Evaluation of effect of probiotics mixture supplementation on growth performance, nutrient digestibility, faecal bacterial enumeration, and noxious gas emission in weaning pigs

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ABSTRACT
A total of 180 twenty-eight-day-old weaning pigs [Duroc × (Yorkshire × Landrace)] with an average body weight of 7.62 ± 1.25 kg were used in a 42-day trial to evaluate the effect of probiotics mixture supplementation on weaning pigs. Pigs were randomly allotted to one of four dietary treatments: (1) CON, basal diet, (2) T1, CON + 0.1% probiotics mixture, (3) T2, CON + 0.2% probiotics mixture, and (4) T4, CON + 0.3% probiotics mixture. Increasing dietary inclusion of probiotics mixture levels linearly increased average daily gain (ADG) and average daily feed intake (ADFI) during day 0–7 as well as ADG and gain to feed ratio (G:F) during day 8–21 (p < .05). There was a quadratic effect in improving ADFI during day 0–7, day 22–42, and ADG during day 8–21 (p < .05). In addition, increasing inclusion of probiotics mixture levels in the diets linearly increased (p < .05) dry matter (DM), nitrogen (N), energy digestibility, and faecal Lactobacillus counts and decreased Escherichia coli counts and ammonia (NH3) emission. However, no significant differences were observed in ADG, ADFI, G:F during day 22–42, and day 0–42 except for a quadratic increase of ADFI for day 22-42 (p < .05). Feeding the pigs with the diets containing different probiotics mixture levels did not affect the faecal hydrogen sulfide emission (p > .05). In conclusion, increasing inclusion of probiotics mixture up to 0.3% linearly improved growth performance during day 0–7 and day 8–21. Pigs fed the diets with probiotics mixture supplementation improved the nutrient digestibility, faecal bacterial enumeration, and decreased NH3 emission.

HIGHLIGHTS
- Probiotics mixture supplementation increased growth performance and nutrient digestibility in weaning pigs.
- Increased fecal Lactobacillus and reduced E. coli counts.
- Reduced fecal ammonia emission that can contribute in reducing environmental pollution.

Introduction
In swine industry, the weaning transition is a complex period during which the piglets have to cope with abrupt separation from their dam, mixing with other litters in a usually new environment and switch from highly digestible feed (milk) to a less digestible more complex solid feed; hence weaning is a stressful experience for the piglets involving nutritional, psychological, environmental, microbiological, and immunological stresses (Lallès 2008), which could result in economic losses due to decreased growth rate, feed efficiency, diarrhoea, and damage to intestinal function and health.

Antibiotics have traditionally been widely administered to nursery pigs for the prevention or treatment of diarrhoea and increasing growth performance in the worldwide (Kong et al. 2009). However, the use of antibiotics in animal feed has been prohibited many countries including European Union since 2006 as well as South Korea since 2011 (Nguyen et al. 2018). Therefore, there is increasing interest in alternatives. It has been recognised that probiotics have received
considerable attention as suitable alternatives of anti-
biotics (Chen et al. 2006; Meng et al. 2010; Yan and Kim 2011). Probiotics are live microorganisms which
have been found to confer health benefits on the host
when administered in adequate amounts (Reid et al.
2003; Weichselbaum 2009).

Many studies have reported that the addition of
probiotics could improve growth performance (Giang
et al. 2012), nutrient digestibility (Dong et al. 2014;
Zhao and Kim 2015), and intestinal eubiosis (Bai and
Ouyang 2006; Rioux and Fedorak 2006; Dong et al.
2014), while decreasing the incidence of diarrhoea in
weaning pigs (Giang et al. 2012). Probiotics also have
roles in promoting immunity function (Yun et al. 2008)
and reducing faecal noxious gas emission (Zhao and
Kim 2015) in weaning pigs, which can result in a
decrease of environmental pollutants (Lee et al. 2001;
Ferket et al. 2002).

However, Sanders and Huis in’t Veld (1999) sug-
gested that the health effects of probiotics are genus-,
species-, and strain-specific. It is reported that multi-
strain probiotics are more beneficial than single-strain
probiotics (Wang et al. 2009; Yan and Kim 2011).
Therefore, we hypothesised that the probiotic mixtures
have greater efficacy than single strains. The objective
of this study was to evaluate the effects of probiotics
mixture (Bacillus coagulans, B. licheniformis, B. subtilis,
and Clostridium butyricum) supplementation on
growth performance, nutrient digestibility, faecal
bacterial enumeration, and noxious gas emission in
weanling pig.

