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ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research
Article Title (within 20 words without abbreviations)	Effects of dietary supplementation of *Pediococcus pentosaceus* strains from kimchi in weaned piglet challenged with *Escherichia coli* and *Salmonella enterica*
Running Title (within 10 words)	Effects of *Pediococcus pentosaceus* isolated from white kimchi in piglet
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Abstract

Escherichia coli (*E. coli*) *and salmonella enterica* (SE) infections in pigs are major source associated with enteric disease such as post weaning diarrhea. The aim of this study was to investigate the effects of *Pediococcus pentosaceus* in weaned piglets challenged with pathogen bacteria. In exp.1 90 weaned piglets with initial body weights of 8.53 ± 0.34 kg were assigned to 15 treatments for 2 weeks. The experiments were conducted 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, respectively and five levels of probiotics (Control, *Lactobacillus plantarum* (LA), *Pediococcus pentosaceus* SMFM2016-WK1 (38W), *Pediococcus acidilactici* K (PK), *Lactobacillus reuteri* PF30 (PF30)). In exp.2 a total of 30 weaned pigs (initial body weight of 9.84 ± 0.85 kg) were used in 4 weeks experiment. Pigs were allocated to 5 groups in a randomized complete way with 2 pens per group and 3 pigs per pen. Supplementation of LA and 38W improved ($p < 0.05$) growth performance, intestinal pathogen bacteria count, fecal noxious odor and diarrhea incidence.

In conclusion, supplementation of 38W strains isolated from white kimchi can act as probiotics by inhibiting *E. coli* and SE.

Keywords (3 to 6): *P. pentosaceus*, *L. plantarum*, growth performance, intestinal pathogen bacteria

Introduction

Weaning is a crucial and stressful period of pig management and usually linked to serious enteric diseases [1]. In the weaning phase, piglets faced great challenges associated with an immature immune and digestive system such as diminished nutrient digestion and changes in intestinal morphology [2]. Although most *Escherichia coli* (*E. coli*) strains are innocuous commensals of the gut microbiome, some types are pathogenic and cause severe intestinal infection such as post weaning diarrhea [3]. Different virulence factors, such as flagella, fimbriae, capsule, lipopolysaccharide and adhesins are involved in their pathogenic mechanisms [4]. Enterotoxigenic *E. coli* (ETEC) and Shigatoxigenic *E. coli* (STEC) are representative types of pathogenic *E. coli*.

Salmonella enterica (SE) has a wide host range, including pigs and humans, causes intestinal diseases. In pigs, SE causes fibrino necrotic enterocolitis, diarrhea, and dehydration [5].

Probiotics are defined as living microorganisms with health benefits for their hosts. Their major effects are gut integrity preservation, antagonism to pathogenic bacteria, immunological modulation and overall health enhancement [6]. Lactic acid bacteria (LAB), commonly used as probiotics, can colonize the digestive tract that increase nutritional digestion and maintain stability of the intestinal flora [7]. Feed fermented by LAB has an antimicrobial effect and withstand vitiation by other microorganisms [8]. Especially, several biological activities

associated with LAB such as *pediococcus* spp. from kimchi, including antioxidative and lipid- lowering properties [9]. According to Wang et al. [10], some species of *Lactobacillus* and *pediococcus* can improve gut health by producing LAB and alleviate pathogen colonization. Especially, *L. plantarum, P. acidilactici* and *L. reuteri* strains improved growth performance with antibacterial activities against pathogens [11,12]. *P. pentosaceus* K10 isolated from kimchi showed inhibitory effect on the bacterial adhesion to intestinal epithelial cells *in vitro* [13]. However, studies about effects of *P. pentosaceus* strains isolated from kimchi *in vivo* are lacking.

Therefore, this study was conducted to determine effects of *P. pentosaceus* strains isolated from white kimchi in weaned piglets.

Materials and Methods

Ethics approval and consent to participate

The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea (approval no. CBNUA-1620-21-02).

Bacterial strains, culture and challenge

STEC F18 and ST was provided in stock form from Dankook University (Cheonan, Korea). The F18 *E. coli* expressed heat labile toxin and Shiga toxin type 2e. Ten microliter of thawed *E. coli* and ST stock were inoculated into 10 mL of nutrient broth and cultured at 37℃ for 24 h and then subcultured [14]. Thereafter, the subcultured *E. coli* and ST were smeared on MacConkey agar (Kisan Biotech Co., Ltd., Seoul, Korea) and Brilliant Green sulfa agar (Kisan Biotech Co., Ltd., Seoul, Korea) to confirm the bacterial enumeration, respectively. A final concentration of 1.2×10^{10} CFU/mL *E. coli* and 2.3×10^{9} CFU/mL SE were used in this study.

Source of probiotics

L. plantarum (LA) was isolated from commercial probiotics supplement (Lactoplan, Genebiotech, Gongju, Korea). *P. pentosaceus* SMFM2016-WK1 (38W) was isolated from White kimchi, *P. acidilactici K* (PK) was isolated from Korean traditional wine and *L. reuteri* (PF30) was isolated from feces of piglets. All of probiotics were received from Sookmyung Women's University (Seoul, Korea). The probiotics were incubated in a stationary state at 37℃ for 48 h in de Man, Rogosa and Sharp (MRS) medium in an anaerobic condition. The viable counts in culture medium were determined by the gradient dilution coating method, stored at 4℃. The final concentration of 2.0×10^{9} CFU/kg probiotics were used in this study.

