and extra intestinal infections [3, 6]. Various antibiotics have been used to prevent and cure pathogens, but the extensive use of antibiotics is known to increase the incidence of antibiotic resistance [7]. Probiotics can be used as alternatives of antibiotics by maintaining health conditions and improving growth performance of weaning pigs [8, 9]. *Bacillus* spp., *Lactiplantibacillus* spp., *Bacillus* spp. and *Saccharomyces* spp. are currently used as probiocs [10]. Especially, *Lactobacillus* spp. and *Pediococcus* spp. belonging to lactic acid bacteria (LAB) are reduced intestinal pathogenic bacteria and have believed beneficial effects on pig nutrition. In order to use LAB as feed additives, a number of challenges must be met, including that the bacteria must be generally recognized as safe, as well as that the microorganisms remain viable during processing, transport, storage and the passage through the digestive system [11]. Additionally, many researchers note that bacteria isolated from the host are more effective probiotics than isolates derived from other sources [12, 13]. In the present study, beneficial microorganisms were isolated from Korean traditional fermented food. Since multi-strain or multi-species probiotics have been found to have more effective and consistent functionality than mono-strain or single-species probiotics [14]. Thus, we hypothesized that dietary supplementation with mono-strain probiotics and multi-strain probiotics such as *Lactiplantibacillus plantarum*, *Pediococcus acidilactici* and *Pediococcus pentosaceus* could improve the growth performance, intestinal morphology and microbiota. Therefore, this study aimed to evaluate the effects of mono- and multi-strain LAB probiotics on the growth performance, nutrient digestibility, blood profiles, fecal noxious gas emission, intestinal microbiota and intestinal morphology of weaning pigs challenged with or without *E. coli* and SE.

Materials and Methods

Animal welfare statement

The experimental protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of the Chungbuk National University, Cheongju, Korea (CBNUA-1696-22-02).

Source of probiotics and bacterial strains

The probiotics used in this study were provided by Sookmyung Women's University and were manufactured by LactoMason Co. (Jinju, Korea). The *L. plantarum* (LP) concentration of 1.0 × 10^8 CFU/g, the *P. acidilactici* K (K) of 1.0 × 10^9 CFU/g, and the *P. pentosaceus* SMFM2016-WK1 (WK1) of 1.0 × 10^9 CFU/g were used in this study. LP was isolated from Lactoplan (Genebiotech, Gongju, Korea), K from Korean traditional wine (Makgeoli) yeast, and WK1 from white kimchi. Shiga toxin-producing *E. coli* (STEC) and *Salmonella enterica* (SE) were provided in stock form. The *E. coli* and SE were thawed and ten microliters mixed with 10 mL of nutrient broth, cultivated at 37 °C for 24 h, and then subcultured at approximately 1 × 10^9 CFU/mL.

Animals and experiment design

Exp. 1

Sixty crossbred ([Landrace × Yorkshire] × Duroc) weaning pigs (initial body weight of 9.01 ± 0.79 kg) were randomly allotted to one of five dietary treatments (three pigs per pen and four replicates per treatment) based on body weight (BW). The experiment was conducted for four weeks. The dietary treatments included NC (negative control; basal diet with no supplement), PC (positive control; basal diet with 0.01% LP containing 1.0 × 10^8 CFU/g), K (basal diet with 0.1% K containing 1.0 × 10^9 CFU/g), WK1 (basal diet with 0.1% WK1 containing 1.0 × 10^9

CFU/g), K-WK1 (basal diet with 0.05% K + 0.05% WK1 containing 1.0 × 10^9 CFU/g). The basal diet was formulated to exceed the NRC requirement (Table 1) [15]. Feed and water were provided *ad libitum*. Each pen was equipped with a single-sided stainless steel automatic feeder and nipple drinker.

Exp. 2

A total of 60 crossbred weaning pigs ([Landrace × Yorkshire] × Duroc) with an initial BW of 8.0 ± 0.55 kg were individually accepted in 45cm × 55cm × 45cm stainless steel metabolism cages. Experiments were conducted with two trials in a 2 × 5 factorial arrangement of treatments consisting of two levels of challenge (challenge and non-challenge) with *E. coli* and SE and five levels of probiotics (Control, LP, K, WK1 and K-WK1). There was one pig in each cage and four replicate cages per treatment and housed in individual pen for 16 days, including 5 days before and 11 days after the first *E. coli* and SE challenge (d 0). All diets were formulated to meet or exceed the NRC requirement [15]. All treatment groups were fed the experimental diet for 16 days, including five days of adaptation. The diets were mixed with water in a 1:1 ratio before feeding and were fed at 08:30 and 17:30 each day. The pigs had *ad libitum* access to water. The experimental environment was maintained a relative humidity of 60 ± 2.3%, temperature of 27 ± 1.5℃ and a wind speed of 0.25 ± 0.03m/s). In the *E. coli* and SE challenge treatments, all pigs were orally inoculated by dividing a total of 10 mL of *E. coli* and SE for three consecutive days from 0 day post-inoculation (DPI) after 5 d of adaptation.

Measurements and sampling

Growth performance and diarrhea score

Exp. 1,

On day 0, week 2, and week 4 weaning pigs body weight (BW) and feed intake were measured, and the average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (G:F) were calculated. Diarrhea scores were individually recorded at 08:00 and 17:00 by the same person during the entire experimental period. Diarrhea score was assigned as follows: 0, hard mass feces; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea.

Exp. 2,

Pigs were individually weighed at the beginning (d -5), d 0 pre-inoculation, and d 7 and 11 DPI. Feed intake was recorded daily the diet supply amount and the remaining amount. The ADG, ADFI, and G:F were calculated for each interval from the adaption period, 0 to 7 DPI, 7 to 11 DPI and d 0 to 11 DPI. Diarrhea scores were individually recorded at 08:00 and 17:00 by the same person during the entire experimental period. Diarrhea score was assigned as follows: 0, hard mass feces; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea. The diarrhea score of each pig was calculated as the average within the period before and after the *E. coli* or *Salmonella* challenge.

Nutrient digestibility

In Exp. 1, fecal samples were collected from each treatment group at weeks 2 and 4, and then immediately analyzed chromium oxide (Cr_2O_3) (0.2%) as an indigestible marker was added to pigs' diet to determine the apparent total tract digestibility (ATTD) of dry matter (DM), crude protein (CP), and gross energy (GE). Cr_2O_3 was measured by acid digestion using a spectrophotometer (Model V-550, Jasco Co., Japan). ATTD was calculated using the following formula: digestibility (%) = $[1 - \{(Nf \times Cd)/(Nd \times Cf)\}] \times 100$, where Nf =nutrient concentration in feces (% dry matter), Nd = nutrient concentration in diet (% dry matter), Cd =chromium concentration in diet (% dry matter), and Cf = chromium concentration in feces (% dry matter).

In Exp. 2, fecal samples were collected from each treatment group at 7 and 11 DPI, and then immediately analyzed Cr_2O_3 (0.2%) as an indigestible marker was added in pigs' diet to determine the ATTD of DM, CP, and GE. Pig diets were mixed with chromic oxide 3 days earlier to collect samples and fresh excreta samples were randomly collected every week and stored at −20 ℃ until analysis. Before starting the chemical analysis, the fecal and feed samples were thawed and dried at 60 ℃ for 72 h, crushed on a 1-mm screen and thoroughly melded before sub-sample collection for chemical analysis. GE was determined by measuring the heat of combustion in the samples, using a bomb calorimeter (Parr 6400; Parr Instrument Co., Moline, IL, USA). Analyses of DM and CP were performed according to the methodology described in AOAC [16] and analysis of AAs was performed using High Performance Liquid Chromatography (HPLC) (SHIMADZU, Model LC-10AT, Shimadzu Corp., Kyoto, Japan) methodology.

Intestinal morphology

At the end of Exp. 2 (11 DPI), pigs were anesthetized with carbon dioxide gas after blood sampling and euthanized by exsanguination. After euthanization, intestinal tissues of about 10 cm from the ileum (close to the ileocecal junction) were collected and fixed in 10% neutral buffered formalin (NBF; Sigma-Aldrich, St. Louis, MO, USA) for intestinal morphology. After cutting the intestinal sample, it was dehydrated and dealcoholized. The samples were then mounted on slides, treated with paraffin, and stained with hematoxylin and eosin. Slides were examined using an Olympus IX51 inverted phase-contrast microscope. Intestinal morphological measurements included villus height (VH), crypt depth (CD), and villus height to crypt depth ratio (VH:CD).

Fecal noxious gas emissions and intestinal bacterial (Exp.1-gas / Exp. 2 intestinal microflora)

At weeks 2 and 4, fresh fecal samples were collected from 2 pigs in each pen using rectal massage (Exp. 1). The feces (300 g) collected per treatment were placed in a plastic box with small holes and the holes were sealed with plaster. The feces in the plastic box were fermented for 24 h and 48 h at room temperature (25 ℃) for fermentation. At room temperature (25 ℃), the samples were fermented for 24 h and 48 h. NH_3 and H_2S concentrations were determined in the ranges of 50.0 to 100.0 ppm (No. 3La, detection tube; Gastec Corp. Kanagawa, Japan). At the end of Exp. 2 (11 DPI), pigs were anesthetized with carbon dioxide gas after blood sampling and euthanized by exsanguination. After euthanization, digestion of the small and large intestine was collected and placed on ice for transportation to the laboratory where analysis was immediately performed. Bacterial colonies were counted using the pour plate method. To measure the number of *Salmonella* and *E. coli*, BG sulfa agar for *Salmonella*, and MacConkey agar for *E. coli* were used, and the agar plates were cultured at 37 ℃ for 24 h.

Blood profiles

In Exp. 1, Blood samples were collected from the jugular vein of 4 pigs each treatment at week 4 to analyze the concentrations of white blood cells (WBC), neutrophils, lymphocytes, monocytes, eosinophils and basophils in whole blood. In Exp. 2, blood samples were collected from the jugular vein of all pigs before the *E. coli* or *Salmonella* challenge (0 DPI), and at 2, 4, 7 and 11 DPI to analyze the concentration of white blood cells (WBC), neutrophils, lymphocytes, monocytes, eosinophils and basophils in whole blood. After collection, the serum samples were centrifuged (3000×g) for 15m at 4 ℃. The WBCs counts were determined using an automatic blood analyzer (ADVIA 120, Bayer, Leverkusen, Germany).

Measurement of serum immunoglobulin and cytokines (Exp. 1)

Blood samples were collected from the jugular vein of all the pigs after 4 weeks of treatment. Blood samples were collected into non-heparinized tubes for serum analysis. After collection, the tubes were centrifuged at 3,000×g at 4 ℃ for 20 min. An automatic biochemistry blood analyzer (Hitachi 747; Hitachi, Tokyo, Japan) was used to measure the immunoglobulin G (IgG) levels. The concentrations of cytokines (TNF-α, IL-4, IL-6, IL-10, and IL-12) in blood samples were determined using commercial ELISA kits (Quantikine; R&D systems, Minneapolis, MN, USA). Briefly, assay diluent (50 µL) was added to 96-well plate. Blood samples (50 µL) were then added to each well and incubated at room temperature for 2 h. Each well was washed 4 times with distilled water. One hundred microliters of conjugate solution were added to each well, incubated at room temperature for 2 h and then washed 5 times with distilled water. One hundred microliters of substrate solution were added to each well and incubated at room temperature for 30 min. The stop solution (100 µL) was added to each well, and the absorbance of the blood samples was measured at 450 nm.

Fecal DNA preparation and metagenome analysis (Exp. 1)

After probiotics treatment for 4 weeks, the fecal samples of weaning pigs were collected. The fecal DNA extraction, library preparation, and pair-end (2×300 bp) sequencing were performed in Macrogen (Seoul, Korea) with the MiSeq™ platform (Illumina, San Diego, USA). The fecal DNA was extracted with DNeasy Powersoil kits (Qiagen, Hilden, Germany) as described by the manufacturer. Briefly, 0.25 g of fecal samples were added to the powerbead tube. Solution C1 (60 µL) was added to the tube, vortexed for 10 min, and centrifuged at 10,000×g for 1 min. The supernatant was transferred to a collection tube, and solution C2 (250 µL) was added and incubated at 4 ℃ for 5 min. After centrifuging the tube at 10,000×g for 1 min, the supernatant (600 µL) was transferred to a new collection tube. Solution C3 (200 µL) was added to the collection tube, incubated 4 ℃ for 5 min, and centrifuged at 10,000×g for 1 min. The supernatant was transferred to a new collection tube and 1,200 µL of solution C4 were added. The solution was transferred to a MB spin column and centrifuged at 10,000×g for 1 min.

