Comparison of efficacy of lactic acid bacteria complex and Enterococcus faecium DSM 7134 in weanling pigs

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**Abstract**
We conducted this study to compare the effects of lactic acid bacteria complex (L. casei, L. rhamnosus, L. lactis, L. plantarum, S. thermophilus, and B. longum) and Enterococcus faecium DSM 7134 in weanling pigs. A total of 120 weanling pigs (24-day-old) were used in a 5-week feeding trial. Pigs were allotted into three dietary treatments: CON, basal diet; LA, CON + 0.1% lactic acid bacteria complex; EF, and CON + 0.1% E. faecium DSM 7134. From days 0 to 14, days 15 to 35, and the overall period, average daily gain was higher (*P < .05*) in EF than that in CON. Higher gain:feed ratio was also observed in EF compared with CON during the overall period. The apparent total tract digestibility of dry matter, nitrogen, and gross energy was greater (*P < .05*) in LA and EF compared with CON on days 14 and 35. Faecal Lactobacillus counts were increased (*P < .05*) and faecal pH was decreased in LA and EF compared with CON on days 14 and 35. Results of the current study indicated that E. faecium DSM 7134 has better effects on growth performance than lactic acid bacteria complex in weanling pigs.

1. **Introduction**
Probiotics which are harmless bacteria or yeast species have been taken into consideration as one alternative of antibiotics in the pig industry (Chen et al. 2006; Meng et al. 2010; Zhao and Kim 2015). Commercial probiotics could be divided into three categories: Bacillus, Lactic acid-producing bacteria, and Saccharomyces (Roselli et al., 2005; Stein and Kil, 2006). Lactic acid-producing bacteria are acids procedures, which can lower the gut pH, benefit gut functions, inhibit enteric pathogens, and improve the host immunity (Guerra et al. 2007; Giang et al. 2010; Choi et al. 2011; Lojanica et al. 2010; Zhang et al. 2014), therefore, it is not surprising substantial interest exists in providing weaning pigs with lactic acid-producing bacteria to restore the gut balance.

Sanders and Veld (1999) reported that multi-strain probiotics may be more effective than single-strain probiotics and could amplify the protective spectrum against microbial infections. Chapman et al. (2011) also reported that multi-strain probiotics appear to show greater efficacy than single-strain probiotics. Therefore, we hypothesis that lactic acid bacteria complex could be more effective than Enterococcus faecium DSM 7134. The aim of this study was to investigate which of them has better effects on growth performance, nutrient digestibility, faecal microflora, and faecal characteristics in weanling pig.

2. **Material and methods**

2.1. **Source of probiotics**
The probiotic preparation used in the current experiment was provided by two commercial companies (Duolac, Cell Biotech Co., Ltd., Seoul, South Korea; Bonvital, Schaumann Agri International GmbH, Pinneberg, Germany). The lactic acid bacteria complex (Duolac) consists of Lactobacillus casei (4.0 × 10⁸ cfu/g), Lactobacillus rhamnosus (2.0 × 10⁸ cfu/g), Lactobacillus lactis (2.0 × 10⁸ cfu/g), Lactobacillus plantarum (2.0 × 10⁸ cfu/g), Streptococcus thermophilus (5.5 × 10⁶ cfu/g), and Bifidobacterium longum (2.5 × 10⁸ cfu/g). The other probiotic (Bonvital) was composed of at least 1.0 × 10¹⁰ cfu/g spray-dried spore-forming E. faecium. The method for cfu determination was based on Sieuwerts et al. (2008).

