



# Effect of direct-fed microbial on growth performance, nutrient digestibility, fecal noxious gas emission, fecal microbial flora and diarrhea score in weanling pigs

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## ABSTRACT

A total of 168 weanling pigs [(Yorkshire × Landrace) × Duroc] with an average body weight of  $7.90 \pm 0.92$  kg (28 d of age) were used in a 28-d trial to determine the effects of dietary *Lactobacillus reuteri* and *Lactobacillus plantarum* complex supplementation on different parameters of piglets after weaning. Pigs were allotted randomly to 1–4 dietary treatments: (1) negative control (NC), basal diet, (2) positive control (PC), NC + 0.01% apramycin, (3) NC + 0.1% probiotics (*L. reuteri* and *L. plantarum* complex) (RP1), and (4) NC + 0.2% probiotics (RP2). There were 7 pens per treatment with 6 pigs/pen (3 gilts and 3 barrows). From d 0 to 14, pigs fed PC diet had a greater ( $P < 0.05$ ) average daily gain (ADG) than pigs fed NC diet. During the overall period, pigs fed PC and RP1 diets had a greater ( $P < 0.05$ ) ADG than pigs fed NC diet. On d 14, the apparent total tract digestibility (ATTD) of nitrogen (N) for pigs fed PC diet was greater ( $P < 0.05$ ) than pigs fed NC, RP1, and RP2 diets. At the end of the experiment, pigs fed RP1 and RP2 diets had a greater ( $P < 0.05$ ) ATTD of N and gross energy (GE) compared with pigs fed NC diet. Additionally, pigs fed PC diet had a greater ( $P < 0.05$ ) ATTD of N compared with pigs fed NC diet. Fecal *Escherichia coli* concentration was decreased ( $P < 0.05$ ) in pigs fed PC and RP1 diets, whereas *Lactobacillus* concentration was increased ( $P < 0.05$ ) in pigs fed RP1 diet compared with NC diet. Diarrhea score was lower ( $P < 0.05$ ) in RP1 treatment compared with NC treatment. On d 5, fecal total mercaptans emission was lower ( $P < 0.05$ ) in PC, RP1, and RP2 treatments compared with NC treatment, and the lowest ( $P < 0.05$ ) was observed in RP1 treatment. On d 10, ammonia and total mercaptans emissions were decreased ( $P < 0.05$ ) by PC, RP1, and RP2 treatments compared with NC treatment. Hydrogen sulfide emission was lower ( $P < 0.05$ ) in RP1 treatment compared with NC treatment. In conclusion, direct-fed 0.1% *L. reuteri* and *L. plantarum* complex ( $1 \times 10^9$  cfu/kg) improved N and GE digestibility, increased fecal *Lactobacillus* concentration, decreased diarrhea score, fecal noxious gas emission, and *E. coli* concentration in weanling pigs.

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**Abbreviations:** ADFI, average daily feed intake; ADG, average daily gain; ATTD, apparent total tract digestibility; BW, body weight; DM, dry matter; GE, gross energy; G:F, gain:feed; H<sub>2</sub>S, hydrogen sulfide; N, nitrogen; NH<sub>3</sub>, ammonia; SE, standard error.

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## 1. Introduction

Antibiotics have been widely used to reduce pathogen infection since the early 1950s. Published research data clearly show that the use of antibiotics during all phases of growth benefits the rate and efficiency of body weight gain (Yokoyama et al., 1982; Zimmerman, 1986), reduces mortality and morbidity (Cromwell, 2002), and improves health in pigs by destroying or slowing down the growth of harmful bacteria, but antibiotic resistance is a growing issue. Thus, it is urgent that we need to find alternatives to antibiotics, especially after the use of antibiotics has been banned in European since 2006. Organic acids, probiotics, prebiotics (oligosaccharides), other carbohydrates, and enzymes have been used as antibiotic alternatives.