Materials and methods

Source of probiotics

The probiotics used in this study were kindly provided
by a commercial company (SynerBig Co. Ltd, Seoul,
Korea) as SynerZymeF10. This product is a mixture of
spray-dried spores of B. coagulans, B. licheniformis,
B. subtilis, and C. butyricum and is guaranteed to con-
tain at least $1 \times 10^{12}$ colony-forming units (CFU) $kg^{-1}$
of B. coagulans, $5 \times 10^{11}$ CFU $kg^{-1}$ of B. licheniformis,

| Table 1. Compositions of basal nursery pig diets (as-fed basis). |
|------------------|------------------|------------------|
| Ingredients, g/kg | Phase 1, day 0–7 | Phase 2, day 8–21 | Phase 3, day 22–42 |
| Extruded corn     | 121.5            | 357.2            | 457.0            |
| Extruded oat      | 100.0            | 0                | 0                |
| Biscuit meal      | 0                | 90.0             | 90.0             |
| Soybean meal, 44% CP | 80.0          | 200.0            | 296.5            |
| Fermented soybean | 78.0             | 82.0             | 0                |
| Meal              |                  |                  |                  |
| Fish meal         | 50.0             | 40.0             | 25.0             |
| Soy oil           | 41.5             | 48.0             | 30.0             |
| Lactose           | 100.0            | 60.0             | 0                |
| Whey              | 165.0            | 100.0            | 62.5             |
| Milk product      | 130.0            | 20.0             | 20.0             |
| Monocalcium       | 12.5             | 10.0             | 6.0              |
| Phosphate         |                  |                  |                  |
| Sugar             | 40.0             | 20.0             | 0                |
| Plasma power      | 65.0             | 0                | 0                |
| L-Lys HCl, 78%    | 1.2              | 2.5              | 1.6              |
| DL-Met, 50%       | 2.6              | 1.5              | 1.4              |
| L-Thr, 89%        | 7.7              | 0.8              | 0                |
| Choline chloride, 25% | 2.0            | 1.0              | 1.0              |
| Vitamin premixa   | 2.0              | 2.0              | 2.0              |
| Limestone         | 0                | 2.0              | 3.0              |
| Salt              | 0                | 2.0              | 3.0              |
| Calculated compo- |                  |                  |                  |
| nent (g/kg)       |                  |                  |                  |
| ME (MJ/kg)        | 14.8             | 14.8             | 14.6             |
| CP                | 220.0            | 210.0            | 205.0            |
| Lys               | 15.7             | 14.1             | 13.3             |
| Met               | 6.0              | 9.9              | 4.7              |
| Thr               | 10.1             | 9.5              | 8.1              |
| Try               | 3.2              | 2.8              | 2.4              |
| Ca                | 8.0              | 7.8              | 7.5              |
| Total P           | 7.6              | 7.6              | 6.4              |

*Provided per kilogram of complete diet: vitamin A, 11 025 U; vitamin D3, 1103 U; vitamin E, 44 U; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic acid, 29 mg; choline, 166 mg; and vitamin B12, 33 μg.

bProvided per kilogram of complete diet: Fe (as FeSO4·7H2O), 80 mg; Cu (as CuSO4·5H2O), 12 mg; Zn (as ZnSO4), 85 mg; Mn (as MnO2), 8 mg; I (as KI), 0.28 mg; and Se (as Na2SeO3·5H2O), 0.15 mg.

ME: metabolizable energy; CP: crude protein.
1 × 10^{12} \text{ CFU kg}^{-1} \text{ of } B. \text{ subtilis, and } 1 \times 10^{11} \text{ CFU kg}^{-1} \text{ of } C. \text{ butyricum.}

**Experimental design, animals, and diets**

A total of 180 twenty-eight-day-old weaning pigs [Duroc × (Yorkshire × Landrace)] with an average body weight (BW) of 7.62 ± 1.25 kg were used in a 42-day trial. All pigs were randomly allotted to 4 experimental diets based on initial BW and sex (9 replicate pens per treatment; 2 gilts, and 3 barrows/pen). Dietary treatments included: (1) Control (CON), basal diet, (2) T1, CON + 0.1% probiotics mixture, (3) T2, CON + 0.2% probiotics mixture, and (4) T4, CON + 0.3% probiotics mixture. Diets (Table 1) were formulated to meet or exceed the nutrient requirements recommended by NRC (2012). All the 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 week 1 was maintained at 32°C and was reduced by 2.5°C each week thereafter.