2.2. **Experimental design, animals and housing**
A total of 120 weanling pigs [(Yorkshire × Landrace) × Duroc] with an average body weight (BW) of 8.47 ± 0.72 kg (24 days of age) were used in a 5-week experiment with two phases (days 0 to 14 and days 15 to 35). Pigs were allotted to 1 of 3
Table 1. Compositions of basal weanling pig diets (as-fed basis).

| Item                        | Phase 1 (days 0 to 14) | Phase 2 (days 15 to 35) |
|-----------------------------|------------------------|-------------------------|
| Extruded corn               | 330.2                  | 546.5                   |
| Soybean meal (440 g CP/kg)  | 260.0                  | 280.0                   |
| Fish meal (640 g CP/kg)     | 45.0                   | 35.0                    |
| Soy oil                     | 55.0                   | 47.0                    |
| Lactose                     | 100.0                  | –                       |
| Whey powder                 | 130.0                  | 60.0                    |
| Plasma powder               | 22.0                   | –                       |
| Calcium phosphate           | 12                     | 15                      |
| Sugar                       | 30.0                   | –                       |
| L-lys-HCl (980 g/kg)        | 2.0                    | 3.5                     |
| DL-Met (500 g/kg)           | 1.0                    | 1.0                     |
| L-Thr (890 g/kg)            | 0.8                    | –                       |
| Choline chloride (250 g/kg) | 1.0                    | 1.0                     |
| Vitamin premix              | 2.0                    | 2.0                     |
| Mineral premix              | 2.0                    | 2.0                     |
| Limestone                   | 5.0                    | 5.0                     |
| Salt                        | 2.0                    | 2.1                     |
| Calculated composition (g/kg) |                       |                         |
| Metabolizable energy (MJ/kg)| 14.32                  | 14.23                   |
| Crude protein               | 211.1                  | 208.8                   |
| Lysine                      | 14.7                   | 14.1                    |
| Methionine                  | 4.3                    | 4.3                     |
| Calcium                     | 8.7                    | 8.7                     |
| Total Phosphorus            | 7.2                    | 7.1                     |

Analysed composition (g/kg)

| Crude protein               | 210.5                  | 208.1                   |
| Lysine                      | 14.6                   | 14.2                    |
| Methionine                  | 4.2                    | 4.3                     |
| Calcium                     | 8.8                    | 8.6                     |
| Total phosphorus            | 7.1                    | 7.1                     |

Notes: Dietary treatments were: CON, basal diet (antibiotics free diet); LA, CON + 0.1% lactic acid bacteria; EF, CON + 0.1% E. faecium. Additives were added by replacing corn; Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D3, 1,103 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 B12, 3 μg. The samples were diluted with distilled water at a ratio of 8:1 per treatment. All nutrients in diets were formulated to meet or exceed the recommendation of NRC (2012) for weanling pigs. The form of our experimental feed is mash. The probiotics were added after grinding the raw material, and then mixed them uniformly. The experimental feed was mixed once and stored in feedbag. Feed was stored in one room that temperature was lower than 15°C and the relative humidity was lower than 70%. The probiotics bioactivities were not affected by the process technology. Treatment additives were added at expense of corn. All pigs were housed in an environmentally controlled room, which provided 0.26 m² for each pig. Each pen was equipped with a one-sided, stainless steel self-feeder and a nipple drinker that pigs were allowed access to feed and water ad libitum throughout the experimental period. Temperature during Week 1 was maintained at 32°C and was lowered 2.5°C each week thereafter.

2.3. Experimental procedures and sampling

Individual pig BW and feed consumption on a pen basis were recorded at days 0, 14, and 35 to calculate ADG, average daily feed intake (ADFI), and gain:feed ratio (G:F). Apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen (N), and gross energy (GE) was determined by adding chromic oxide (0.2%) as an inert indicator (Fenton and Fenton 1979) in the diet. Pigs were fed diets mixed with chromic oxide from days 8 to 14 and days 29 to 35.

Fresh faecal grab samples collected from 2 pigs (1 gilt and 1 barrow) per pen via rectal massage on days 14 and 35 were mixed and pooled, and a representative sample was placed on ice for transportation to the laboratory where analysis was immediately carried out.

Subjective diarrhoea scores were recorded daily from days 0 to 7 by the same person and were based on the following: 1 = dry pellet, 2 = formed faeces, 3 = moist stool that retains shape, 4 = unformed stool that assumes shape of container, and 5 = watery liquid that can be poured. Scores were recorded on a pen basis following observations of individual pig and signs of stool consistency in the pen. The score is reported as average daily diarrhoea of individual pig score.