Experimental design and sample collection

***Exp.* 1.** A total of 90 male (Duroc × Yorkshire × Landrace) weaned pigs (initial body weight of 8.53 ± 0.34 kg and 28 ± 3 d old) were used in 2 weeks experiment. Pigs were individually placed in 45 × 55 × 45 cm stainless steel metabolism cages in an environmentally controlled room. Pigs were allotted to 1 of 15 treatments (6 replication for each treatment) in a completely randomized block design based on initial BW. 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, respectively and five levels of probiotics (Control, *L. plantarum*, *P. pentosaceus* SMFM2016-WK1, *P. acidilactici* K and *L. reuteri* PF30). Corn and soybean meal basal diets were formulated to meet or exceed the nutrient requirements for the weaned piglets [15]. For probiotic treatments, piglets fed the basal diet supplemented with 0.1% of probiotics, respectively. The pigs were fed daily at 8:30 and 17:30 h and had *ad libitum* access to water. Feed residues were removed before the next meal and considered in feed intake calculations. In the *E. coli* and SE challenge treatments, all pigs were orally inoculated with a total of 10 mL of *E. coli* F18 or SE for 3 consecutive days.

***Exp.* 2.** A total of 30 weaned pigs (initial body weight of 9.84 ± 0.85 kg) were used in 4 weeks experiment. Pigs were allocated to 5 groups in a randomized complete way with 2 pens per group and 3 pigs per pen. Dietary treatments included: 1) NC, 2) LA (NC + 0.1% *L. plantarum*), 3) 38W (NC + 0.1% *P. pentosaceus* SMFM2016-WK1), 4) PK (NC + 0.1% *P. acidilactici* K), 5) PF30 (NC + 0.1% *L. reuteri* PF30). The basal diet was formulated to exceed the NRC requirement (Table 1) [15]. Pigs had free access to diets and water.

Growth performance and chemical analysis

Growth performance

All piglets were weighed every week during the experiment period and feed consumption was recorded to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F).

Intestinal microbiota shedding

For intestine *E. coli*, *salmonella* and *lactobacillus* population analysis, samples of small intestine and large intestine were taken 6 pigs per treatment at the end of experiment. The samples were immediately packaged in plastic bags and transferred to the laboratory freezer (-20℃) for the duration of the experiment. To count the number of *Lactobacillus* and *E. coli*, 1 g of samples from each treatment were diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co, Franklin Lakes, NJ, USA) and homogenized. In 6-fold to 4-fold dilution (1% peptone solution) samples were used to analyze the viability of *E. coli* on MacConkey agar plates and

Lactobacillus on de Man, Rogosa, and Sharpe (MRS) agar plates (Kisan Biotech Co., Ltd., Seoul, Korea), BG sulfa agar for *Salmonella*, respectively. *E. coli* and *salmonella* were incubated at 37℃ for 24h and *Lactobacillus* were incubated for 48h.

Nutrient digestibility

To estimate the digestibility, 0.2% chromium oxide (Cr_2O_3) was supplemented with diets as an indigestible marker. Pigs were fed diets mixed with chromium oxide for 4 consecutive days from d 11 to 14 and d 25 to 28, fresh excreta samples were collected in that period. At the end of the experiment, fecal samples were stored at -20℃ and dried at 70°C for 72 h, and then, ground to pass through a 1 mm screen. All analysis items (feed and fecal) were analyzed for dry matter (DM) and crude protein (CP). The procedures utilized for the determination of DM and CP digestibility were conducted with the methods by the [16]. Chromium was analyzed with an ultraviolet absorption spectrophotometer (UV-1201, Shimadzu, Kyoto, Japan). The digestibility was calculated using the following formula: digestibility (%) = [1–(Nf×Cd)/ (Nd×Cf)] ×100, where Nf is the nutrient concentration in feces (% DM), Nd is the nutrient concentration in diet (% DM), Cd is the chromium concentration in diet (% DM), and Cf is the chromium concentration in feces (% DM).

Diarrhea scores

The diarrhea scores were individually recorded at 08:00 and 17:00 by the same person during the entire experimental period. The diarrhea score was scored using a method used by Zhao et al. [17]. The diarrhea scores were as follows: 0, Normal feces; 1, Soft feces; 2, Mild diarrhea; and 3, Severe diarrhea.

Fecal noxious gas emissions

The fecal samples were allowed to ferment for 12 h and 1 day at room temperature (25℃), after which 100 mL of the headspace air was sampled from approximately 2 cm above the fecal sample. Prior to measurement, the fecal samples were manually shaken for approximately 30 s to disrupt any crust formation on the surface of the fecal sample and to homogenize the samples. Ammonium (NH_3) concentrations were determined within the scope of 5.0 - 100.0 ppm (No.3La, detection tube, Gastec Corp., Kanagawa, Japan), hydrogen sulfide (H_2S) concentrations were determined within scope of 2.0 - 20.0 ppm (No.4LK, detection tube, Gastec Corp., Kanagawa, Japan)

Blood profile

Blood samples were obtained from jugular vein 6 pigs per each treatment at the end of experiment. At the time of collection, blood samples were collected into vacuum tubes containing K_3EDTA for complete blood count (CBC) analysis, and nonheparinized tubes for serum analysis, respectively. After collection, blood samples were centrifuged at 3,000 rpm for 15 min at 4℃. The white blood cells (WBC), basophils, neutrophils and lymphocyte levels in the whole blood were measured using an automatic blood analyzer (ADVIA 120, Bayer, NY, USA).