Solution C5 (500 µL) was added to the spin column and centrifuged for 30 s at 10,000×g. The spin column was transferred to a 1.5-mL tube, and 50 µL of solution C6 were added into the tube. It was then centrifuged at 10,000×g for elution. The fecal microbiota sequencing library was amplified with the Illumina 16s metagenomic sequencing protocol to amplify the V3-V4 regions of the 16S rRNA gene as follows. The fecal DNA was amplified with a reaction buffer, 1 mM dNTP mix, 500 nM PCR primer, and 2.5 U of Herculase II fusion DNA polymerase (Agilent Technologies, Santa Clara, CA, USA), and purified using AMPure beads (Agencourt Bioscience, Beverly, MA, USA) as described by the manufacturer. The paired-end (2×300 bp) sequencing was then performed with the MiSeq™ platform (Illumina, San Diego, USA). For amplicon sequence variant (ASV) analysis and taxonomic information, the National Center for Biotechnology Information 16s Microbial DB (Bethesda, MD, USA) was used. The Shannon index and Chao1 were used to assess microbial species evenness and richness for α-diversity [17]. In the case of β-diversity, community dissimilarity among samples was measured by unweighted Unifrac distance, and microbial differences among samples were visualized by principal coordinates analysis (PCoA) [18, 19]. The relative classification frequency table represented differential abundance tests at specific taxonomic levels was created using collapse and feature-table within the QIIME2 plugins. The “diversity” QIIME2 plugin was used to estimate alpha diversity measurements

Statistical analysis

In Exp. 1, The data were statistically analyzed by the generalized linear model (GLM) procedure in SAS® (SAS Institute Inc., Cary, NC, USA). Cages were used for each experimental unit. A significant difference in the least-squares means between the samples was determined using a pairwise t-test at α=0.05.

In Exp. 2, The data were analyzed by two-way ANOVA, with the general linear model (GLM) procedure in SAS® (SAS Institute Inc., Cary, NC, USA) as a 2 (non-challenge or challenge *E. coli* and *salmonella*) × 5 (mono or multi-strain LAB probiotics) factorial design. Differences between treatment groups were determined using Tukey's honest significant difference (HSD) test with a *P*-value of < 0.05 indicating significance and $0.05 < P$-value < 0.10 indicating a tendency.

Results

Exp. 1

Growth performance

The growth performance results of the Exp. 1 are in Table 2. There was no difference between treatments in BW of weaning pigs. In phase 1 (0-2w), ADG was higher ($P < 0.05$) in K group than WK1 group. ADFI was higher ($P < 0.05$) in the NC and K group than WK1 group. In phase 2 (2-4w), there was no difference between treatments in ADG. ADFI was higher ($P < 0.05$) in WK1 and K-WK1 groups than NC and PC. K-WK1 group was lower ($P < 0.05$) in G:F than other treatments. In the overall period, ADG and ADFI were no difference between the treatments. But G:F was higher ($P < 0.05$) in PC and K groups than K-WK1 group.

Diarrhea score

Diarrhea score data are shown in Table 3. The diarrhea score from phase 1 was significantly lower ($P < 0.05$) in K, WK1, and K-WK1 groups than NC. In phase 2, the diarrhea score was lower ($P < 0.05$) in PC, K, WK1 and K-WK1 groups than NC. Also, in the overall period, NC was significantly higher ($P < 0.05$) diarrhea score than other treatments.

Nutrient digestibility

The ATTD data are shown in Table 4. There was no difference between the treatments in DM, CP, and GE.

Blood profiles

The blood profile data are shown in Table 5. There was no difference between the treatments in the blood profiles.

Fecal noxious gas emissions

The fecal noxious gas emissions data are shown in Table 6. On week 2, the fecal NH_3 emission in the NC was higher (P < 0.05) than other treatment groups. There was no significant difference between the treatments to which the probiotic was added. In week 4, the fecal NH_3 emission in NC and K groups were higher (P < 0.05) than PC, WK1, and K-WK1 groups. There was no significant difference between treatments in the fecal H_2S emission on week 2 and 4.

Gut microbial diversity and taxonomic composition comparison

The number of the observed species in the NC, PC, K, WK1, and K-WK1 groups was 339.75, 403.25, 346.25, 384.00, and 328.25, respectively, indicating that the microflora of the PC and WK1 groups were more diverse than in the other probiotic-fed groups (K and K-WK1) (Fig. 1a). Among the α-diversity indexes, Chao1 represents the abundance of intestinal flora [20]. The Chao 1 indexes of the PC and WK1 groups were 404.1 and 386.1, respectively, indicating greater richness than the other groups (NC, K, and K-WK1) (Fig. 1b). The Shannon index of the PC and WK1 groups were 7.260 and 7.233, respectively, which were higher than the other groups (NC, K, and K-WK1) (Fig. 1c). In the PCoA plot of unweighted UniFrac distance to analyze β-diversity, the PC group and WK1 groups were clustered due to the high similarity of the intestinal flora among samples, but NC, K, and K-WK1 groups were not clustered due to the low similarity of the intestinal flora among samples (Fig. 2). Consequently, probiotic strains of PC and WK1 may help to regulate similarly the gut flora of weaning pigs with probiotic supplementation.

As a result of analyzing the gut microbiota of weaning pigs by OTU clustering, the bacterial phyla with the highest abundance in all groups were Firmicutes and Bacteroidetes, followed by Proteobacteria, Spirobacteria, and Actinobacteria at the phylum level (Fig.3). The

Firmicutes:Bacteroidetes (F:B) ratios of PC and WK1 were calculated to be 1.83 and 1.68, respectively, which was higher than the F:B ratio of NC (1.58). At the genus level, the *Roseburia* abundance in WK1 was higher ($P < 0.05$) than in other treatment groups (Table 7). In the case of *Weisella* abundance, the four probiotic treatment groups showed higher abundance than the NC group. *Olsenella* was more abundant in K and K-WK1 fed groups than in the other groups. Especially, *Succinivibrio*, which is the core microbiome of the swine, was the most abundant in the WK1 among the experimental groups ($P < 0.05$). At the species level, *Blautia wexlerae* abundance was lower in the WK1 than in the other groups, while *Succinivibrio dextrinosolvens* abundance was higher ($P < 0.05$) in the WK1 (Table 8). The abundance of *Roseburia faecis* was higher in the probiotic-fed groups (K, WK1 and K-WK1) than in the NC and PC groups. Even within the probiotic-fed groups, the K-WK1 group had higher ($P < 0.05$) abundance of *R. faecis* than the K and WK1 groups. The abundance of *Eubacterium coprostanoligenes* was lower ($P < 0.05$) in the K-WK1 group than in the other groups, while WK1 group showed slightly higher abundance. *Lactobacillus delbrueckii* was abundant in the WK1 group (0.13%), but not in the NC or K groups, and was present in the PC and K-WK1 groups with an abundance of less than 0.1%.

Serum cytokine and immunoglobulin profiles

To assess the immune response of weaned piglets to probiotic feeding, serum IgG level, as well as pro-inflammatory (TNF-α, IL-6 and IL-12) and anti-inflammatory (IL-4, and IL-10) cytokine levels were measured. The IgG concentration of the K-WK1 group was significantly higher ($P < 0.05$) than that of the NC group, and there were no significant differences among the PC, WK1 and K-WK1 groups (Fig. 4a). The concentrations of TNF-α, IL-12, IL-4 and IL-10 were not significantly different among all experimental groups, but the concentrations of the pro-inflammatory cytokines TNF-α and IL-12 in the K and K-WK1 groups were higher than the other groups (NC, PC, and WK1) (Fig. 4b-e). The WK1 group had significantly higher (P < 0.05) IL-6 concentration than the NC group (Fig. 4f).

Exp. 2

Growth performance

Table 9, 10 and figure 5 and 6 show the growth performance of weaning pigs challenged with *E. coli* and SE. BW was not affected by oral challenge and probiotics. On 0 to 7 DPI and overall period, ADG, ADFI and G:F were lower (P < 0.05) in challenged groups than non-challenged groups. On 7 to 11 DPI, the ADG and ADFI were lower (P < 0.05) in the SE challenged groups than non-challenged group. On 0 to 11 DPI, supplementation of LP, K and WK1 groups showed higher (P < 0.05) ADG than NC and supplementation of K-WK1 group. On adaption period, supplementation of LP group showed lower (P < 0.05) ADFI than other probiotics groups and supplementation of K group showed higher (P < 0.05) G:F than other supplementation of probiotic groups. On 0 to 7 DPI, supplementation of LP, K and WK1 groups showed higher (P < 0.05) G:F than NC and supplementation of W-KW1 groups. On 0 to 7 DPI, supplementation of LP, K and WK1 groups showed higher (P < 0.05) ADG than NC and supplementation of W-KW1 groups and supplementation of WK1 group showed higher (P < 0.05) ADFI than other probiotics groups. On 7 to 11 DPI, supplementation of WK1 group showed significantly higher (P < 0.05) ADG and G:F than other supplementation of probiotic groups. In overall period, supplementation of WK1 showed significantly higher (P < 0.05) ADG and G:F than other probiotics groups.

Diarrhea score

The diarrhea score data are shown in Table 11 and 12. The diarrhea scores from all periods were significantly higher (P < 0.05) in the challenged groups than in the non-challenged groups.

On 0 to 7 DPI, NC groups was higher ($P < 0.05$) than supplementation of probiotic groups. In the overall period, the supplementation of LP and W-KW1 groups were significantly lower ($P < 0.05$) diarrhea score than other groups.

Nutrient digestibility

Table 13 and 14 show the nutrient digestibility of weaning pigs challenged *E. coli* and SE. Nutrient digestibility was not affected ($P > 0.05$) by different probiotics and challenges.

Blood profiles

Table 15 and 16 show the blood profiles of weaning pigs challenged *E. coli* and SE.
In *E. coli* and SE challenge, monocyte and eosinophil levels were increased ($P < 0.05$) at 2 and 4 DPI. Also, on 7 DPI, neutrophil levels were also increased. There were no significant differences between probiotics groups.

Intestinal morphology

Table 17 shows the intestinal morphology of weaning pigs challenged *E. coli*. When *E. coli* was challenged, VH and VH:CD was lower ($P < 0.05$) than non-challenged groups. But CD was higher ($P < 0.05$) than non-challenged groups. There was an interaction between the *E. coli* challenge and probiotics in VH. Table 18 shows the intestinal morphology of weaning pigs challenged SE. As with the challenge with *E. coli*, there was an interaction between the SE challenge and the probiotics in VH. The probiotics did not affect the intestinal morphology of weaning pigs.

Small and large intestinal microbial

Table 19 and 20 show the small and large intestinal microbial of exp 2. *E. coli* and SE were not affected ($P > 0.05$) by different probiotics and challenges.

Discussion

Exp. 1 was conducted to evaluate effects of mono and multi-strain LAB. Results of this study showed that mono-strain probiotics had positive effects on growth performance of weaning pigs whereas multi-strain probiotic did not. LAB can improve growth performance because lactic acid and digestive enzymes, which are metabolites of LAB, can promote gastrointestinal peristalsis and feed digestion [21]. In previous studies, supplementation of *P. acidilactici* and PC improved feed conversion ratio [14, 22]. Results of the present study conformed to those of previous studies. Supplemented multi-strain probiotics K-WK1 had no effect on growth performance compared with NC. However, many studies have shown that LAB complex probiotics can enhance the growth performance of weaning pigs [23-25]. This reason is because multi-strain probiotics might broaden the range of protection against microbial infections [26]. These inconsistent results might be due to several factors such as differences in age of pigs, the type of probiotics, the amount of addition, and feed composition [27].

When weaning piglets were fed WK1, the α-diversity (Chao 1 and Shannon) of the WK1 group increased as much as that in PC group, indicating an increase in both the richness and evenness of the gut microbial composition. These increases in the diversity of gut microbiota indicate that the intestinal environment might be stable, and it might be related to the host health because Chang et al. [28] and Pozuelo et al. [29] suggested that low diversity of intestinal flora is correlated with inflammatory bowel disease, allergies, and immune disorders. Moreover, the more diverse the intestinal microbiota, the more nutrition metabolism happens via numerous processes, which can help the host maintaining health [28-30]. Furthermore, in the PCoA plot for β-diversity, the WK1 group had better clustering than the PC group. It indicates that feeding WK1 to weaning pigs might make the piglets have more similar gut microbiota composition among the piglets than other probiotics. Probiotics can help maintain health of the host by increasing proportion and diversity of beneficial bacteria in the intestine

[31, 32]. As a result, feeding WK1 to weaning piglets could enhance both α-diversity and β-diversity of weaning piglets' intestinal microbiota, which could improve weaning piglets' intestinal environment.