2.4. Laboratory analysis

One gram of the composite faecal sample from each pen for faecal microbial flora was diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co.) and then homogenized. Viable counts of bacteria in the faecal samples were then conducted by plating serial 10-fold dilutions (in 1% peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI, USA), lactobacilli medium III agar plates (Medium 638; DSMZ, Braunschweig, Germany), and Salmonella shigella agar plates (Becton, Dickinson and Company, Sparks, MD, USA) to isolate the Escherichia coli, Lactobacillus, and Salmonella, respectively. The lactobacilli medium III agar plates were then incubated for 48 h at 37°C under anaerobic conditions. The MacConkey agar plates were incubated for 24 h at 37°C. The Salmonella shigella agar plates were incubated for 12 h at 37°C. The E. coli, Lactobacillus, and Salmonella colonies were counted immediately after removal from the incubator.

The samples were diluted with distilled water at a ratio of 1:9, then the pH value was measured using a Fisher Scientific Accumet 1001 pH metre (Fisher Scientific, Pittsburgh, PA) with an MI-410 microcombination pH electrode probe attached (Microelectrodes, Londonderry, NH). The same samples were then used to determine faecal moisture. Samples were first air-dried at 60°C, followed by an equilibration and moisture determination at 105°C (Harris 1970). Before chemical analysis, the other half of faecal samples were thawed and dried at 57°C for 72 h, after which they were finely ground to pass through a 1-mm screen. All feed and faecal samples were analysed for DM (method 930.15, AOAC 2007) and crude protein (method 990.03, AOAC 2007). Chromium was analysed via UV absorbance spectrophotometry (Shimadzu UV-1201, Shimadzu, Kyoto, Japan). The GE was determined by measuring the heat of combustion in the samples using a Parr 6100 oxygen bomb calorimeter (Parr instrument Co., Moline, IL). The ATTD was calculated using the following formula: ATTD = 1 – ([Nf × Cd]/[Nd × Cf]), where Nf is the nutrient concentration in faeces (% DM), Nd is the nutrient concentration in diet (% DM), Cd is the chromium concentration in diet (% DM), and Cf is the chromium concentration in faeces (% DM).
2.5. Statistical analysis

All data were subjected to the statistical analysis as a randomized complete block design using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). The pen was used as the experimental unit. Differences among treatment means were determined using the Tukey’s multiple range tests with a P < .05 indicating a significance.

3. Results

3.1. Growth performance and nutrient digestibility

From days 0 to 14 and days 15 to 35, ADG was higher (P < .05) in EF treatment than that in CON treatment (Table 2). From days 15 to 35, ADFI and G:F were not affected by dietary treatments. During the whole period, pigs fed EF had greater (P < .05) ADG and G:F than those fed the CON diet. No difference was observed on ADFI during any period of the experiment. The ATTD of DM, N and GE was greater (P < .05) in LA and EF treatments compared with CON treatment at both days 14 and 35 (Table 3).

3.2. Faecal microflora and faecal characteristics

The faecal Lactobacillus counts were increased (P < .05), whereas faecal pH was decreased (P < .05) by LA and EF treatments compared with CON treatment on both days 14 and 35 (Table 4). There is no difference in E. coli and Salmonella population on day 14 or 35 among treatments. Diarrhoea score and faecal moisture values were not affected by dietary treatments on day 14 or 35 (Table 5).