Statistical analysis

Data for effects of different levels of probiotics added with challenge or not. Data were subjected to two-way ANOVA in *Exp*.1 and one way ANOVA in *Exp*.2. Parametric data were statistically analyzed with PROC General Linear Models (GLM) of SAS 9.4 (SAS Institute, Cary, NC, USA). Differences between treatment groups were measured using Duncan's multiple range test with a *p*-value of less than 0.05 designating statistical significance. Non-parametric data (diarrhea score) were analysed using contingency analysis with graphpad prism 8 software (GraphPad Software, San Diego, CA, USA) to test the relationship between categorical variables (scores) and the different combinations tested in this study. A Chi-square test was performed to determine if the different combinations had an effect on the categorical variables repartition with significance accepted at $p < 0.05$.

Results

***Exp.*1**

Growth performance

***E. coli* challenge**

Table 2 shows the results of growth performance of piglets challenged with *E. coli*. *E. coli* challenge decreased ($p = 0.009$) BW on d 14 compared to non-challenge group. Also, *E. coli* challenge decreased ($p < 0.05$) ADG, ADFI, G:F in whole experimental period compared to non-challenge group. Piglets supplemented with LA increased ($p < 0.05$) BW on d 14, ADG and G:F on d 0 to 14 compared to supplementation of CON, PK and PF30. However, supplementation of 38W had no difference on BW, ADG and G:F compared to supplementation of LA except ADG 0 to 14. There was no interaction between supplementation of probiotics and *E. coli* challenge.

SE challenge

Table 3 shows the results of growth performance of piglets challenged with SE. SE challenge decreased ($p < 0.05$) BW on d 7 and 14 compared to non-challenged group. Also, SE challenge decreased ($p < 0.05$) ADG, ADFI, G:F compared to non-challenge group. Supplementation of LA increased ($p < 0.05$) the BW on d 14,

ADG and G:F compared to supplementation of CON, PK and PF30. However, supplementation of 38W had no difference on BW, ADG on d 0 to 7 and G:F compared to supplementation of LA. There was no interaction between supplementation of probiotics and SE challenge.

Intestinal microbiota

***E. coli* challenge**

Table 4 shows the results of intestinal pathogen bacteria counts of piglets challenged with *E. coli*. *E. coli* challenge increased ($p < 0.05$) the counts of *E. coli* in small intestine and large intestine compared to non-challenge groups. Supplementation of probiotic groups showed lower ($p < 0.05$) counts of *E. coli* in small intestine than NC group. Also, supplementation of LA, 38W and PK showed lower ($p < 0.05$) *salmonella* counts in small intestine. In large intestine, supplementation of LA and 38W showed lower ($p < 0.05$) counts of *E. coli* and *salmonella* than other groups. There was an interaction between supplementation of LA, 38W and *E. coli* challenge. Piglets supplemented with LA and 38W with *E. coli* challenge decreased ($p < 0.05$) the counts of *E. coli* compared with piglets supplemented no probiotics with *E. coli* challenge.

SE challenge

Table 5 shows the results of intestinal pathogen bacteria counts of piglets challenged with SE. SE challenge increased ($p < 0.05$) the counts of *salmonella* compared to non-challenge groups. Supplementation of probiotic groups showed lower ($p < 0.05$) counts of *salmonella* in small intestine. Supplementation of LA and 38W groups showed lower ($p < 0.05$) counts *salmonella* than other groups in large intestine and counts of *E. coli* in small intestine. There was an interaction between supplementation of LA, 38W and SE challenge. Piglets supplemented with LA and 38W with SE challenge decreased ($p < 0.05$) the counts of *salmonella* compared to piglets supplemented with no probiotics with SE challenge.

***Exp.*2**

Growth performance

Table 6 shows the results of growth performance of piglets supplemented with probiotics. Supplementation of LA and 38W showed higher ($p < 0.05$) BW than CON group and supplementation of PF30 group on d 28. Also, supplementation of LA and 38W showed higher ($p < 0.05$) ADG and G:F than CON group on d 14 to 28 and d 0 to 28. There was no difference between supplementation of 38W and supplementation of PK group.

Nutrient Digestibility

Table 7 shows the results of nutrient digestibility of piglets supplemented with probiotics. Supplementation of LA and 38W increased ($p < 0.05$) DM and CP digestibility compared to CON group on 2w. Also, supplementation of LA increased ($p < 0.05$) GE digestibility compared to CON group on 2w. In addition, piglets supplemented with 38W increased ($p < 0.05$) CP digestibility compared to CON group on 4w.