As a result of analyzing distribution of the gut microbiome at the phylum level by ASV clustering, F:B ratios of PC and WK1 groups were higher than that of NC. The F:B ratio is associated with energy absorption and storage after dietary fat intake and obese pigs have higher F:B ratios than normal weight pigs. It was presented in the studies by Guo et al. [33] and Wang et al. [34] using obese pigs and normal weight pigs [33 34]. Because Firmicutes are related to many bacteria that produce short-chain fatty acids, they might be involved in maintaining energy balance [35]. Thus, feeding PC and WK1 could be beneficial to pig farms for increasing productivity. At the genus level, the WK1 group was shown to have higher *Roseburia* and *Eubacterium* ratios than other groups. These bacteria are known to produce butyrate [36]. Butyrate, a short chain fatty acid, is an important energy substrate of colonocyte [37]. Furthermore, as butyrate can lower the pH of the colon, it may inhibit pathogenic bacterial growth [38]. Thus, WK1 could inhibit the growth of pathogen bacteria and reduce diarrhea of weaning pigs. The WK1 group had higher ratio of *Succinivibrio*, a major intestinal bacterium of swine, than other groups. *Succinivibrio* is primarily involved in the production of acetate and succinate, both of which play important roles in the synthesis of propionate and thus, in improving feed efficiency [39]. At the species level, *Roseburia faecis* was abundant in probiotics-treated groups (PC, K, WK1, and K-WK1). It can produce short-chain fatty acids, particularly lactate, known to be beneficial to intestinal health of the host [40]. *Eubacterium coprostanoligenes* was abundant in the WK1 group. It might influence fat metabolism in pigs by converting cholesterol to coprostanol [41, 42]. Because *E. coprostanoligenes* can reduce the amount of total cholesterol in pigs, pork from these pigs might be considered healthier for those at risk of cardiovascular diseases [43, 44]. *Lactobacillus delbrueckii*, a beneficial bacterium for mammals such as humans and pigs, was found to be more abundant (0.13%) in the WK1 group than in other groups. Furthermore, *L. delbrueckii* has been shown to have antioxidant and immune-improving effects to piglets before 4 weeks of age, and these effects were maintained even after weaning [45]. Thus, WK1 could benefit weaning pig gut health by producing SCFAs and increasing the ratio of beneficial bacteria in their gut.

Immunoglobulin is a substance released by plasma cells as a marker of immunological function of the body [46]. In the present study, PC, WK1, and K-WK1 groups showed increased IgG levels. IgG plays a role as a physiological barrier to protect piglet intestinal epithelium, and as a result, it could minimize intestinal epithelial cell detachment caused by diarrhea during weaning transition [47]. As for pro-inflammatory cytokines, serum TNF-α, and IL-12 levels of PC and WK1 groups were as low as those of the NC group. High levels of these cytokines might result in symptoms such as fever, anorexia, and anxiety [48-50]. These results suggest that WK1 supplementation might enhance the immune function of weaning pigs.

Exp. 2 was conducted to investigate effects of mono and multi-strain probiotics supplementation in weaning pigs following *E. coli* or SE challenge, with respect to growth performance, diarrhea score, nutrient digestibility, intestinal morphology, blood profiles, and intestinal microbiome. Overall effects revealed that PC, K and WK1 supplementation improved ADG and G:F of piglets, similar to previous studies reporting that *Lactobacillus* supplementation could increase daily weight gain of piglets [51]. This advantageous effect of *Lactobacillus* supplementation on growth performance might be related to improved villus height of piglets as demonstrated in this study. *Lactobacillus* might also modulate intestinal environment and growth of intestinal microflora, thus decreasing diarrhea [52]. Other previous studies suggested that *Lactobacillus* species supplementation might stimulate the secretion of mucus which can promote the growth of intestinal microflora [53, 54]. In general, probiotics are intended to maintain the intestinal ecosystem and improve animal health [55]. Probiotic bacteria produce several anti-microorganism substances such as bacteriocin, hydrogen peroxide, carbon dioxide, and acetic acid [56], which can support gut health. For example, bacteriocin can inhibit peptidoglycan of pathogenic bacteria and interfere with the function of cell membranes, resulting in inhibition of bacteria pathogens [57]. Enhancement of epithelial barrier [58] and concomitant inhibition of pathogen adhesion [59] by *Lactobacillus* might also prevent intestinal damage, thus improving gut health and growth performance [56]. However, multi-strain probiotics failed to improve growth performance of weaning pigs.

PWD is the most frequent disease in weaning piglet. It is a main economic problem because it can increase dehydration and mortality, and lower growth performance of weaning pigs [60, 61]. Probiotics such as *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* can prevent PWD due to their antagonistic activities against hazardous bacteria, ability to modulate gut microbiome balance, effects on the digestive processes, and ability to improve the immunity of pigs [1, 62] Supporting this mechanism, many previous studies have shown that mono and multi-strain probiotics can improve diarrhea score [25, 60, 63, 64]. In the present study, treatments with mono and multi-strain probiotics improved diarrhea score compared to NC treatment with or without challenge, consistent with previous studies. Thus, mono and multi-strain LAB probiotics are considered effective for decreasing diarrhea in weaning pigs.

Lan et al. [26] reported that supplementation of probiotics (*B. coagulance*, *B. lichenformis*, *B. subtilis* and *C. butyricum* complex) has positive effects on DM and GE digestibility. The addition of *L. reuteri* and *L. plantarum* complex probiotics (0.1%) and *P. acidilactici* increased the digestibility of CP and GE [65, 66]. Probiotics can improve nutrient digestibility of pig by producing metabolites, stimulating gastrointestinal peristaltic movement and promoting apparent nutrient digestibility [66]. In contrast, this study showed no effect of probiotics on nutrient digestibility of DM, CP and GE of weaning pig with or without challenge. These differences in results might be affected by the type, amount and combination of probiotics. More studies are needed to clarify this.

One of the objectives of the present study was to determine whether addition of mono and multi-strain probiotics could affect blood profiles, including WBC, neutrophil, lymphocyte, monocyte, eosinophil, and basophil of weaning pig. However, there were no significant differences in blood profiles. Likewise, Tufarelli et al. [67] and Dowarah et al. [68] reported that probiotics have no effect on blood profiles of pigs. Moreover, Wang and Kim [69] reported that supplementation of *L. plantarum* has no effect on WBC. Effects of *P. acidilactici*, *P. pentosaceus* and *L. plantarum* on blood and action mechanisms have not been clearly elucidated yet. WBCs, which circulate in the blood, fulfil most of their functions outside circulation. To achieve this, they have systems that can respond to specific stimuli and enable them to enter and traffic through the extravascular milieu. In Exp. 2, after oral challenge with *E. coli* or SE, monocyte and eosinophil levels increased at 2 DPI and 4 DPI but gradually stabilized over time. Neutrophil levels increased on 7 DPI, but then stabilized. As part of the inflammatory response, neutrophils' main function is to consume and eliminate bacteria found in the extravascular area [70] Both allergic responses and a parasite infection can result in increased eosinophil levels [71]. This mechanism might increase the neutrophil and eosinophil levels after challenge inoculation.

Fecal noxious gas emission has become one of the major air pollutions in modern concentrative pig production [72]. Excessive harmful gas emissions can disrupt ecological balance [73]. We found that dietary supplementation with *L. plantarum*, *P. acidilactici*, and *P. pentosaceus* affected harmful gas emission in feces. However, probiotic supplementation in pig diet did not affect H_2S. In addition, fecal noxious gas emission is associated with nutrient digestibility because a higher digestibility may result in a lower substrate for microbial fermentation in the large intestine, consequently decreasing fecal noxious.

After weaning, impaired intestinal barrier function cause decreased VH and mucin levels [74, 75]. Epithelial cells in the gastrointestinal tract play crucial roles in digestion, nutrient absorption, and protection from pathogens and toxins [76]. Hence, morphology of the intestine can be a useful indicator for assessing the gastrointestinal system's health and function [77]. A secretory mucin glycoprotein is secreted by goblet cells at the intestinal mucus layer act as a line of defense against enteric pathogens as well as microbial adhesion and invasion [78, 79]. Ng et al. [80] suggested that probiotics may influence intestinal microflora by facilitating antibody production, promoting epithelial barrier integrity and activating Toll-like receptor signaling, as well as some other mechanisms. In the current study, dietary supplementation with mono and multi-strain probiotics had no effect on *E. coli* or *Salmonella* counts. Microflora in the gastrointestinal tract plays a crucial role in anti-bacterial, physiological and immunological functions of host animals [81]. Therefore, the absence of a significant difference in nutrient digestibility could be explained by the absence of a significant difference in intestinal microbials.

Conclusion

In weaning pigs infected with *E. coli* and SE, the supplement of mono-strain probiotics reduced the negative effect of *E. coli* and SE and improved growth performance and diarrhea score. Multi-strain probiotics had no effect on growth performance but were effective in improving diarrhea. However, supplementation of WK1 showed a particularly positive effect on growth performance and diarrhea, villus height and intestinal microbiota in oral challenge experiment and feeding trial. Therefore, WK1 might be the most effective among the probiotics used in this experiment.

Disclosure statement

There are no potential conflicts of interest.

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Table 1. Compositions of basal diets (as-fed-basis)

Items	content
Ingredients, %	
corn	34.43
extruded corn	15.00
lactose	10.00
Dehulled soybean meal, 51% CP[a]	13.50
Soy protein concentrate, 65% CP[a]	10.00
Plasma powder	6.00
Whey	5.00
Soy oil	2.20
Monocalcium phosphate	1.26
Limestone	1.40
L-Lysine-HCl, 78%	0.06
DL-Methionine, 50%	0.15
Choline chloride, 25%	0.10
Vitamin premix[b]	0.25
Trace mineral premix[c]	0.25
Salt	0.40
Total	100
Calculated value	
ME, Kcal/kg	3433
CP, %	20.76
Lysine, %	1.35
Methionine, %	0.39
Ca	0.82
P	0.65
Analyzed value	
ME, kcal/kg	3512
CP, %	20.92

[a]Abbreviation: CP, Crude protein

[b]Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D_3, 1103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; ribofavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic, 29 mg; choline, 166 mg; and vitamin B12, 33 mg

[c]Provided per kg of complete diet without Zinc: Cu (as $CuSO_4{\bullet}5H_2O$), 12mg; Mn (as MnO_2), 8mg; I (as KI), 0.28mg; and Se (as $Na_2SeO_3{\bullet}5H_2O$), 0.15mg

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Table 2. Effects of different probiotics on growth performance of weaning pigs (Exp.1)

Items	NC	PC	K	WK1	K-WK1	SE	*P*-value
BW, kg							
Initial	9.04	9.00	9.00	9.05	9.00	0.07	0.998
2W	12.06	11.85	12.53	11.71	12.04	0.11	0.190
4W	18.60	18.82	19.43	19.01	18.66	0.17	0.560
Phase 1 0-2 week, g							
ADG	216^{ab}	204^{ab}	252^{a}	190b	218^{ab}	6.00	0.018
ADFI	365^{a}	315^{ab}	369^{a}	285^{b}	340^{ab}	7.00	0.001
G:F	0.59	0.68	0.68	0.67	0.64	0.03	0.189
Phase 2 2-4 week, g							
ADG	467	498	493	522	473	7.00	0.064
ADFI	798^{b}	755^{b}	776^{ab}	828^{a}	831^{a}	8.00	0.001
G:F	0.59^{a}	0.66^{a}	0.64^{a}	0.63^{a}	0.57^{b}	0.01	0.001
Overall 0-4 week, g							
ADG	342	351	373	356	346	5.00	0.267
ADFI	582	535	573	557	586	6.00	0.094
G:F	0.59^{ab}	0.66^{a}	0.65^{a}	0.64^{ab}	0.59^{b}	0.01	0.001

NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed ratio

[a,b]Means within column with different superscripts differ significantly ($P < 0.05$).

Table 3. Effects of different probiotics on diarrhea of weaning pigs $^{(Exp.1)}$

Items	NC	PC	K	WK1	K-WK1	SE	*P*-value
0-2 week							
Diarrhea score	1.63^{a}	1.19^{ab}	1.04^{b}	1.05^{b}	0.98^{b}	0.06	0.001
2-4 week							
Diarrhea score	1.35^{a}	0.58^{b}	0.59^{b}	0.69^{b}	0.75^{b}	0.06	< 0.001
0-4 week							
Diarrhea score	1.51^{a}	0.89^{b}	0.80^{b}	0.88^{b}	0.88^{b}	0.05	< 0.001

NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1

^{a,b}Means within column with different superscripts differ significantly ($P < 0.05$).