4. Discussion

4.1. Growth performance

Many strains of bacteria have been used as probiotics, and the most commonly used species are lactic acid bacteria such as Lactobacillus, Enterococcus, Streptococcus, and Bifidobacteria (Dunne et al. 2001). Lactic acid bacteria can relieve weaning stress, promote growth and prevent diarrhea of piglets after weaning (Fuller 1989; Kanitz et al. 1998; Ross et al. 2010). Yu et al. (2008) demonstrated that both different concentration of Lactobacillus fermentum and 3.4 × 10^3 cfu/g lacticbacci complex supplements (Lactobacillus gasseri, Lactobacillus reuteri, Lactobacillus acidophilus, and L. fermentum) increased ADG from week 0–3 in weanling pigs without influencing feed conversion ratio. Our previous study reported that 0.1% L. reuteri and L. plantarum (1 × 10^5 cfu/g) complex improved ADG from week 0 to 4, but did not affect ADFI and G:F (Zhao et al. 2015). However, in this study, 0.1% different concentration of lactic acid bacteria complex did not influence ADG, ADFI, and G:F from days 0 to 14, days 15 to 35, or the overall period, but there was an increasing tendency. Different results may be caused by not only different strain and concentration of Lactobacillus, but also animal health condition and environment. Mohana Devi and Kim (2014) demonstrated that

### Table 2. Effects of lactic acid bacteria complex and Enterococcus faecium DSM 7134 on growth performance in weanling pigs.

| Items (%) | CON | LA | EF | SEM | P-value |
|-----------|-----|----|----|-----|---------|
| D 0–14    |     |    |    |     |         |
| ADG (g)   | 279b | 298ab | 304a | 6    | <.05    |
| ADFI (g)  | 359  | 369 | 361 | 4    | .75     |
| G:F       | 0.777| 0.807| 0.843| 0.021| .22     |
| D 15–35   |     |    |    |     |         |
| ADG (g)   | 534b | 576ab | 591a | 14   | <.05    |
| ADFI (g)  | 864  | 854 | 857 | 14   | .64     |
| G:F       | 0.618| 0.676| 0.691| 0.021| .18     |
| D 0–35    |     |    |    |     |         |
| ADG (g)   | 432b | 465ab | 476a | 12   | <.05    |
| ADFI (g)  | 662  | 660 | 659 | 8    | .52     |
| G:F       | 0.653b| 0.705ab | 0.724a | 0.017| <.05    |

Notes: CON, basal diet; LA, CON + 0.1% lactic acid bacteria; EF, CON + 0.1% E. faecium; Standard error of means. a,b Means in the same row with different superscripts differ (P < .05).

### Table 3. Effects of lactic acid bacteria complex and Enterococcus faecium DSM 7134 on nutrient digestibility in weanling pigs.

| Items (%) | CON | LA | EF | SEM | P-value |
|-----------|-----|----|----|-----|---------|
| D 14      |     |    |    |     |         |
| DM        | 78.3b | 81.4a | 82.0a | 2.14| <.05    |
| N         | 76.4b | 80.9a | 80.3a | 0.81| <.05    |
| GE        | 77.5b | 80.7a | 81.5a | 0.79| <.05    |
| D 35      |     |    |    |     |         |
| DM        | 80.1b | 82.1a | 81.9a | 0.65| <.05    |
| N         | 77.9b | 81.6a | 81.7a | 0.76| <.05    |
| GE        | 80.0b | 83.4a | 83.9a | 0.68| <.05    |

Notes: CON, basal diet; LA, CON + 0.1% lactic acid bacteria; EF, CON + 0.1% E. faecium; Standard error of means. a,b Means in the same row with different superscripts differ (P < .05).

### Table 4. Effects of lactic acid bacteria complex and Enterococcus faecium DSM 7134 on faecal microflora in weanling pigs.

| Items (log10 cfu/g) | CON | LA | EF | SEM | P-value |
|--------------------|-----|----|----|-----|---------|
| D 0–14             |     |    |    |     |         |
| Lactobacillus      | 7.45b| 7.64a| 7.59a| 0.04| <.05    |
| Escherichia coli   | 6.77 | 6.73| 6.69 | 0.05| .16     |
| Salmonella         | 1.34 | 1.25| 1.19 | 0.07| .20     |
| D 35               |     |    |    |     |         |
| Lactobacillus      | 7.54b| 7.67a| 7.70a| 0.03| <.05    |
| Escherichia coli   | 6.67 | 6.71| 6.67 | 0.05| .23     |
| Salmonella         | 1.30 | 1.22| 1.14 | 0.06| .18     |

Notes: CON, basal diet; LA, CON + 0.1% lactic acid bacteria; EF, CON + 0.1% E. faecium; Standard error of means. a,b Means in the same row with different superscripts differ (P < .05).