Diarrhea scores

Table 8 and figure 1 show the results of diarrhea score and diarrhea incidence of piglets supplemented with probiotics. Piglets supplemented with probiotic decreased ($p < 0.05$) diarrhea scores compared to CON group in whole experimental period. Among these probiotics, supplementation of LA and 38W showed lower ($p < 0.05$) diarrhea score than supplementation of PK and PF30 groups.

Fecal noxious gas emissions

Table 9 shows the results of fecal noxious odor of piglets supplemented with probiotics. Supplementation of LA and PK groups showed lower ($p < 0.05$) NH_3 concentration than NC group on 2w fermented for 12 and 24h. Also, supplementation of LA and 38W groups showed lower ($p < 0.05$) NH_3 concentration than NC group. supplementation of LA decreased ($p < 0.05$) H_2S compared with other groups on 4w fermented for 12h.

Intestinal microbiota

Table 10 shows the results of intestinal pathogen bacteria of piglets supplemented with probiotics. Piglets supplemented with LA showed higher ($p < 0.05$) counts of *Lactobacillus* than other groups on 2w and 4w. There was no difference between supplementation of probiotic groups.

Blood profile

Table 11 shows the results of blood profile of piglets supplemented with probiotics. White blood cell (WBC) including neutrophils, lymphocytes, monocytes, eosinophils and basophils were not affected ($p > 0.05$) by supplementation of probiotics.

Discussion

Weaning stress can cause poor growth performance, with diarrhea being a common issue in weaned piglets [18, 19]. In our experiment, inoculation with *E. coli* and SE to induce weaning stress resulted in poor growth performance and diarrhea, respectively. These results are similar to those of a previous study using a harmful bacterial pathogen to inoculate pigs [20]. Intestinal epithelium functions as a barrier of defense and promotes in nutrition absorption [18]. However, stresses associated with early weaning commonly impair the intestinal barrier and have a negative impact on the growth performance and feed efficiency [21]. Bacterial pathogens can interrupt the release of fluid and electrolytes in the intestine, leading to diarrhea [22].

In the present study, challenged with *E. coli* and SE increased pathogen shedding, respectively. Previous studies reported that *E. coli* and SE challenge had higher shedding compared than non-challenge group [23, 24]. These results confirmed that the challenge model was successful in the present study.

Probiotics are defined as "live microorganism that, when given in sufficient quantities, improve the host's health" [25]. To colonize and cause illness, pathogenic bacteria adhere to the intestinal epithelial membrane [23]. It is hypothesized that probiotics will promote colonization of beneficial microbes, thus preventing harmful bacteria from adhering to the gut epithelium [26]. Production of bacteriocins, proteins with antibacterial characteristics that can limit the function of harmful bacteria, by members of the *Bacillus*, *Lactobacillus* and *Pediococcus* species also has been frequently demonstrated [27, 28].

While *E. coli* and SE challenge exacerbated poor growth performance and pathogen bacteria shedding of challenged pigs, supplementation of probiotics alleviated such poor growth performance and pathogen bacteria shedding in the current metabolic trial. A variety of strains of *L. plantarum* have been shown to be resistant acid and bile and they can be found in gastrointestinal tracts as a useful probiotics [29]. *P. pentosaceus* is the main species used in probiotic supplements for animals [30]. *Lactobacillus* spp. and *Pediococcus* spp. produce antibacterial substances such as organic acid and hydrogen peroxide to inhibit the growth of pathogenic bacteria [31]. In the current experiment, supplementation of LP and 38W improved growth performance and pathogen bacteria shedding in weaned piglets challenged with *E. coli* and SE. Previous studies showed that the *L. plantarum* and *P. pentosaceus* can be used as growth stimulators [32, 33]. Yang et al. [34] have also reported that supplementation of *L. plantarum* could decreased *E. coli* counts. Some strains of *P. pentosaceus* showed antibacterial activity and lower the pH in the intestine by releasing organic acid [35]. In addition, Lan et al. [36] reported that supplementation of *P. pentosaceus* alleviated counts of SE in the cecum of broiler infected with SE. These results of growth performance and pathogen bacteria shedding indicate that supplementation of LA and 38W could enhance intestinal microflora and growth of piglets infected with pathogenic bacteria.

In feeding trial, supplementation of LA and 38W improved growth performance, diarrhea incidence, nutrient digestibility, fecal noxious odor and intestinal microbiome in weaned piglets. In our study, supplementation of LA and 38W increased the BW, ADG and G:F compared to non-supplementation group. These results are consistent with previous studies showing that supplementation of *Lactobacillus* species improved growth performance of pigs [37]. This result might be a link between growth performance and increased nutrient digestibility. Our results showed that supplementation of LA and 38W increased the digestibility of CP, DM and GE on 2w and CP digestibility on 4w.

Furthermore, supplementation of LA and 38W decreased the diarrhea incidence compared with non-supplementation group. Consistent with results of diarrhea incidence, the intestinal microbiome such as *E. coli* and SE was decreased in piglets supplemented with LA and 38W. These results are also in agreement with previous study showing anti-diarrheal activity and anti-pathogenic activity of *L. plantarum* and *L. reuteri* in weaned piglets [38]. *Lactobacillus* species generally reduce pH in the presence of carbohydrate fermentation by

producing lactic acid, suppressing pathogenic bacteria as a result [39]. In addition, fecal noxious gases are affected by the nutrient digestibility and intestinal microbiota [40]. Consistent with our results of digestibility and counts of intestinal pathogen bacteria, supplementation of LA and 38W decreased the concentration of NH_3 and H_2S. *Lactobacillus* based probiotics feed for weaned piglets reduce the emission of total mercaptans, NH_3 and H_2S, because more nutrients are digestible and less substrate for microbial fermentation in the colon [37].