Table 4. Effects of different probiotics on the nutrient digestibility of weaning pigs (Exp.1)

Items	NC	PC	K	WK1	K-WK1	SE	*P* -value
2W							
DM, %	89.17	89.38	90.02	89.33	89.18	0.20	0.659
CP, %	67.32	67.29	68.08	67.35	68.01	0.23	0.676
GE, %	72.91	72.35	72.15	72.74	73.02	0.35	0.932
4W							
DM, %	89.80	89.79	89.80	89.71	89.46	0.09	0.752
CP, %	70.91	70.83	68.80	70.38	70.48	0.30	0.150
GE, %	72.88	71.15	72.32	72.27	72.13	0.24	0.250

NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1; DM, dry matter; CP, crude protein; GE, gross energy

Table 5. Effect of different probiotics on blood profiles in weaned pigs (Exp.1)

Items	NC	PC	K	WK1	K-WK1	SE	*P*-value
Final WBC, 10^3/ $\mu\ell$	17.26	24.11	23.19	24.22	28.38	1.60	0.304
Neu, %	36.65	22.88	22.93	21.50	16.58	3.69	0.553
Lym, %	46.40	64.98	52.40	65.20	68.48	3.60	0.232
Mon, %	6.70	3.43	7.10	3.50	4.18	1.04	0.717
Eos, %	10.18	8.63	17.43	9.73	10.63	1.29	0.207
Bas, %	0.08	0.10	0.15	0.08	0.15	0.03	0.901

NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1; WBC, white blood cell; Neu, neutrophil; Lym, lymphocyte; Mon, monocyte; Eos, eosinophil; Bas, basophil

Table 6. Effect of different probiotics on gas emission of weaning pigs (Exp.1)

Items, ppm	NC	PC	K	WK1	K-WK1	SE	*P* -value
2W							
NH_3	78.13[a]	16.80[b]	12.25[b]	19.10[b]	9.83[b]	4.01	<0.001
H_2S	5.03	4.85	4.98	6.20	6.98	1.07	0.563
4W							
NH_3	35.30[a]	10.40[b]	46.03[a]	6.68[b]	4.13[b]	4.51	<0.001
H_2S	9.10	9.35	11.13	9.70	9.03	1.09	0.654

NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1

Abbreviation: NH_3, ammonia; H_2S, hydrogen sulfide

[a,b]Means within column with different superscripts differ significantly ($P < 0.05$)

Table 7. Taxonomy abundance of the microbial genus among groups (%)

Genus	NC	PC	K	WK1	K-WK1
Prevotella	23.00±4.19^{ab}	19.16±5.92^{b}	24.91±5.62^{ab}	21.37±2.93^{ab}	26.53±2.98^{a}
Clostridium	13.74±10.42	15.57±3.39	14.45±9.67	12.90±7.86	13.72±7.89
Megasphaera	4.39±8.72	0.28±0.55	0.28±0.38	0.42±0.42	0.22±0.36
Blautia	3.95±2.80	2.35±0.71	4.54±4.13	2.79±0.78	6.71±4.99
Barnesiella	3.22±2.16	1.46±0.58	2.37±1.86	2.89±4.12	2.79±3.08
Parabacteroides	2.48±1.08	2.72±1.53	1.87±1.65	2.10±0.35	2.98±3.18
Faecalibacterium	1.62±1.83	0.70±0.63	4.59±8.07	1.14±0.67	4.97±7.21
Lactobacillus	1.86±1.59	5.22±7.07	1.65±1.20	1.50±1.26	2.04±1.52
Oscillibacter	2.24±1.51	2.10±0.39	2.67±1.50	2.73±0.96	1.87±0.83
Gemmiger	1.53±1.30	0.73±0.14	1.07±0.55	1.37±0.45	0.88±0.46
Prevotellamassilia	1.34±1.09^{a}	1.03±0.39^{ab}	1.59±1.24^{ab}	1.14±0.71^{ab}	0.56±0.45^{b}
Butyricicoccus	0.93±0.67	1.22±0.54	1.09±0.74	0.81±0.51	0.47±0.48
Succinivibrio	1.69±2.27^{b}	2.05±1.41^{b}	0.85±0.88^{b}	6.45±5.79^{a}	0.35±0.53^{b}
Roseburia	2.07±1.16^{b}	1.64±0.88^{b}	2.90±1.0^{b}	3.30±0.86^{a}	4.43±2.57^{b}
Weissella	0.02±0.01	0.03±0.02	0.03±0.02	0.05±0.01	0.03±0.03
Helicobacter	0.03±0.07	0.03±0.04	0.02±0.02	0.01±0.02	0.00±0.01
Methanomassiliicoccus	0.04±0.04	0.04±0.03	0.02±0.02	0.02±0.01	0.02±0.03
Olsenella	0.00±0.00	0.00±0.00	0.05±0.09	0.00±0.00	0.08±0.15
Eubacterium	0.87±0.46^{ab}	1.31±0.49^{a}	1.11±0.27^{a}	1.30±0.41^{a}	0.53±.0.06^{b}

NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1

^{a,b}different letters in a same row indicate a significant difference ($P < 0.05$)

Table 8. Taxonomy abundance of the microbial species among groups (%)

Species	NC	PC	K	WK1	K-WK1
Blautia wexlerae	2.55±2.05	1.05±0.35	2.85±3.23	0.98±0.48	4.59±4.03
Succinivibrio dextrinosolvens	1.69±2.27^{b}	2.05±1.41^{b}	0.85±0.88^{b}	6.45±5.79^{a}	0.35±0.53^{b}
Roseburia faecis	1.57±0.98^{b}	1.39±0.57^{b}	2.79±1.03^{ab}	3.01±0.69^{ab}	3.76±2.2^{a}
Oscillibacter ruminantium	1.29±1.29	1.36±0.29	1.68±1.07	1.86±0.93	1.25±0.78
Eubacterium coprostanoligenes	0.55±0.29^{a}	0.77±0.29^{a}	0.84±0.42^{a}	0.95±0.16^{a}	0.34±0.09^{b}
Blautia obeum	0.45±0.46	0.11±0.08	0.53±0.77	0.56±0.35	0.83±0.64
Blautia luti	0.41±0.40	0.26±0.18	0.33±0.53	0.38±0.2	0.63±0.52
Blautia faecicola	0.32±0.09	0.54±0.20	0.44±0.22	0.56±0.24	0.39±0.18
Blautia faecis	0.18±0.11	0.36±0.41	0.34±0.22	0.28±0.13	0.22±0.18
Helicobacter apri	0.03±0.07	0.01±0.02	0.02±0.02	0.00±0.00	0.00±0.01
Lactobacillus delbrueckii	0.00±0.00	0.02±0.03	0.00±0.00	0.13±0.27	0.03±0.05

NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1

^{a,b}different letters in a same row indicate a significant difference ($P < 0.05$)

Table 9. Effects of different probiotics on growth performance in weaned piglets challenged *E.coli* (Exp.2)

Items		BW, kg				ADG, g				ADFI, g				G:F			
CHAL	PRO	d -5	d 0	d 7	d 11	d-5 to 0	d 0 to 7	d 7 to 11	d 0 to 11	d-5 to 0	d 0 to 7	d 7 to 11	d 0 to 11	d-5 to 0	d 0 to 7	d 7 to 11	d 0 to 11
-	NC	7.95	8.16	9.49	10.57	43	190^{de}	269^{b}	219^{e}	216^{ab}	345^{cd}	415^{abc}	367^{bcd}	0.20	0.55^{bc}	0.65	0.60^{cd}
-	PC	7.98	8.38	10.16	11.30	80	255^{ab}	284^{ab}	265^{b}	212^{ab}	356^{bc}	389^{cd}	360^{cde}	0.36	0.72^{a}	0.73	0.74^{a}
-	K	8.00	8.55	10.34	11.52	110	256.5^{ab}	294^{ab}	270^{b}	210^{abc}	353^{bc}	403^{bcd}	368^{bcd}	0.52	0.73^{a}	0.73	0.74^{a}
-	WK1	8.05	8.32	10.34	11.66	55	289^{a}	329^{a}	303^{a}	215^{ab}	393^{a}	445^{a}	411^{a}	0.26	0.74^{a}	0.74	0.74^{a}
-	K-WK1	8.10	8.72	9.85	10.97	124	161.5^{e}	282^{ab}	205^{ef}	218^{ab}	314^{ef}	424^{ab}	361^{cde}	0.57	0.51^{bc}	0.67	0.57^{de}
+	NC	8.03	8.47	9.59	10.58	89	160^{e}	249^{b}	192^{f}	215^{ab}	332^{de}	403^{bcd}	352^{e}	0.41	0.48^{c}	0.62	0.55^{e}
+	PC	8.05	8.17	9.83	10.99	25	238^{bc}	291^{ab}	256^{bc}	188^{c}	343^{cd}	424^{ab}	370^{bc}	0.13	0.69^{a}	0.68	0.69^{ab}
+	K	8.04	8.68	10.12	11.32	128	206^{cd}	302^{ab}	241^{cd}	230^{a}	302^{f}	441^{a}	356^{de}	0.56	0.68^{a}	0.68	0.68^{b}
+	WK1	8.09	8.24	10.04	11.13	30	258^{ab}	273^{b}	263.5^{b}	205^{bc}	372^{b}	383^{d}	376^{b}	0.15	0.69^{a}	0.71	0.70^{ab}
+	K-WK1	7.98	8.32	9.72	10.85	67	201^{d}	289^{ab}	233^{d}	209^{abc}	353^{bc}	426^{ab}	371^{bc}	0.32	0.57^{b}	0.68	0.63^{c}
CHAL																	
-		8.01	8.42	10.04	11.20	82.40	230.40	291.60	252.40	214.20	352.20	415.20	373.40	0.38	0.65	0.70	0.68
+		8.03	8.37	9.86	10.97	39.00	212.60	280.80	237.10	209.40	340.40	415.40	363.50	0.31	0.62	0.68	0.65
	PRO																
	NC	7.99	8.32	9.54	10.57	66.00^{ab}	175.00^{c}	259.00^{b}	205.50^{d}	215.50^{a}	338.50^{bc}	409.00	359.50^{b}	0.30^{ab}	0.51^{b}	0.63^{b}	0.57^{b}
	PC	8.01	8.28	10.00	11.14	52.50^{b}	246.50^{b}	287.50^{ab}	260.50^{b}	200.00^{b}	349.50^{b}	406.50	365.00^{b}	0.25^{b}	0.70^{a}	0.71^{ab}	0.72^{a}
	K	8.02	8.61	10.23	11.42	119.00^{a}	231.25^{b}	298.00^{a}	255.50^{b}	220.00^{a}	327.50^{c}	422.00	362.00^{b}	0.54^{a}	0.71^{a}	0.71^{ab}	0.71^{a}
	WK1	8.07	8.28	10.19	11.39	42.50^{b}	273.50^{a}	301.00^{a}	283.25^{a}	210.00^{ab}	382.50^{a}	414.00	393.25^{a}	0.20^{b}	0.72^{a}	0.73^{a}	0.72^{a}
	K-WK1	8.04	8.52	9.78	10.91	95.50^{ab}	181.25^{c}	285.50^{ab}	219.00^{c}	213.50^{ab}	333.50^{c}	425.00	361.00^{b}	0.45^{ab}	0.54^{b}	0.67^{ab}	0.60^{b}
P-value	CHAL		0.787	0.387	0.260	0.274	0.001	0.126	<0.001	0.123	<0.001	0.924	<0.001	0.252	0.033	0.088	<0.001
	PRO		0.706	0.189	0.050	0.005	<0.001	0.005	<0.001	0.003	<0.001	0.038	<0.001	0.004	<0.001	0.012	<0.001
	CHAL×PRO		0.776	0.964	0.918	0.071	<0.001	0.026	<0.001	0.001	<0.001	<0.001	<0.001	0.103	0.019	0.863	<0.001
SE		0.08	0.09	0.10	0.10	8.09	7.03	4.42	5.26	2.09	4.17	3.63	2.63	0.04	0.02	0.01	0.01

NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1; CHAL -, non-challenge with *E. coli*; CHAL +, challenge with *E. coli*; PRO, probiotics; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed ratio

[a,b,c,d,e,f,g]different letters in a same row indicate a significant difference ($P < 0.05$)

Table 10. Effects of different probiotics on growth performance in weaned piglets challenged *Salmonella*$^{(Exp.2)}$