### Table 5. Effects of lactic acid bacteria complex and Enterococcus faecium DSM 7134 on faecal moisture and faecal pH in weanling pigs.

| Items (%) | CON | LA | EF | SEM | P-value |
|-----------|-----|----|----|-----|---------|
| D 0–7     |     |    |    |     |         |
| Faecal scorea | 3.15 | 3.14| 3.13| 0.03| .31     |
| D 14      |     |    |    |     |         |
| Faecal moisture (%) | 69.71 | 69.03| 70.93| 1.53| .28     |
| pH        | 6.09b| 5.96b| 5.92b| 0.02| <.05    |
| D 35      |     |    |    |     |         |
| Faecal moisture (%) | 68.62 | 69.19| 69.67| 0.99| .21     |
| pH        | 5.85b| 5.74b| 5.71b| 0.02| <.05    |

Notes: CON, basal diet; LA, CON + 0.1% lactic acid bacteria; EF, CON + 0.1% E. faecium; Standard error of means. a,b Means in the same row with different superscripts differ (P < .05).

*aStandard error of means; Faecal scores were determined using the following faecal scoring system: 1 = dry pellet, 2 = formed stool, 3 = moist stool that retains shape, 4 = unformed stool that assumes shape of container, 5 = watery liquid that can be poured.
weanling pigs fed the diets with *E. faecium* (10⁶ cfu/g) had greater ADG and G:F from days 0 to 14, days 15 to 42, and the overall period. The inclusion of *E. faecium* (10⁶ cfu/g) improved ADG and G:F of weanling pigs in a 28-day trial (Mallo et al. 2010). Results of this study were consistent with the two studies mentioned above. Increased ADG was observed from days 0 to 14, days 15 to 35, and the overall period compared with CON, and G:F was found higher during the overall period as well.

The natural adaptation of lactic acid bacteria to the gut environment and their production of antimicrobial substances, such as organic acids and bacteriocins, which functions as a natural antimicrobial, decreases the intestinal pH, inhibits the growth of pathogenic bacteria, such as *Salmonella* spp. or strains of *E. coli* (Mallett et al. 1989; Mack et al. 1999; Alakomi et al., 2000; Cernauskiene et al. 2011). The improved microbiota balance in the weaning pigs' intestine plays an important role in improving animal health and nutrient utilization (Giang et al., 2010). Additionally, the increased ATTD of nutrient may also improve growth performance of weanling pigs.

### 4.2. Nutrient digestibility

Yu et al. (2008) demonstrated that both *Lactobacillus fermentum* and lactobacilli complex supplements (*Lactobacillus gasseri, Lactobacillus reuteri, Lactobacillus acidophilus and Lactobacillus fermentum*) increased the ATTD of CP in weanling pigs. Zhao and Kim (2015) also reported that the ATTD of N and GE was improved by 0.1% *L. reuteri* and *L. plantarum* (10⁶ cfu/g) complex. Moreover, the ATTD of DM, N, and GE fed with *E. faecium* (10⁶ cfu/g) diets was increased in weanling pigs (Mohana Devi and Kim, 2014). In this study, both lactic acid bacteria complex and *E. faecium* supplementation improved the ATTD of DM, N, and GE on days 14 and 35. Lactic acid bacteria are known to produce lactic acid and proteolytic enzymes, which can enhance nutrient digestion in the gastrointestinal tract (Yu et al. 2008).