Experiments have been conducted for a long time on of *Lactobacillus* spp. and *P. acidilactici*, but there is lack of study about *P. pentosaceus.* Various LAB, including *Pediococcus* spp. and *Lactobacillus* spp., participate in the fermentation of kimchi and produce an antimicrobial bacteriocin called pediocin, which is produced by *P. pentosaceus* derived from kimchi [41]. Thus, we conducted an experiment about effects of *P. pentosaceus* strains isolated from white kimchi, which is Korean traditional fermented food, as a potential probiotics. In our study, supplementation of 38W enhanced the microbial community and improved the growth performance. As mentioned above, our current study showed that supplementation of 38W had similar effects to LA, used as commercial probiotics. In conclusion, *P. pentosaceus* isolated from white kimchi should be viewed as a candidate medication with antibacterial effects.

CONCLUSION

Weaning pigs supplemented with *P. pentosaceus* isolated from kimchi had improved the growth performance and enhanced the microbial community. Supplementation of *P. pentosaceus* SMFM2016-WK1 have achieved similar effects as *L. plantarum*, which is being used as a commercial probiotics. Therefore, we considered that *P. pentosaceus* SMFM2016-WK1 could be used as a growth stimulator and medication with antibacterial effects in pigs.

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405 **Tables and Figures**

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Table.1 Ingredient composition of the experimental diets in *Exp*.1 and 2

Items	Content
Ingredients, %	
Corn	34.43
Extruded corn	15.00
Lactose	10.00
Dehulled soybean meal, 51% CP[1]	13.50
Soy protein concentrate, 65% CP[1]	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[2]	0.25
Trace mineral premix[3]	0.25
Salt	0.40
Total	100.00
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

[1] CP, crude protein.
[2] 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; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic, 29 mg; choline, 166 mg; and vitamin B_{12}, 33 mg.
[3] Provided per kg of complete diet without Zinc: Cu (as $CuSO_4 \cdot 5H_2O$), 12 mg; Mn (as MnO_2), 8 mg; I (as KI), 0.28 mg; and Se (as $Na_2SeO_3 \cdot 5H_2O$), 0.15 mg.

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Table 2. Effects of dietary probiotics on growth performance of piglets challenged with *E. coli* in *Exp*.1

Items		BW, kg		d 0 to 7				d 7 to 14			d 0 to 14		
CHAL	PRO	d 0	d 7	d14	ADG, g	ADFI, g	G:F	ADG, g	ADFI, g	G:F	ADG, g	ADFI, g	G:F
-	NC	8.53	11.33	14.50ab	400.43	591.67	0.68	452.38	745.33	0.61	426.40	668.50	0.64
-	LA	8.47	11.93	16.23a	494.76	618.33	0.80	614.29	803.33	0.76	554.52	710.83	0.78
-	38W	8.58	11.73	15.17ab	450.48	583.33	0.77	490.48	745.00	0.66	470.48	664.17	0.71
-	PK	8.52	11.70	14.97ab	454.29	600.00	0.76	466.67	755.00	0.62	460.48	677.50	0.68
-	PF30	8.48	11.20	14.40ab	388.10	540.00	0.72	457.14	690.00	0.66	422.62	615.00	0.69
+	NC	8.51	10.67	13.50b	308.57	566.67	0.54	404.76	825.00	0.49	356.67	695.83	0.51
+	LA	8.50	11.83	15.47ab	476.19	665.00	0.72	519.05	791.67	0.66	497.62	728.33	0.68
+	38W	8.50	11.47	14.53ab	424.29	678.33	0.63	438.10	795.00	0.55	431.19	736.67	0.59
+	PK	8.59	11.20	14.03b	373.33	635.00	0.59	404.76	765.00	0.53	389.05	700.00	0.56
+	PF30	8.58	11.10	14.00b	360.48	595.00	0.61	414.29	780.00	0.53	387.38	687.50	0.56
CHAL													
-		8.52	11.58	15.05	437.61	586.67	0.75	496.19	747.73	0.66	466.90	667.20	0.70
+		8.53	11.25	14.31	388.57	628.00	0.62	436.19	791.33	0.55	412.38	709.67	0.58
	PRO												
	NC	8.52	11.00	14.00b	354.50d	579.17	0.61b	428.57b	785.17	0.55	391.54b	682.17	0.57b
	LA	8.49	11.88	15.85a	485.48a	641.67	0.76a	566.67a	797.50	0.71	526.07a	719.58	0.73a
	38W	8.54	11.60	14.85ab	437.38ab	630.83	0.69ab	464.29ab	770.00	0.60	450.83b	700.42	0.64ab
	PK	8.55	11.45	14.5b	413.81bc	617.50	0.67ab	435.71b	760.00	0.57	424.76b	688.75	0.62b
	PF30	8.53	11.15	14.2b	374.29cd	567.50	0.66ab	435.71b	735.00	0.59	405.00b	651.25	0.62b
p-value	CHAL	0.939	0.178	0.009	<0.001	0.034	<0.001	0.044	0.048	0.021	<0.001	0.035	<0.001
	PRO	1.000	0.156	0.001	<0.001	0.068	0.002	0.021	0.418	0.183	<0.001	0.275	0.003
	CHAL × PRO	0.999	0.926	0.961	0.165	0.394	0.876	0.981	0.513	1.000	0.877	0.809	0.998
SE[1]		0.341	0.378	0.435	18.724	30.037	0.037	45.902	34.033	0.064	21.669	30.988	0.038