Items		BW, kg				ADG, g				ADFI, g				G:F			
CHAL	PRO	d -5	d 0	d 7	d 11	d-5 to 0	d 0 to 7	d 7 to 11	d 0 to 11	d-5 to 0	d 0 to 7	d 7 to 11	d 0 to 11	d-5 to 0	d 0 to 7	d 7 to 11	d 0 to 11
-	NC	7.95	8.16	9.49	10.57	43	190^{cde}	269^{b}	219^{cde}	216^{abc}	345^{bc}	415	367^{b}	0.20	0.55^{bcd}	0.65	0.60^{bcd}
-	PC	7.98	8.38	10.16	11.30	80	255^{ab}	284^{ab}	265^{ab}	212^{abc}	356^{b}	389	360^{b}	0.36	0.72^{ab}	0.73	0.74^{a}
-	K	8.00	8.55	10.34	11.52	110	257^{ab}	294^{ab}	270^{ab}	210^{bc}	353^{b}	403	368^{b}	0.52	0.73^{a}	0.73	0.74^{a}
-	WK1	8.05	8.32	10.34	11.66	55	289^{a}	329^{a}	303^{a}	215^{abc}	393^{a}	445	411^{a}	0.26	0.74^{a}	0.74	0.74^{a}
-	K-WK1	8.10	8.72	9.85	10.97	124	162^{e}	282^{ab}	205de	218^{abc}	314^{d}	424	361^{b}	0.57	0.51^{cd}	0.67	0.57^{cd}
+	NC	8.03	8.41	9.58	10.56	75	168de	244^{b}	195^{e}	228^{ab}	349^{bc}	401	365^{b}	0.33	0.48^{d}	0.61	0.54^{d}
+	PC	7.91	8.50	10.16	11.26	117	237^{abc}	277^{ab}	252^{bc}	220^{abc}	350^{bc}	389	365^{b}	0.54	0.68^{abc}	0.71	0.69^{ab}
+	K	7.98	8.13	9.71	10.80	30	227^{abcd}	273^{b}	243^{bcd}	215^{abc}	332^{cd}	405	364^{b}	0.14	0.68^{ab}	0.67	0.67^{abc}
+	WK1	7.97	8.49	10.09	11.27	105	229^{abcd}	296^{ab}	253^{bc}	230^{a}	351^{bc}	414	369^{b}	0.45	0.65^{abc}	0.71	0.69^{ab}
+	K-WK1	7.96	8.39	9.92	11.00	86	219^{bcde}	269^{b}	237^{bcd}	208^{c}	340^{bc}	399	369^{b}	0.42	0.64^{abcd}	0.67	0.64^{abc}
CHAL																	
-		8.01	8.42	10.04	11.20	82.40	230.60	291.60	252.40	214.20	352.20	415.20	373.40	0.38	0.65	0.70	0.68
+		7.97	8.38	9.89	10.98	82.60	216.00	280.67	236.00	220.20	344.40	401.60	366.40	0.38	0.63	0.68	0.65
	PRO																
	NC	7.99	8.28	9.54	10.56	59.00	179.00^{b}	269.00^{b}	207.00^{b}	222.00	347.00^{b}	408^{ab}	366.00^{b}	0.26	0.52^{b}	0.63^{b}	0.57^{b}
	PC	7.95	8.44	10.16	11.28	98.50	246.00^{a}	280.50^{ab}	258.50^{a}	216.00	353.00^{b}	389.00^{b}	362.50^{b}	0.45	0.70^{a}	0.72^{a}	0.71^{a}
	K	7.99	8.34	10.03	11.16	70.00	242.00^{a}	294.00^{ab}	256.50^{a}	212.50	342.50^{b}	404.00^{b}	366.00^{b}	0.33	0.70^{a}	0.70^{ab}	0.70^{a}
	WK1	8.01	8.41	10.22	11.46	80.00	259.00^{a}	312.50^{a}	278.00^{a}	222.50	372.00^{a}	429.50^{a}	390.00^{a}	0.36	0.69^{a}	0.73^{a}	0.71^{a}
	K-WK1	8.03	8.55	9.88	10.98	105.00	190.50^{b}	275.50^{b}	221.00^{b}	213.00	327.00^{c}	411.50^{ab}	365.00^{b}	0.49	0.58^{b}	0.67^{ab}	0.61^{b}
P-value	CHAL		0.839	0.503	0.318	0.991	0.095	0.008	0.005	0.019	0.006	0.014	0.001	0.940	0.317	0.098	0.037
	PRO		0.946	0.291	0.130	0.444	<0.001	0.001	<0.001	0.027	<0.001	0.001	<0.001	0.380	<0.001	0.004	<0.001
	CHAL×PRO		0.799	0.801	0.791	0.110	0.002	0.785	0.001	0.031	<0.001	0.211	<0.001	0.107	0.029	0.823	0.012
SE		0.08	0.10	0.10	0.11	9.12	7.15	4.56	5.46	1.55	3.25	3.41	2.43	0.04	0.02	0.01	0.01

NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1; CHAL -, non-challenge with *Salmonella*; CHAL +, challenge with *Salmonella*; PRO, probiotics; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed ratio

[a,b,c,d]different letters in a same row indicate a significant difference ($P < 0.05$)

Table 11. Diarrhea score of *E. coli* challenged pigs fed diets supplemented with different probiotics(Exp.2)

Items		Fecal score			
CHAL	PRO	d -5 to 0	d 0 to 7	d 7 to 11	d 0 to 11
-	NC	1.50	1.44[b]	0.38	1.08
-	PC	1.20	0.94[cd]	0.50	0.79
-	K	1.10	1.19[bcd]	0.63	1.00
-	WK1	0.90	1.07[bcd]	0.63	0.92
-	K-WK1	0.80	0.82[d]	0.50	0.71
+	NC	1.80	2.07[a]	0.88	1.67
+	PC	1.60	1.32[bc]	0.88	1.17
+	K	1.50	1.38[b]	1.00	1.25
+	WK1	1.60	1.38[b]	0.75	1.17
+	K-WK1	1.40	1.44[b]	0.38	1.08
CHAL					
-		1.10	1.09	0.53	0.90
+		1.58	1.52	0.78	1.27
	PRO				
	NC	1.65	1.75	0.63	1.38
	PC	1.40	1.13	0.69	0.98
	K	1.30	1.28	0.82	1.13
	WK1	1.25	1.23	0.69	1.05
	K-WK1	1.10	1.13	0.44	0.90
P-value	CHAL	0.001	<0.001	0.059	<0.001
	PRO	0.050	<0.001	0.412	<0.001
	CHAL×PRO	0.695	0.033	0.495	0.110
SE		0.08	0.08	0.06	0.06

NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1; CHAL -, non-challenge with *E. coli*; CHAL +, challenge with *E. coli*; PRO, probiotics

[a,b,c,d]different letters in a same row indicate a significant difference ($P < 0.05$)

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Table 12. Diarrhea score of *Salmonella* challenged pigs fed diets supplemented with different probiotics$^{(Exp.2)}$

Items		Fecal score			
CHAL	PRO	d -5 to 0	d 0 to 7	d 7 to 11	d 0 to 11
-	NC	1.50	1.44^{bc}	0.38	1.08^{de}
-	PC	1.20	0.94^{d}	0.50	0.79^{fg}
-	K	1.10	1.19^{bcd}	0.63	1.00^{e}
-	WK1	0.90	1.07^{cd}	0.63	0.92^{ef}
-	K-WK1	0.80	0.82^{d}	0.50	0.71^{g}
+	NC	2.20	2.32^{a}	0.88	1.83^{a}
+	PC	1.60	1.57^{b}	1.13	1.42^{b}
+	K	1.60	1.44^{bc}	0.75	1.21^{cd}
+	WK1	1.20	1.13^{cd}	0.63	0.96^{ef}
+	K-WK1	1.20	1.57^{b}	0.75	1.29^{bc}
CHAL					
-		1.10	1.09	0.53	0.90
+		1.56	1.61	0.83	1.34
	PRO				
	NC	1.85	1.88	0.63	1.46
	PC	1.40	1.26	0.82	1.11
	K	1.35	1.32	0.69	1.11
	WK1	1.05	1.10	0.63	0.94
	K-WK1	1.00	1.20	0.63	1.00
P-value	CHAL	0.008	<0.001	0.009	<0.001
	PRO	0.019	<0.001	0.667	<0.001
	CHAL×PRO	0.859	0.025	0.265	0.001
SE		0.08	0.08	0.06	0.06

NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1; CHAL -, non-challenge with *Salmonella*; CHAL +, challenge with *Salmonella*; PRO, probiotics

[a,b,c,d]different letters in a same row indicate a significant difference ($P < 0.05$)

Table 13. Effects of different probiotics on nutrient digestibility in weaned piglets challenged *E. coli*(Exp.2)

Items		d 0 to 7			d 7 to 11		
CHAL	PRO	DM	CP	GE	DM	CP	GE
-	NC	87.06	70.52	72.26	89.28	74.71	79.55
-	PC	87.56	71.22	71.36	89.67	74.22	73.52
-	K	87.40	71.49	72.29	89.85	74.68	74.30
-	WK1	87.09	70.08	72.62	89.81	75.39	73.89
-	K-WK1	87.89	70.43	72.81	89.60	74.30	73.64
+	NC	86.76	71.70	69.14	89.47	74.99	79.87
+	PC	87.02	70.72	70.07	89.33	73.67	73.57
+	K	87.47	70.50	70.59	89.32	74.69	73.13
+	WK1	87.08	71.24	71.00	89.33	74.38	74.57
+	K-WK1	87.03	70.15	69.51	89.23	73.84	73.20
CHAL							
-		87.40	70.75	72.27	89.64	74.66	74.98
+		87.07	70.86	70.06	89.34	74.31	74.87
	PRO						
	NC	86.91	71.11	70.70	89.38	74.85	79.71
	PC	87.29	70.97	70.72	89.50	73.95	73.55
	K	87.44	71.00	71.44	89.59	74.69	73.72
	WK1	87.09	70.66	71.81	89.57	74.89	74.23
	K-WK1	87.46	70.29	71.16	89.42	74.07	73.42
P-value	CHAL	0.188	0.871	0.068	0.107	0.316	0.825
	PRO	0.564	0.941	0.987	0.939	0.261	0.872
	CHAL×PRO	0.739	0.785	0.912	0.744	0.777	0.795
SE[1]		0.12	0.31	0.51	0.09	0.17	0.23

NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1; CHAL -, non-challenge with *E. coli*; CHAL +, challenge with *E. coli*; PRO, probiotics; DM, dry matter; CP, crude protein; GE, gross energy

Table 14. Effects of different probiotics on nutrient digestibility in weaned piglets challenged *Salmonella*(Exp.2)

Items		d 0 to 7			d 7 to 11		
CHAL	PRO	DM	CP	GE	DM	CP	GE
-	NC	87.06	70.52	72.26	89.28	74.71	73.55
-	PC	87.56	71.22	71.36	89.67	74.22	73.52
-	K	87.40	71.49	72.29	89.85	74.68	74.30
-	WK1	87.09	70.08	71.62	89.81	75.39	73.89
-	K-WK1	87.89	70.43	72.81	89.6	74.30	73.64
+	NC	86.72	70.08	71.87	90.11	74.33	73.58
+	PC	87.42	70.18	72.35	89.66	74.36	73.34
+	K	86.59	71.39	71.26	89.57	74.04	74.50
+	WK1	87.64	70.30	71.23	89.05	74.73	73.20
+	K-WK1	87.03	70.29	72.25	88.76	74.04	74.11
CHAL							
-		87.40	70.75	72.07	89.64	74.66	73.78
+		87.08	70.45	71.79	89.43	74.30	73.75
	PRO						
	NC	86.89	70.30	72.07	89.70	74.52	73.57
	PC	87.49	70.70	71.86	89.67	74.29	73.43
	K	87.00	71.44	71.78	89.71	74.36	74.40
	WK1	87.37	70.19	71.43	89.43	75.06	73.55
	K-WK1	87.46	70.36	72.53	89.18	74.17	73.88
p-value	CHAL	0.187	0.426	0.795	0.261	0.431	0.859
	PRO	0.372	0.225	0.975	0.327	0.756	0.443
	CHAL×PRO	0.341	0.855	0.981	0.053	0.979	0.609
SE		0.12	0.18	0.47	0.10	0.21	0.22

NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1; CHAL -, non-challenge with *Salmonella*; CHAL +, challenge with *Salmonella*; PRO, probiotics; DM, dry matter; CP, crude protein; GE, gross energy

Table 15. Effects of different probiotics on intestinal morphology in weaned piglets challenged *E. coli*(Exp.2)