### 4.3. Faecal microflora

Weaning is a great challenge to piglets, and the normal gut microflora balance may be influenced, which leads to decrease growth performance, diarrhoea, and other problems. The gut microflora can be re-established afterwards, and the oral administration of beneficial bacterial species such as *lactobacilli and bifidobacteria* may accelerate this process (Metchnikoff 1910; Goldin et al. 1980; Alm 1991). Faecal *Lactobacillus* concentration was increased in weanling pigs fed 0.1% *L. reuteri* and *L. plantarum* (10⁶ cfu/g) complex diet. Faecal lactobacilli in weanling pigs fed high energy diets with *E. faecium* (10⁶ cfu/g) were increased on days 14 and 28 (Zhang et al. 2014). Mallo et al. (2010) reported that *E. faecium* (10⁶ cfu/g) increased faecal lactobacilli population, but did not influence *E. coli* counts on day 56 in weanling pigs, which were similar with our results that *Lactobacillus* counts were increased, but *E. coli* and *Salmonella* counts were not affected by *E. faecium* (10⁷ cfu/g) addition on days 14 and 35 in weanling pigs. Those results indicated that lactic acid bacteria complex and *E. faecium* could increase faecal lactobacilli counts, but have no effect on *E. coli* counts. However, Zhang et al. (2014) found that *E. coli* counts were decreased by *E. faecium* (10⁶ cfu/g) supplementation. The results are not always consistent, Mohana Devi and Kim (2014) reported that *E. faecium* (10⁶ cfu/g) supplementation did not influence faecal *Lactobacillus* and *E. coli* counts in weanling pigs. While, Zhao and Kim (2015) found that *E. coli* concentration was decreased by 0.1% lactobacillus complex (10⁶ cfu/g). The difference may due to the probiotic concentration (Zhao and Kim 2015), health condition of weanling pigs, and feeding environment.

### 4.4. Faecal score, moisture, and pH

Lactic acid bacteria including *E. faecium* are used as probiotics which can maintain the balance of intestinal microbiota and control of diarrhoea (Arvola et al. 1999), because they prevent *E. coli* from binding to the gut wall and causing damage. Maia et al. (2001) also reported that the presence of *E. faecium* is beneficial to prevent infection by *Salmonella*. In our previous study, 0.1% *L. reuteri* and *L. plantarum* (10⁶ cfu/g) complex decreased diarrhoea score from days 0 to 7 in weanling pigs (Zhao and Kim 2015). Huang et al. (2004) detected that diarrhoea index and diarrhoea incidence were decreased from days 7 to 14 by addition of *Lactobacillus* complex (*L. gasseri, L. reuteri, L. acidophilus*, and *L. fermentum*). However, Mohana Devi and Kim (2014) reported that no effects were detected by the supplementation of *E. faecium* (10⁶ cfu/g) in weanling pigs from days 0 to 7 after weaning. It is consistent with our results that there was no difference in diarrhoea from days 0 to 7 among treatments.

There was a saturation point for the faecal material at around 70–73% moisture, and that loose stools were observed above this percentage (Zhao et al. 2015). Percentage moisture could possibly be used as an index for diarrhoea severity (Etheridge et al. 1984). As there is no research on the influence of lactic acid bacteria in diets fed to pigs, and thus no comparisons could be made. In this study, no effect was observed by the supplementation of lactic acid bacteria complex and *E. faecium*. The mechanism of how probiotics affecting faecal moisture remains unclear. From diarrhoea score and faecal moisture data in this study, we hypothesize that probiotics will not influence faecal moisture content so much, or it will lead to diarrhoea. Sarmad et al. (2011) found that *L. rhamnosus* fed mice decreased the faecal pH on week 2, 4, and 6. Foo et al. (2003) also reported that faecal pH was lower in rats when given lactic acid bacteria via drinking water. As previous studies, weanling pigs fed lactic acid bacteria complex and *E. faecium* decreased faecal pH on week 2 and 5 in this study. The decrease in faecal pH was due to the lactic acid produced by lactic acid bacteria (Shah 2001).

### 5. Conclusion

*E. faecium* DSM 7134 has better effect on ADG at any period and G:F at the end of experiment than lactic acid bacteria complex. Moreover, both lactic acid bacteria complex and *E. faecium* can increase nutrient digestibility, faecal *Lactobacillus* concentration, and decrease faecal pH in weanling pigs.
Disclosure statement

No potential conflict of interest was reported by the authors.

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