Probiotics are harmless bacteria or yeast species, which have been demonstrated to be useful in equilibrating intestinal microflora and improve animal health (Fuller, 1989; Meng et al., 2010). Previous investigations have already used direct-fed microbial complex such as *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and *Lactobacillus reuteri* for growth promoter in weanling pigs (Kyriakis et al., 1999; Chang et al., 2000; Xuan et al., 2001), growing pigs (Fialho et al., 1998), finishing pigs (Hong et al., 2002), and reduced fecal gas emissions (Chiang and Hsieh, 1995; Hong et al., 2002). However, other studies reported that no significant effects were observed on growth performance in growing-finishing pigs fed 0.2% *L. reuteri* complex (Shon et al., 2005) and 0.1% *Lactobacillus* (Harper et al., 1983). We hypothesize that the addition of *Lactobacillus* complex as probiotics can improve nutrient digestibility and decrease diarrhea score in weanling pig. In addition, due to the different results and limited studies in pigs, the principal objective of this study was to evaluate effects of dietary *L. reuteri* and *Lactobacillus plantarum* complex supplementation on growth performance, nutrient digestibility, blood profile, fecal noxious gas emission, fecal microbial flora, and diarrhea score in weanling pigs.

## 2. Materials and methods

The experimental protocol used in this study was approved by the Animal Care and Use Committee of Dankook University.

### 2.1. Experimental design, animals, and housing

A total of 168 weanling pigs [(Yorkshire × Landrace) × Duroc] with an average body weight (BW) of  $7.90 \pm 0.92$  kg (28 d of age) were used in a 28-d experiment. Pigs were randomly allotted to four experimental diets according to their initial BW and gender (7 pens; 3 gilts; and 3 barrows/pen). Dietary treatment groups were: (1) negative control (NC), basal diet, (2) positive control (PC), NC + 0.01% apramycin, (3) NC + 0.1% probiotics (*L. reuteri* and *L. plantarum* complex) (RP1), and (4) NC + 0.2% probiotics (RP2). The diets were formulated to meet or exceed the NRC (2012) nutrient requirements (Table 1). The concentrations of *L. reuteri* and *L. plantarum* are both  $1 \times 10^9$  cfu/kg. The form of our experimental feed is mash. The probiotics complex was added after grinding the raw material, and then mixed them uniformly. The probiotics bioactivities were not affected by the process technology. Treatment additives were included in the diet by replacing the same amount of corn. All pigs were housed in an environmentally controlled room with a slatted plastic floor. Each pen was equipped with a one-sided self feeder and a nipple waterer to allow the pig *ad libitum* access to feed and water throughout the experimental period. Temperature during wk 1 was maintained at 32 °C and was lowered 2.5 °C each week thereafter.

### 2.2. Experimental procedures and sampling

Individual pig BW was recorded at the beginning, middle (d 14), and end (d 28) of the experimental period, and feed consumption was recorded on a pen basis during the experiment to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain:feed ratio (G:F). From d 8 to 14 and d 22 to 28, pigs were fed diets mixed with chromic oxide (0.2%) as an indigestible marker to determine apparent total tract digestibility (ATTD) for dry matter (DM) and nitrogen (N) (Fenton and Fenton, 1979). On d 14 and 28, fecal samples were collected from at least 2 pigs in each pen via rectal massage. All feed and fecal samples were stored at –20 °C until analysis.

Fresh feces and urine samples were collected randomly from at least 2 pigs (1 gilt and 1 barrow) in each pen on the last 2 d of the experiment. Fresh feces were collected directly via massaging the rectum. Fresh urine was collected in a bucket via a funnel below the cage. For fecal microbial flora, fecal samples collected in each pen were pooled and placed on ice for transportation to the laboratory where analysis was immediately carried out. For fecal gas emission, samples were kept in sealed containers and were immediately stored at –4 °C for the duration of the period. After the collection period, feces and urine samples were pooled and each mixed well for each pen. As described by Zhao et al. (2013), subsamples of slurry (150 g feces and 150 g of urine were mixed well; 1:1 on the wet weight basis) were taken and stored in 2.6-L plastic boxes in duplicate. Each box had a small hole in the middle of one side wall, which was sealed with adhesive plaster.

Subjective diarrhea scores were recorded daily from d 0 to 7 by the same person and were based on the following: 1 = well-formed feces, 2 = sloppy feces, 3 = diarrhea (Marquardt et al., 1999). 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 diarrhea of individual pig score.

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