Items,	C-					C+					C		PRO					SE	*P*-value		
	NC	PC	K	WK1	K-WK1	NC	PC	K	WK1	K-WK1	-	+	NC	PC	K	WK1	K-WK1		C	PRO	C×PRO
Pre (D-5)																					
WBC, $10^3/\mu\ell$	17.81	17.52	17.64	18.42	17.85	18.64	17.29	17.05	18.58	16.92	17.85	17.70	18.23	17.41	17.35	18.50	17.39	0.18		0.118	
Neu, %	38.18	41.18	40.58	41.02	39.57	40.90	41.38	40.93	42.50	40.15	38.96	41.17	39.54	41.28	40.76	41.77	39.87	0.57		0.993	
Lym, %	47.23	44.03	45.15	44.08	46.13	44.12	44.40	44.68	43.48	45.28	44.46	44.39	45.68	44.22	44.92	43.78	45.71	0.56		0.896	
Mon, %	7.13	7.74	7.83	7.40	7.40	7.28	6.90	7.13	7.18	6.98	7.16	7.09	7.21	7.34	7.48	7.29	7.19	0.30		0.997	
Eos, %	7.36	6.65	6.40	7.40	6.85	7.50	7.27	7.21	6.81	7.54	6.94	7.26	7.43	7.10	6.80	7.10	7.19	0.33		0.995	
Bas, %	0.10	0.10	0.04	0.10	0.05	0.20	0.05	0.05	0.03	0.05	0.08	0.08	0.15	0.07	0.05	0.07	0.05	0.02		0.311	
Post																					
D2																					
WBC, $10^3/\mu\ell$	19.97	14.66	13.44	17.35	19.94	28.08	15.59	17.86	22.16	21.55	17.07	21.05	24.03	15.13	15.65	19.76	20.75	1.06	0.045	0.032	0.768
Neu, %	42.83	42.65	41.68	42.55	41.85	44.05	41.50	41.63	41.80	41.85	42.31	42.17	43.44	42.08	41.66	42.18	41.85	0.47	0.890	0.834	0.961
Lym, %	43.93	44.93	45.25	45.33	45.25	40.95	43.60	43.50	43.65	43.00	44.94	42.94	42.44	44.27	44.38	44.49	44.13	0.49	0.060	0.697	0.988
Mon, %	6.28	5.60	6.20	6.05	5.90	7.43	7.28	7.20	7.10	7.30	6.01	7.26	6.86	6.44	6.70	6.58	6.60	0.27	0.048	0.995	0.996
Eos, %	6.95	6.63	6.78	6.03	6.90	7.56	7.38	7.58	7.40	7.85	6.65	7.55	7.25	7.00	7.17	6.71	7.38	0.15	0.002	0.581	0.921
Bas, %	0.01	0.19	0.09	0.05	0.10	0.01	0.24	0.09	0.05	0.00	0.09	0.06	0.01	0.21	0.09	0.04	0.04	0.02	0.454	<0.01	0.218
D4																					
WBC, $10^3/\mu\ell$	20.91	15.09	16.56	16.77	16.38	29.21	19.72	20.79	22.08	22.75	17.14	22.91	25.06	17.41	18.68	19.43	19.57	0.92	0.001	0.033	0.919
Neu, %	43.68	43.03	43.95	43.83	42.65	47.15	44.95	45.80	44.93	45.15	43.43	45.60	45.42	43.99	44.88	44.38	43.90	0.35	0.002	0.537	0.817
Lym, %	42.23	42.55	43.35	44.55	42.20	41.95	43.08	43.55	44.65	43.45	42.98	43.34	42.09	42.82	43.45	44.60	42.83	0.36	0.632	0.301	0.975
Mon, %	6.65	7.90	5.55	4.95	7.45	5.10	6.10	5.13	4.73	5.83	6.50	5.38	5.88	7.00	5.34	4.84	6.64	0.26	0.022	0.036	0.733
Eos, %	7.30	6.48	7.10	6.63	7.55	5.68	5.30	5.45	5.65	5.48	7.01	5.61	6.49	6.13	6.28	6.13	6.51	0.21	0.000	0.831	0.904
Bas, %	0.14	0.04	0.05	0.04	0.14	0.12	0.07	0.05	0.04	0.09	0.08	0.07	0.12	0.06	0.05	0.05	0.12	0.01	0.802	0.022	0.706

D7																					
WBC, $10^3/\mu\ell$	21.17	18.30	17.25	18.15	18.52	24.60	18.34	19.68	21.60	22.43	18.67	21.33	22.89	18.32	18.46	19.87	20.47	0.81	0.121	0.426	0.950
Neu, %	44.05	41.98	43.31	43.68	42.10	45.20	44.58	46.38	45.38	46.10	43.02	45.53	44.63	43.28	44.85	44.53	44.10	0.47	0.012	0.842	0.884
Lym, %	44.13	45.78	43.88	44.80	44.75	44.38	45.08	44.18	45.10	44.00	44.67	44.55	44.26	45.43	44.03	44.95	44.38	0.39	0.891	0.843	0.987
Mon, %	6.23	6.38	6.50	6.05	6.90	4.95	4.73	3.93	4.18	4.40	6.41	4.44	5.59	5.56	5.22	5.12	5.65	0.21	0.000	0.670	0.546
Eos, %	5.43	5.73	6.15	5.33	6.10	5.30	5.50	5.38	5.20	5.33	5.74	5.33	5.35	5.60	5.76	5.26	5.71	0.12	0.117	0.657	0.830
Bas, %	0.16	0.13	0.16	0.14	0.15	0.17	0.11	0.13	0.14	0.17	0.16	0.15	0.17	0.13	0.14	0.14	0.16	0.01	0.940	0.967	0.992
D11																					
WBC, $10^3/\mu\ell$	18.55	18.83	17.63	18.55	18.15	19.10	19.33	19.13	19.30	20.33	18.34	19.44	18.83	19.08	18.38	18.93	19.24	0.48	0.313	0.989	0.984
Neu, %	44.90	43.55	43.45	43.65	45.00	43.53	41.73	43.35	43.45	45.00	44.11	43.41	44.22	42.64	43.40	43.55	45.00	0.67	0.639	0.881	0.992
Lym, %	47.15	48.20	47.85	46.60	45.45	48.53	49.15	48.35	47.75	46.15	47.05	47.99	47.84	48.68	48.10	47.18	45.80	0.58	0.464	0.658	1.000
Mon, %	4.08	3.30	4.80	5.30	4.90	4.15	4.33	4.78	4.68	4.33	4.47	4.44	4.11	3.81	4.78	4.98	4.61	0.19	0.949	0.308	0.674
Eos, %	3.80	4.85	3.78	4.38	4.58	3.75	4.73	3.48	4.03	4.45	4.28	4.09	3.78	4.79	3.63	4.21	4.52	0.24	0.715	0.586	1.000
Bas, %	0.07	0.10	0.12	0.07	0.07	0.04	0.06	0.04	0.09	0.07	0.09	0.07	0.05	0.08	0.09	0.08	0.07	0.01	0.401	0.946	0.736

NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1; C-, non-challenge with *E. coli*; C+, challenge with *E. coli*; PRO, probiotics; Pre, pre-inoculation; Post, post-inoculation; WBC, white blood cell

[a,b,c]different letters in a same row indicate a significant difference ($P < 0.05$)

Table 16. Effects of different probiotics on intestinal morphology in weaned piglets challenged *Salmonella*(Exp.2)

Items,	C-					C+					C		PRO					SE	*P*-value		
	NC	PC	K	WK1	K-WK1	NC	PC	K	WK1	K-WK1	-	+	NC	PC	K	WK1	K-WK1		C	PRO	C×PRO
Pre (D-5)																					
WBC, $10^3/\mu\ell$	17.81	17.52	17.64	18.42	17.85	17.33	17.78	18.30	17.72	17.28	17.85	17.68	17.57	17.65	17.97	18.07	17.57	0.45		0.996	
Neu, %	38.18	41.18	40.58	41.03	39.58	40.95	41.10	40.40	41.33	41.50	40.11	41.06	39.57	41.14	40.49	41.18	40.54	0.61		0.933	
Lym, %	47.23	44.03	45.15	44.08	46.13	44.23	43.73	45.45	43.75	44.13	45.32	44.26	45.93	43.88	45.30	43.92	45.13	0.56		0.814	
Mon, %	7.13	7.78	7.83	7.40	7.40	7.55	7.03	7.18	7.28	7.13	7.53	7.23	7.34	7.41	7.51	7.34	7.27	0.22		0.999	
Eos, %	7.38	6.65	6.40	7.40	6.85	7.25	8.05	6.88	7.60	7.20	6.98	7.40	7.32	7.29	6.64	7.50	7.03	0.24		0.845	
Bas, %	0.08	0.06	0.04	0.09	0.04	0.02	0.09	0.09	0.04	0.04	0.06	0.05	0.04	0.08	0.08	0.08	0.05	0.01		0.866	
Post																					
D2																					
WBC, $10^3/\mu\ell$	19.97	14.66	13.44	17.35	19.94	23.81	14.54	17.34	21.19	20.27	17.07	19.43	21.89	14.60	15.39	19.27	20.11	0.95	0.208	0.078	0.905
Neu, %	42.83	42.65	41.68	42.55	41.85	45.23	44.28	44.18	44.13	45.10	42.31	44.58	44.03	43.47	42.93	43.34	43.48	0.47	0.026	0.970	0.981
Lym, %	43.93	44.93	45.25	45.33	45.25	44.73	45.03	44.78	45.55	44.68	44.94	44.95	44.33	44.98	45.02	45.44	44.97	0.44	0.988	0.971	0.992
Mon, %	6.28	5.60	6.20	6.05	5.90	4.78	4.70	4.78	4.75	4.73	6.01	4.75	5.53	5.15	5.49	5.40	5.32	0.28	0.041	0.995	0.998
Eos, %	6.95	6.63	6.78	6.03	6.90	5.23	5.80	6.15	5.43	5.40	6.66	5.60	6.09	6.22	6.47	5.73	6.15	0.19	0.008	0.803	0.814
Bas, %	0.03	0.20	0.10	0.05	0.10	0.05	0.20	0.13	0.15	0.10	0.10	0.13	0.04	0.20	0.12	0.10	0.10	0.01	0.183	0.002	0.599
D4																					
WBC, $10^3/\mu\ell$	20.91	15.09	16.56	16.77	16.38	29.30	19.44	20.71	21.63	22.24	16.20	22.66	29.30	17.27	18.64	19.20	19.31	0.96	0.002	0.049	0.923
Neu, %	43.68	43.03	43.95	43.83	42.65	46.14	45.28	45.05	45.38	45.60	43.43	45.49	44.91	44.16	44.50	44.61	44.13	0.36	0.007	0.952	0.927
Lym, %	42.23	42.55	43.35	44.55	42.20	43.65	42.04	44.38	43.10	42.88	42.98	43.21	42.94	42.30	43.87	43.83	42.54	0.37	0.772	0.621	0.774
Mon, %	6.65	7.90	5.55	4.95	7.45	5.40	6.55	5.15	4.90	5.45	6.50	5.49	6.03	7.23	5.35	4.93	6.45	0.26	0.035	0.028	0.675
Eos, %	7.30	6.48	7.10	6.63	7.55	4.70	6.08	5.38	6.55	5.98	7.01	5.74	6.00	6.28	6.24	6.59	6.77	0.23	0.006	0.816	0.361

Bas, %	0.15	0.05	0.05	0.05	0.15	0.11	0.06	0.05	0.08	0.10	0.09	0.08	0.13	0.06	0.05	0.07	0.13	0.01	0.659	0.064	0.769
D7																					
WBC, $10^3/\mu\ell$	21.17	18.30	17.25	18.15	18.52	23.26	18.72	18.52	19.74	21.99	18.67	20.44	22.21	18.51	17.88	18.94	20.25	0.82	0.319	0.549	0.987
Neu, %	44.05	41.98	43.31	43.68	42.10	45.33	45.85	45.88	46.28	45.50	43.02	45.77	44.69	43.92	44.60	44.98	43.80	0.59	0.033	0.966	0.970
Lym, %	44.13	45.78	43.88	44.80	44.75	45.30	44.50	44.63	44.28	43.35	44.67	44.41	44.72	45.14	44.26	44.54	44.05	0.50	0.820	0.976	0.924
Mon, %	6.23	6.38	6.50	6.05	6.90	4.25	4.68	4.48	4.30	5.65	6.41	4.67	5.24	5.53	5.49	5.18	6.28	0.21	0.000	0.214	0.941
Eos, %	5.43	5.73	6.15	5.33	6.10	4.93	4.90	4.93	5.05	5.40	5.75	5.04	5.18	5.32	5.54	5.19	5.75	0.16	0.035	0.750	0.906
Bas, %	0.18	0.15	0.16	0.15	0.15	0.20	0.08	0.10	0.10	0.10	0.16	0.12	0.19	0.11	0.13	0.13	0.13	0.01	0.086	0.327	0.711
D11																					
WBC, $10^3/\mu\ell$	18.55	18.83	17.63	18.55	18.15	18.75	19.10	18.68	19.20	18.68	18.34	18.88	18.65	18.97	18.16	18.88	18.42	0.52	0.651	0.992	0.999
Neu, %	44.90	43.55	43.45	43.65	45.00	44.20	43.68	45.13	43.65	44.13	44.11	44.16	44.55	43.62	44.29	43.65	44.57	0.69	0.977	0.989	0.986
Lym, %	47.15	48.20	47.85	46.60	45.45	46.78	48.53	47.53	46.60	47.38	47.05	47.36	46.97	48.37	47.69	46.60	46.42	0.57	0.809	0.858	0.978
Mon, %	4.08	3.30	4.80	5.30	4.90	4.43	3.43	4.23	5.10	4.20	4.48	4.28	4.26	3.37	4.52	5.20	4.55	0.22	0.648	0.139	0.930
Eos, %	3.80	4.85	3.78	4.38	4.58	4.53	4.33	3.08	4.58	4.18	4.28	4.14	4.17	4.59	3.43	4.48	4.38	0.17	0.696	0.269	0.697
Bas, %	0.08	0.10	0.13	0.08	0.08	0.08	0.05	0.05	0.08	0.08	0.09	0.07	0.08	0.08	0.09	0.08	0.08	0.01	0.332	0.997	0.814

NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1; C-, non-challenge with *Salmonella*; C+, challenge with *Salmonella*; PRO, probiotics; Pre, pre-inoculation; Post, post-inoculation; WBC, white blood cell
[a,b,c]different letters in a same row indicate a significant difference ($P < 0.05$)

Table 17. Effects of different probiotics on intestinal morphology in weaned piglets challenged *E. coli*(Exp.2)