Abbreviation: -, non-challenged with *E. coli*; +, challenged with *E. coli*; NC, non-supplemented with probiotics; LA, *L. plantarum;* 38W, *P. pentosaceus* SMFM2016-WK1; PK, *P. acidilactici* K*;* PF30, *L. reuteri*; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, feed efficiency

a,b.c.d Values within a row with different superscripts are significantly different.

[1]SE, standard error

Table 3. Effects of dietary probiotics on growth performance of piglets challenged with *Salmonella* in *Exp*.1

Items		BW, kg			d 0 to 7			d 7 to 14			d 0 to 14		
CHAL	PRO	d 0	d 7	d14	ADG, g	ADFI, g	G:F	ADG, g	ADFI, g	G:F	ADG, g	ADFI, g	G:F
-	NC	8.53	11.33	14.50	400.43	591.67	0.68	452.38	745.33	0.61	426.40	668.50	0.64
-	LA	8.47	11.93	16.23	494.76	618.33	0.80	614.29	803.33	0.76	554.52	710.83	0.78
-	38W	8.58	11.73	15.17	450.48	583.33	0.77	490.48	745.00	0.66	470.48	664.17	0.71
-	PK	8.52	11.70	14.97	454.29	600.00	0.76	466.67	755.00	0.62	460.48	677.50	0.68
-	PF30	8.48	11.20	14.40	388.10	540.00	0.72	457.14	690.00	0.66	422.62	615.00	0.69
+	NC	8.58	10.73	13.57	308.10	565.00	0.55	404.76	810.00	0.50	356.43	687.50	0.52
+	LA	8.58	11.67	15.23	440.48	665.00	0.66	509.52	825.00	0.62	475.00	745.00	0.64
+	38W	8.48	11.27	14.47	397.62	645.00	0.62	457.14	830.00	0.55	427.38	737.50	0.58
+	PK	8.50	10.97	14.03	351.90	615.00	0.57	438.10	835.00	0.52	395.00	725.00	0.54
+	PF30	8.51	10.97	13.93	350.33	625.00	0.56	423.81	815.00	0.52	387.07	720.00	0.54
CHAL													
-		8.52	11.58	15.05	437.61	586.67	0.75	496.19	747.73	0.66	466.90	667.20	0.70
+		8.53	11.12	14.25	369.69	623.00	0.59	446.67	823.00	0.54	408.18	723.00	0.56
	PRO												
	NC	8.55	11.03	14.03b	354.26d	578.33	0.61	428.57b	777.67	0.55b	391.42c	678.00	0.58b
	LA	8.53	11.80	15.73a	467.62a	641.67	0.73	561.90a	814.17	0.69a	514.76a	727.92	0.71a
	38W	8.53	11.50	14.82ab	424.05ab	614.17	0.69	473.81b	787.50	0.60ab	448.93b	700.83	0.64ab
	PK	8.51	11.33	14.50b	403.10bc	607.50	0.66	452.38b	795.00	0.57b	427.74bc	701.25	0.61b
	PF30	8.50	11.08	14.17b	369.21cd	582.50	0.63	440.48b	752.50	0.59ab	404.85bc	667.50	0.61b
p-value	CHAL	0.941	0.047	0.001	<0.001	0.089	<0.001	0.015	0.001	<0.001	<0.001	0.010	<0.001
	PRO	1.000	0.201	<0.001	<0.001	0.319	0.107	0.001	0.496	0.014	<0.001	0.414	0.002
	CHAL × PRO	0.998	0.948	0.948	0.270	0.495	0.983	0.719	0.679	0.977	0.688	0.717	0.997
SE[1]		0.330	0.357	0.370	17.047	33.126	0.046	30.963	34.705	0.040	17.489	33.105	0.032

Abbreviation: CHAL, challenge; PRO, probiotics; -, non-challenged with *E. coli*; +, challenged with *E. coli*; NC, non-supplemented with probiotics; LA, *L. plantarum*; 38W, *P. pentosaceus* SMFM2016-WK1; PK, *P. acidilactici* K*;* PF30, *L. reuteri*

a,b,c,d Values within a row with different superscripts are significantly different.