Items, μm		Intestinal morphology		
CHAL	PRO	VH	CD	VH:CD
-	NC	297.22	136.10	2.28
-	PC	369.51	171.43	2.20
-	K	325.27	140.61	2.34
-	WK1	391.36	168.89	2.38
-	K-WK1	366.25	198.32	1.90
+	NC	299.05	189.37	1.58
+	PC	340.62	201.18	1.68
+	K	337.38	188.21	1.79
+	WK1	291.86	163.85	1.92
+	K-WK1	301.39	209.78	1.45
CHAL				
-		349.92	163.07	2.22
+		314.06	190.48	1.68
	PRO			
	NC	298.14	162.74	1.93
	PC	355.07	186.31	1.94
	K	331.33	164.41	2.07
	WK1	341.61	166.37	2.15
	K-WK1	333.82	204.05	1.68
P-value	CHAL	0.024	0.016	< 0.001
	PRO	0.213	0.087	0.170
	CHAL×PRO	0.138	0.403	0.968
SE		8.51	6.05	0.07

NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1; CHAL -, non-challenge with *E. coli*; CHAL +, challenge with *E. coli*; PRO, probiotics; VH, villus height; CD, crypt depth; VH/CD, villus height to crypt depth ratio

[a,b,c,d]different letters in a same row indicate a significant difference ($P < 0.05$)

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Table 18. Effects of different probiotics on intestinal morphology in weaned piglets challenged *Salmonella* (Exp.2)

Items, μm		Intestinal morphology		
CHAL	PRO	VH	CD	VH/CD
-	NC	297.22	136.10	2.28
-	PC	369.51	171.43	2.20
-	K	325.27	140.61	2.34
-	WK1	391.36	168.89	2.38
-	K-WK1	366.25	198.32	1.90
+	NC	300.89	197.17	1.58
+	PC	348.14	175.60	2.02
+	K	350.91	171.69	2.05
+	WK1	307.40	165.64	1.88
+	K-WK1	346.66	204.30	1.71
CHAL				
-		349.92	163.07	2.22
+		330.80	182.88	1.85
	PRO			
	NC	299.06	166.64	1.93
	PC	358.83	173.52	2.11
	K	338.09	156.15	2.20
	WK1	349.38	167.27	2.13
	K-WK1	356.46	201.31	1.81
P-value	CHAL	0.162	0.085	0.002
	PRO	0.051	0.141	0.156
	CHAL×PRO	0.138	0.361	0.481
SE		7.58	5.99	0.06

NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1; CHAL -, non-challenge with *Salmonella*; CHAL +, challenge with *Salmonella*; PRO, probiotics; VH, villus height; CD, crypt depth; VH/CD, villus height to crypt depth ratio

[a,b,c]different letters in a same row indicate a significant difference ($p < 0.05$)

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Table 19. Effects of different probiotics on intestinal bacterial in weaned piglets challenged

Items, log_{10}CFU/g		Small intestine		Large intestine	
CHAL	PRO	*E. coli*	*Salmonella*	*E. coli*	*Salmonella*
-	NC	5.89	3.75	6.63	4.15
-	PC	5.77	3.69	6.59	4.09
-	K	5.84	3.7	6.62	4.07
-	WK1	5.84	3.73	6.65	4.11
-	K-WK1	5.86	3.73	6.65	4.15
+	NC	6.01	3.88	6.73	4.23
+	PC	5.74	3.71	6.61	4.15
+	K	5.77	3.71	6.67	4.19
+	WK1	5.83	3.77	6.67	4.2
+	K-WK1	5.85	3.74	6.68	4.16
CHAL					
-		5.84	3.72	6.63	4.11
+		5.84	3.76	6.67	4.19
	PRO				
	NC	5.95	3.82	6.68	4.19
	PC	5.76	3.70	6.60	4.12
	K	5.81	3.71	6.65	4.13
	WK1	5.84	3.75	6.66	4.16
	K-WK1	5.86	3.74	6.67	4.16
p-value	CHAL	0.995	0.436	0.579	0.203
	PRO	0.297	0.751	0.955	0.947
	CHAL×PRO	0.850	0.970	0.997	0.974
SE		0.03	0.02	0.03	0.03

NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1; CHAL -, non-challenge with *E. coli*; CHAL +, challenge with *E. coli*; PRO, probiotics

Table 20. Effects of different probiotics on intestinal bacterial in weaned piglets challenged *Salmonella*(Exp.2)

Items, log_{10}CFU/g		Small intestine		Large intestine	
CHAL	PRO	*E. coli*	*Salmonella*	*E. coli*	*Salmonella*
-	NC	5.89	3.75	6.63	4.15
-	PC	5.77	3.69	6.59	4.09
-	K	5.84	3.70	6.62	4.07
-	WK1	5.83	3.73	6.65	4.11
-	K-WK1	5.86	3.73	6.65	4.15
+	NC	5.93	3.86	6.81	4.17
+	PC	5.74	3.70	6.54	4.09
+	K	5.79	3.70	6.61	4.09
+	WK1	5.82	3.72	6.62	4.15
+	K-WK1	5.89	3.79	6.71	4.14
CHAL					
-		5.84	3.72	6.63	4.11
+		5.83	3.76	6.66	4.13
	PRO				
	NC	5.91	3.81	6.72	4.16
	PC	5.76	3.70	6.57	4.09
	K	5.82	3.71	6.62	4.08
	WK1	5.83	3.73	6.64	4.13
	K-WK1	5.88	3.76	6.68	4.15
p-value	CHAL	0.900	0.522	0.665	0.792
	PRO	0.548	0.760	0.608	0.848
	CHAL×PRO	0.986	0.967	0.814	0.998
SE		0.03	0.03	0.03	0.02

NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1; CHAL -, non-challenge with *Salmonella*; CHAL +, challenge with *Salmonella*; PRO, probiotics

Figure legend

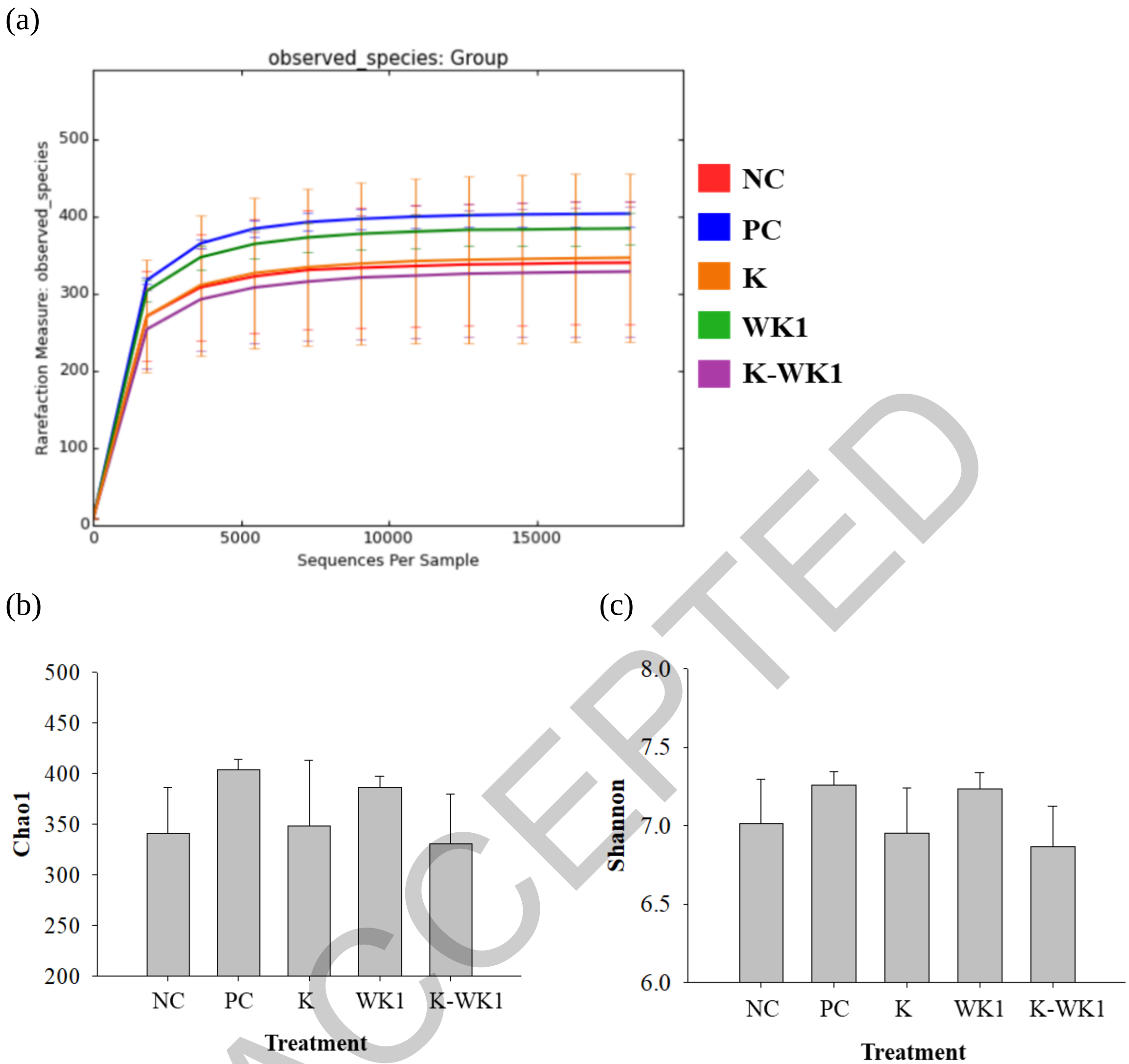

Fig. 1. *α*-diversity for weaned pig fecal microbiota after treatments. (a) Observed species. (b) Chao 1. (c) Shannon. NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1

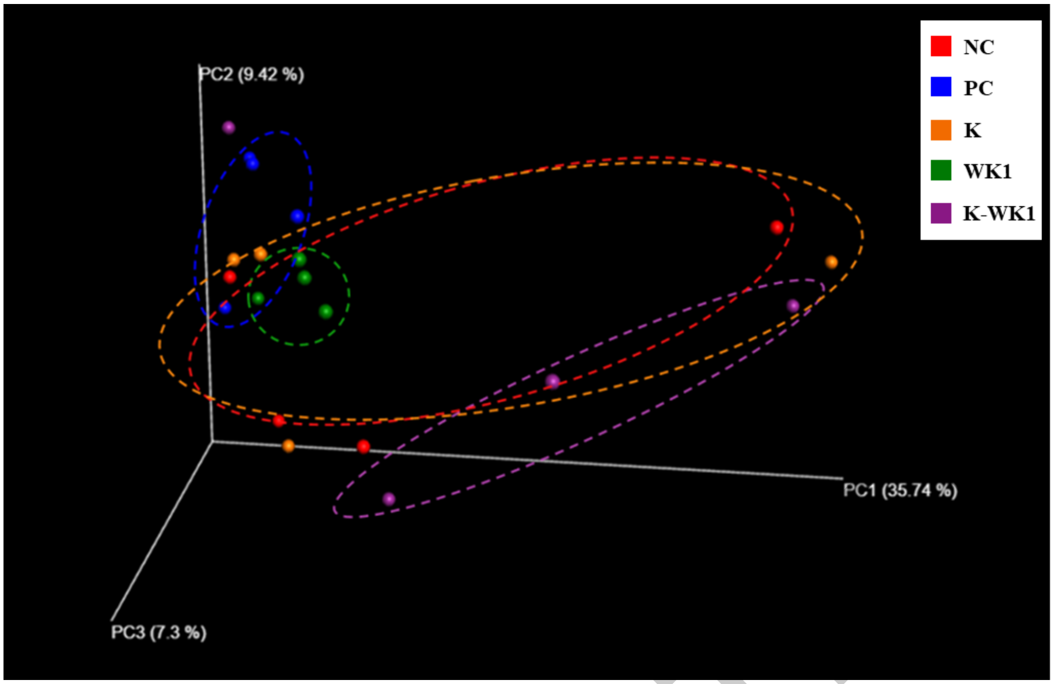

Fig. 2. Principal coordinates analysis for weaned pig fecal microbiota after treatments.

NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1

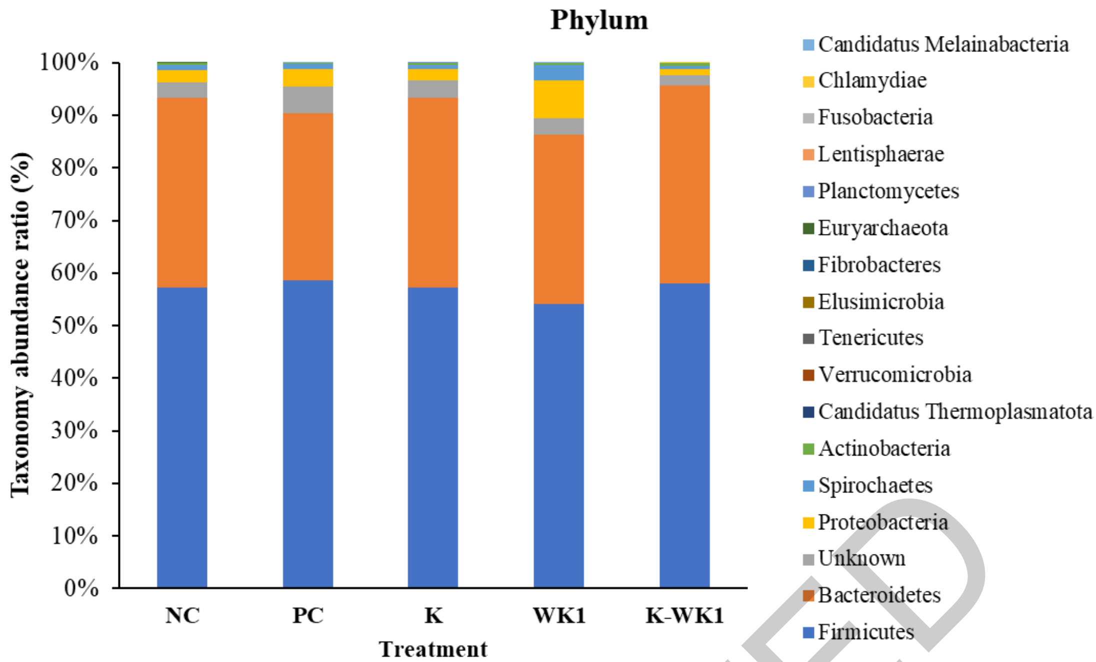

Fig. 3. Taxonomy abundance of the microbial phylum among treatment groups. NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1

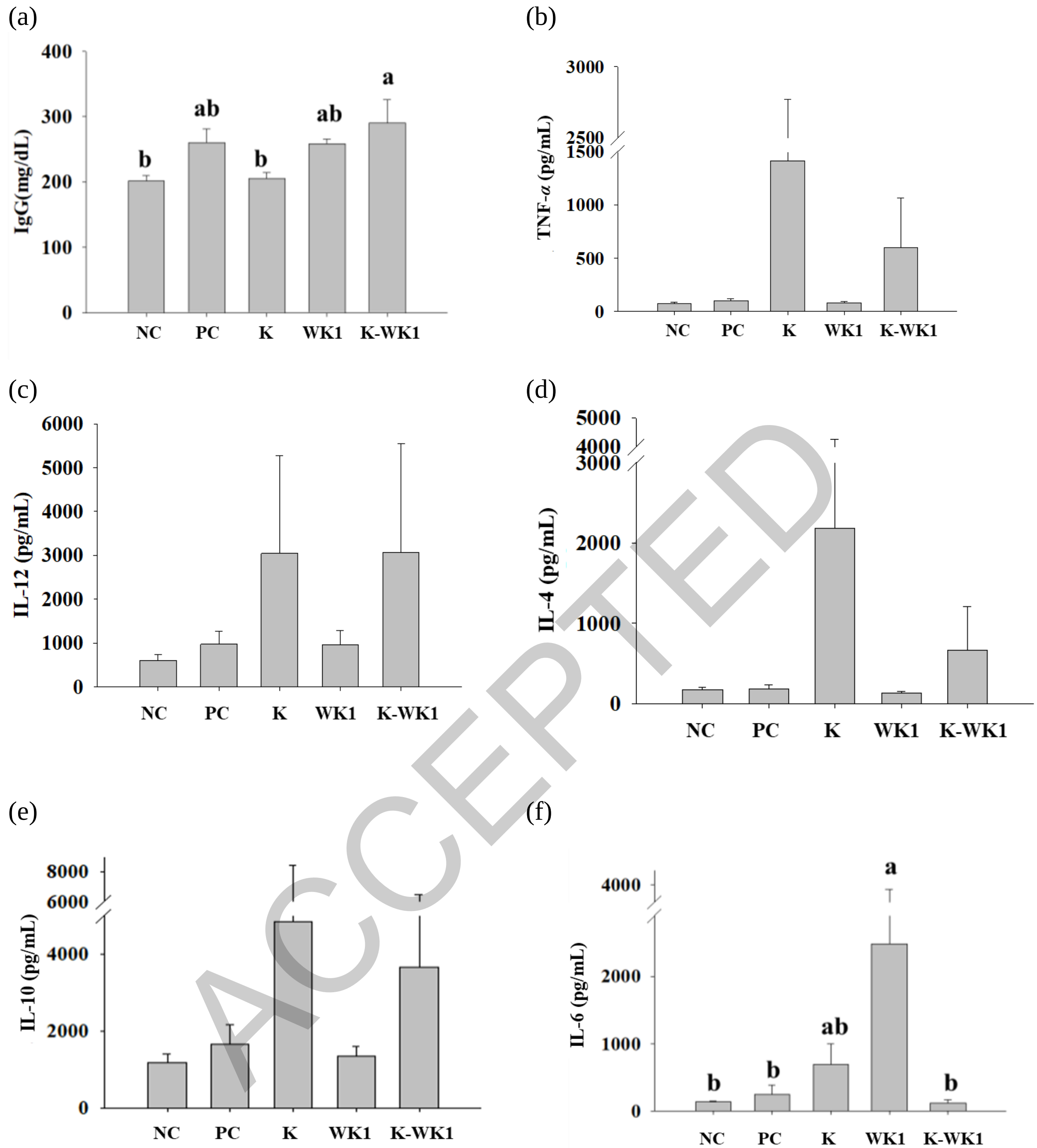

Fig. 4. Concentration of immunoglobulin G and the cytokines in the serum of piglets treated with probiotics. (a) immunoglobulin G. (b) TNF- *α*. (c) IL-12. (d) IL-4. (e) IL-10. (f) IL-6. NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1

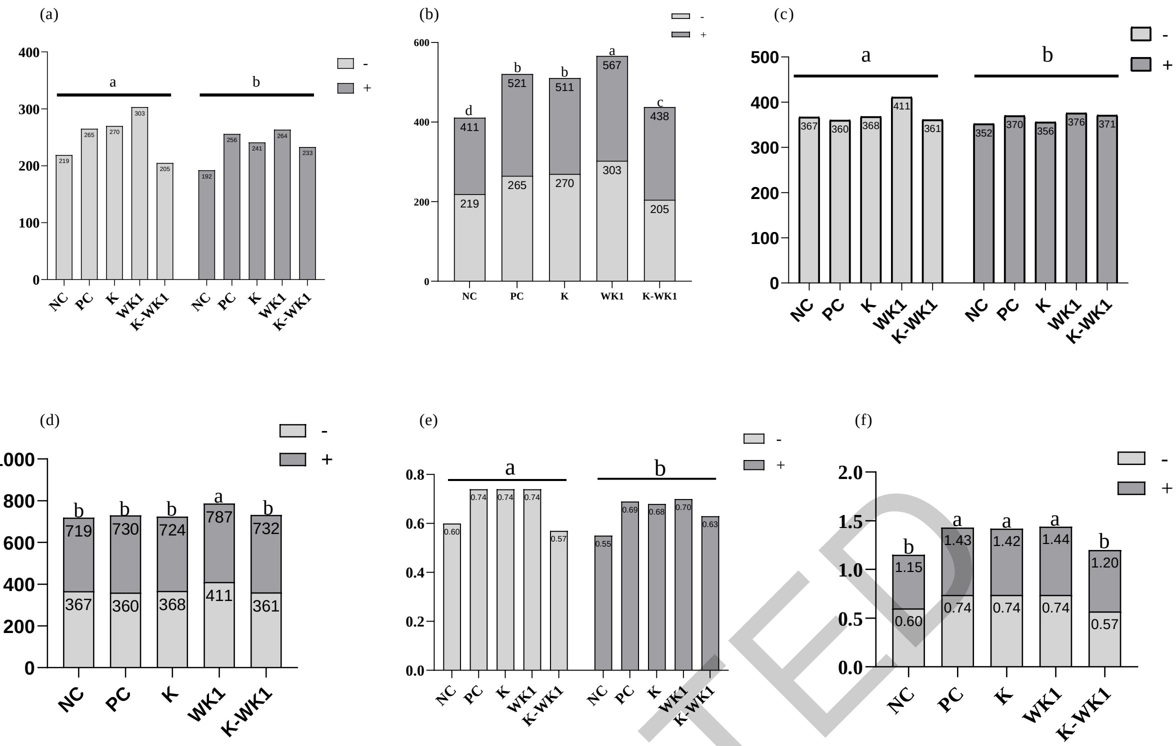

Fig. 5. Growth performance of weaned piglets challenged with *E.coli*. (a) ADG 0 to 11 by *E. coli* challenge (b) comparison of ADG 0 to 11 by different probiotics (c) comparison of ADFI 0 to 11 by *E. coli* challenge (d) comparison of ADFI 0 to 11 by different probiotics (e) comparison of G:F 0 to 11 by *E. coli* challenge (f) comparison of G:F 0 to 11 by different probiotics

NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1; -, non-challenge with *Salmonella*; CHAL +, challenge with *Salmonella*

a,b,c,dMeans scores followed by different superscript in the bar graph indicates statistically significant by the Student′s T test ($P < 0.05$)

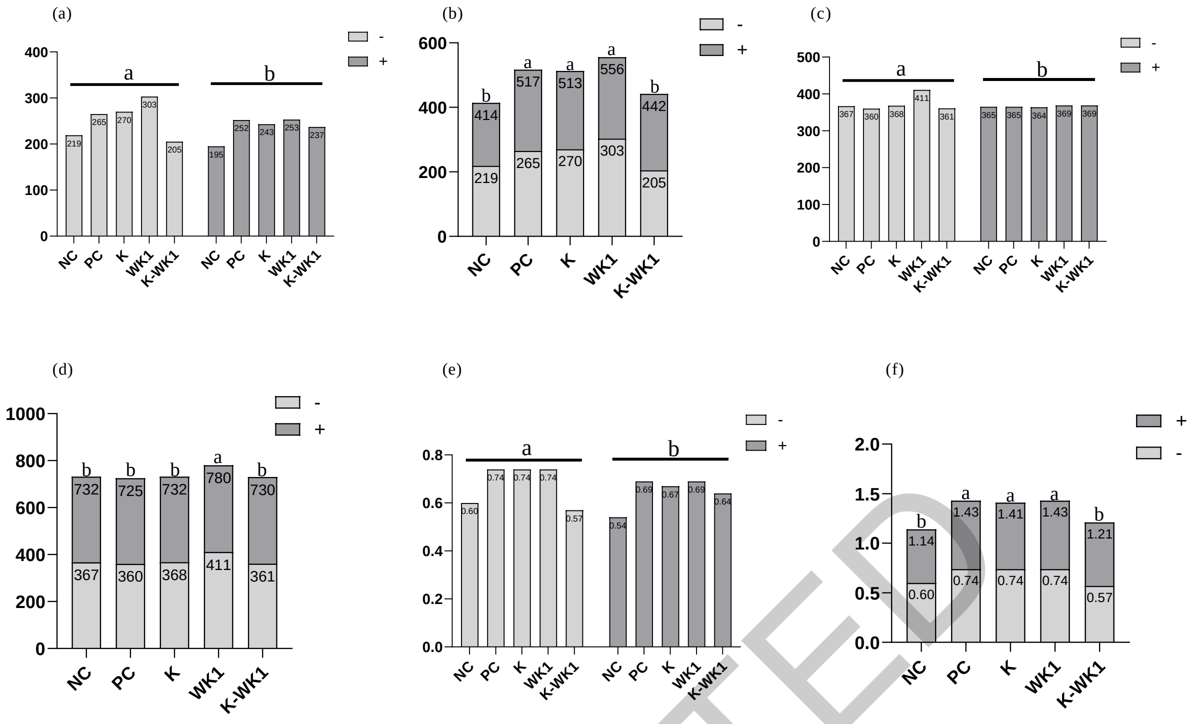

Fig. 6. Growth performance of weaned piglets challenged with *Salmonella*. (a) ADG 0 to 11 by *Salmonella* challenge (b) comparison of ADG 0 to 11 by different probiotics (c) comparison of ADFI 0 to 11 by *Salmonella* challenge (d) comparison of ADFI 0 to 11 by different probiotics (e) comparison of G:F 0 to 11 by *Salmonella* challenge (f) comparison of G:F 0 to 11 by different probiotics

NC, basal diet; PC, NC + 0.01% *Lactiplantibacillus plantarum*; K, NC + 0.1% *Pediococcus acidilactic* K; WK1, NC + 0.1% *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, NC + 0.05% *P. acidilactici* K + 0.05% *P. pentosaceus* SMFM2016-WK1; -, non-challenge with *Salmonella*; CHAL +, challenge with *Salmonella*

a,b,cMeans scores followed by different superscript in the bar graph indicates statistically significant by the Student′s T test ($P < 0.05$)