[1]SE, standard error

Table 4. Effects of dietary probiotics on intestinal microbiota of piglets challenged with *E. coli* in *Exp*.1

Items, log_{10}CFU/g		Small intestine		Large intestine	
CHAL	PRO	*Escherichia coli*	*Salmonella*	*Escherichia coli*	*Salmonella*
-	NC	4.71c	2.68	5.54d	2.91
-	LA	4.59c	2.53	5.49d	2.79
-	38W	4.58c	2.50	5.55d	2.75
-	PK	4.66c	2.48	5.59d	2.92
-	PF30	4.67c	2.51	5.51d	2.97
+	NC	6.95a	2.59	7.55a	2.98
+	LA	6.33b	2.41	6.83c	2.78
+	38W	6.34b	2.49	7.02bc	2.79
+	PK	6.58ab	2.55	7.46c	2.92
+	PF30	6.49b	2.60	7.39ab	2.96
CHAL					
-		4.64	2.54	5.54	2.87
+		6.54	2.53	7.25	2.89
	PRO				
	NC	5.83a	2.63a	6.55a	2.95a
	LA	5.46b	2.47b	6.16c	2.79b
	38W	5.46b	2.50b	6.29bc	2.77b
	PK	5.62b	2.52b	6.53a	2.92a
	PF30	5.58b	2.56ab	6.45ab	2.97a
p-value	CHAL	<0.001	0.710	<0.001	0.651
	PRO	0.002	0.035	0.001	0.013
	CHAL × PRO	0.048	0.178	0.004	0.954
SE[1]		0.085	0.050	0.087	0.064

Abbreviation: CHAL, challenge; PRO, probiotics; -, non-challenged with *E. coli*; +, challenged with *E. coli*; NC, non-supplemented with probiotics; LA, *L. plantarum*; 38W, *P. pentosaceus* SMFM2016-WK1; PK, *P. acidilactici* K*;* PF30, *L. reuteri*

a,b,c,d Values within a row with different superscripts are significantly different.

[1]SE, standard error

Table 5. Effects of dietary probiotics on intestinal microbiota of piglets challenged with *Salmonella* in *Exp*.1

Items, log_{10}CFU/g		Small intestine		Large intestine	
CHAL	PRO	*Escherichia coli*	*Salmonella*	*Escherichia coli*	*Salmonella*
-	NC	4.71	2.68c	5.54	2.91c
-	LA	4.59	2.53c	5.49	2.79c
-	38W	4.58	2.5c	5.55	2.75c
-	PK	4.66	2.48c	5.59	2.92c
-	PF30	4.67	2.51c	5.51	2.97c
+	NC	4.80	3.98a	5.49	4.46a
+	LA	4.54	3.66b	5.56	4.12b
+	38W	4.53	3.65b	5.60	4.07b
+	PK	4.71	3.91a	5.62	4.65a
+	PF30	4.77	3.81ab	5.57	4.61a
CHAL					
-		4.64	2.54	5.54	2.87
+		4.67	3.80	5.57	4.38
	PRO				
	NC	4.76a	3.33a	5.52	3.69a
	LA	4.57b	3.10bc	5.53	3.46b
	38W	4.56b	3.08c	5.58	3.41b
	PK	4.69a	3.20b	5.61	3.79a
	PF30	4.72a	3.16bc	5.54	3.79
p-value	CHAL	0.402	<0.001	0.627	<0.001
	PRO	0.002	<0.001	0.896	<0.001
	CHAL × PRO	0.423	0.031	0.977	0.007
SE[1]		0.052	0.048	0.102	0.060

Abbreviation: CHAL, challenged; PRO, probiotics; -, non-challenged with *Salmonella*; +, challenged with *Salmonella*; NC, non-supplemented with probiotics; LA, *L. plantarum;* 38W, *P. pentosaceus* SMFM2016-WK1; PK, *P. acidilactici* K*;* PF30, *L. reuteri*

a,b,c Values within a row with different superscripts are significantly different.

[1]SE, standard error

Table 6. Effects of dietary probiotics on growth performance of weaned piglets in *Exp*.2

Items	NC	LA	38W	PK	PF30	SE[1]	*p* -value
BW, kg							
D0	9.80	9.77	9.92	9.82	9.88	0.379	0.999
D14	14.38ab	15.30a	14.75ab	15.23ab	14.23b	0.246	0.014
D28	21.18c	24.75a	23.45ab	22.03bc	20.92c	0.514	<0.001
ADG, g							
D0 to 14	327.33	394.17	345.33	386.83	310.67	22.834	0.063
D14 to 28	485.00b	676.33a	621.50a	485.67b	477.33b	25.318	<0.001
D0 to 28	406.17c	535.00a	483.33ab	436.33bc	394.00c	14.273	<0.001
ADFI, g							
D0 to 14	545.00	545.67	486.67	606.67	558.00	34.085	0.212
D14 to 28	1120.00	1125.00	1040.00	1010.00	1030.00	33.342	0.061
D0 to 28	832.50	835.33	763.33	808.83	794.33	25.318	0.271
G:F							
D0 to 14	0.60b	0.73a	0.72a	0.64ab	0.56b	0.026	<0.001
D14 to 28	0.44b	0.60a	0.60a	0.48b	0.46b	0.025	<0.001
D0 to 28	0.49b	0.64a	0.63a	0.54b	0.50b	0.016	<0.001

Abbreviation: NC, non-supplemented with probiotics; LA, *L. plantarum;* 38W, *P. pentosaceus* SMFM2016-WK1; PK, *P. acidilactici* K*;* PF30, *L. reuteri*; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, feed efficiency

a,b,c Values within a row with different superscripts are significantly different.

[1]SE, standard error

Table 7. Effects of dietary probiotics on nutrient digestibility of weaned piglets in *Exp*.2

Items, %	NC	LA	38W	PK	PF30	SE[1]	*p* -value
2W							
DM	79.15b	80.62a	80.58a	79.13b	79.09b	0.344	0.002
CP	72.16c	75.70a	74.71ab	73.52abc	73.14bc	0.538	0.001
GE	74.40b	76.51a	75.74ab	74.44b	74.62b	0.410	0.003
4W							
DM	77.14	77.24	77.58	76.61	77.12	0.264	0.173
CP	71.38b	73.63ab	74.17a	72.36ab	72.66ab	0.584	0.021
GE	71.84	72.25	72.61	71.48	72.13	0.302	0.130

Abbreviation: NC, non-supplemented with probiotics; LA, *L. plantarum;* 38W, *P. pentosaceus* SMFM2016-WK1; PK, *P. acidilactici* K*;* PF30, *L. reuteri*; DM, dry matter; CP, crude protein; GE, gross energy

a,b,c Values within a row with different superscripts are significantly different.

[1]SE, standard error

Table 8. Effects of dietary probiotics on diarrhea score of weaned piglets in *Exp*.2

Items	NC	LA	38W	PK	PF30	SE[1]	*p*-value
Diarrhea score							
D 1 to 14	1.190a	0.917b	0.917b	1.214a	1.178a	0.049	<0.001
D 15 to 28	0.893a	0.440c	0.464c	0.655b	0.631b	0.016	<0.001
D 1 to 28	1.042a	0.679c	0.690c	0.935b	0.905b	0.024	<0.001

Abbreviation: NC, non-supplemented with probiotics; LA, *L. plantarum;* 38W, *P. pentosaceus* SMFM2016-WK1; PK, *P. acidilactici* K*;* PF30, *L. reuteri*

a,b,c Values within a row with different superscripts are significantly different.

[1]SE, standard error

Table 9. Effects of dietary probiotics on fecal noxious odor of weaned piglets in *Exp*.2

Items, ppm		NC	LA	38W	PK	PF30	SE[1]	*p*-value
2W								
12h	NH_3	27.43a	18.37c	22.87b	23.43b	23.50b	0.379	<0.001
	H_2S	6.73	3.97	5.67	5.13	6.57	0.785	0.115
24h	NH_3	34.63a	31.13bc	34.53ab	30.60c	32.27abc	0.826	0.004
	H_2S	10.33	8.57	8.87	9.57	8.83	0.612	0.271
4W								
12h	NH_3	22.80a	15.43c	19.43b	20.97ab	20.60ab	0.581	<0.001
	H_2S	4.80a	3.20b	4.50a	4.03a	4.30a	0.195	<0.001
24h	NH_3	32.87a	28.23b	28.57b	28.40b	29.47ab	0.907	0.007
	H_2S	9.17	7.73	8.30	8.67	8.40	0.466	0.311

Abbreviation: NC, non-supplemented with probiotics; LA, *L. plantarum;* 38W, *P. pentosaceus* SMFM2016-WK1; PK, *P. acidilactici* K*;* PF30, *L. reuteri*
a,b,c Values within a row with different superscripts are significantly different.
[1]SE, standard error

Table 10. Effects of dietary probiotics on intestinal microbiota of weaned piglets in *Exp*.2

Items, log_{10}CFU/g	NC	LA	38W	PK	PF30	SE[1]	*p*-value
2w							
Escherichia coli	5.35	5.25	5.29	5.40	5.37	0.068	0.551
Lactobacillus	7.00c	7.59a	7.22b	7.16bc	7.18bc	0.045	<0.001
4w							
Escherichia coli	4.54	4.44	4.44	4.48	4.50	0.064	0.748
Lactobacillus	7.11c	7.68a	7.43b	7.25bc	7.30bc	0.055	<0.001

Abbreviation: NC, non-supplemented with probiotics; LA, *L. plantarum;* 38W, *P. pentosaceus* SMFM2016-WK1; PK, *P. acidilactici* K*;* PF30, *L. reuteri*
a,b,c Values within a row with different superscripts are significantly different.
[1]SE, standard error

Table 11. Effects of dietary probiotics on blood profile of weaned piglets in *Exp*.2

Items	NC	LA	38W	PK	PF30	SE[1]	*p*-value
WBC, $10^3/\mu\ell$	16.90	18.10	18.21	18.01	18.35	1.463	0.958
Neutrophil, %	44.35	44.08	45.58	44.83	45.62	1.242	0.865
Lymphocyte, %	43.95	44.58	43.90	43.87	43.40	1.039	0.954
Monocyte, %	9.48	8.90	8.37	8.96	8.78	1.096	0.969
Eosinophil, %	1.15	1.32	1.08	1.11	1.07	0.212	0.917
Basophil, %	1.07	1.12	1.08	1.23	1.13	0.241	0.990

Abbreviation: NC, non-supplemented with probiotics; LA, *L. plantarum;* 38W, *P. pentosaceus* SMFM2016-WK1; PK, *P. acidilactici* K; PF30, *L. reuteri*; WBC, white blood cell

[1]SE, standard error