**Growth performance and apparent total tract digestibility**

Individual pig BW was recorded at the beginning and on days 7, 21, and 42 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 to feed ratio (G:F). Chromium oxide was added to the diet as an indigestible marker at 0.20% of the diet for 7 d before faecal collection at the end of experiment to calculate the apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen (N), and energy digestibility. Faecal grab samples were collected at random from at least 2 pigs in each pen (1 gilt and 1 barrow). All feed and faeces samples were stored immediately at −20°C until analysis. Faecal samples were dried at 70°C for 72 h and finely ground to pass through a 1-mm screen. The procedures used for the determination of DM, N, and energy digestibility were in accordance with the methods established by the AOAC (2002). Chromium concentrations were determined via UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan). The ATTD was then calculated using the following formula: Digestibility (%) = \{1 − [(Nf × Cd)/ (Nd × Cf)]\} × 100, where Nd = nutrient concentration in diet (% DM), Nf = nutrient concentration in faeces (% DM), Cd = chromium concentration in faeces (% DM), and Cf = chromium concentration in faeces (% DM).

**Faecal bacterial enumeration**

At the end of the experiment, faecal samples were collected directly via massaging the rectum of 2 pigs (1 gilt and 1 barrow) in each pen and then pooled and placed on ice for transportation to the laboratory where analysis was immediately performed. A calibrated, glass-electrode pH metre (WTW pH 340- A; WTH Measurement Systems Inc., Ft. Myers, FL) was used to measure the pH of the faecal samples, which were diluted with deionised water at a ratio of 1:7.5 (wt/wt). One gram of the composite faecal sample from each pen was diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co.) and then homogenised. Then 10-fold dilutions of faecal sample were placed onto MacConkey agar plates (Difco Laboratories, Detroit, MI) and Lactobacilli medium III agar plates (Medium 638; DSMZ, Braunschweig, Germany) to isolate the *Escherichia coli* and *Lactobacillus*, respectively. The *Lactobacilli* medium III agar plates were then incubated for 48 h at 39°C under anaerobic conditions. The MacConkey agar plates were incubated for 24 h at 37°C. The *E. coli* and *Lactobacillus* colonies were counted immediately after removal from the incubator. The microbial populations were log transformed before statistical analysis.

**Faecal noxious gas emission**

At the end of the experiment, fresh faeces and urine samples were collected randomly from at least two pigs in each pen for analysis of the faecal NH3, H2S, and total mercaptans concentrations. The urine was collected in a bucket via a funnel below the cage. Samples were kept in sealed containers and were immediately stored at −4°C for the duration of the period. After the collection period, faeces and urine samples were pooled and each mixed well for each pen. Then the slurry was prepared by mixing urine and faeces in 1:1 ratio. 300 g slurry were taken and stored in 2.6-L plastic boxes in duplicate as described by Cho et al. (2008). Each box had a small hole in the middle of one side wall, which was sealed with adhesive plaster. The samples were permitted to ferment for 7 d at room temperature (25°C). The concentrations of gas were determined one time after 7 d of fermentation. A gas sampling pump (Model GV-100; Gastec Corp., Ayase, Japan) was utilised for gas detection (Gastec detector tube No. 3La for ammonia (NH3),...
No. 4LK for hydrogen sulfide (H₂S), and No. 70 for mercaptans; Gastec Corp.). The adhesive plasters were punctured, and 100 mL of headspace air was sampled approximately 2.0 cm above the slurry surface.

**Statistical analysis**

All data were analysed as a completely randomised design using mixed procedures of SAS (SAS Institute 2004) with pen as the experimental unit. Inclusion level of probiotics was the fixed effect and block was the random factor in the statistical model. The initial BW was used as a covariate for the growth performance. Single-degree of freedom orthogonal contrasts were used to determine linear or quadratic effects of increasing probiotics inclusion on growth performance, nutrient digestibility, faecal bacterial enumeration, and noxious gas emission and to compare means of parameters for each treatment with CON for day 0–42 (Littell et al. 2006). Probability level of less than 0.05 was considered as statistically significant.

**Results**

**Growth performance**

The results of growth performance are summarised in Table 2. Increasing dietary inclusion of probiotics mixture up to 0.3% did not affect (p > 0.05) ADG for the entire trial and for day 22–42. Increasing inclusion of probiotics mixture linearly increased ADG for day 0–7 and 8–21. A quadratic effect also was seen in improvement of the ADG for day 8–21 (p < 0.05). For the entire trial, day 8–21, and day 22–42, increasing inclusion of probiotics mixture did not affect ADFI, but linearly and quadratically increased (p < 0.05) ADFI for day 0–7. However, no influence of probiotics mixture supplementation was found G:F for day 0–7, 8–21, 22–42, and the whole experimental period. Among diets with 0.1%, 0.2%, and 0.3% probiotics mixture, inclusion of 0.1% probiotics mixture in the diet was the sole inclusion for which each of ADFI, ADG, and G:F for day 0–7, 8–21, 22–42, and 0–42 did not differ (p > 0.05) from the diet without probiotics mixture except for a significant increase of ADFI for day 22–42 (p < 0.05).

**Nutrient digestibility**

The data presented in Table 3 shows the results of nutrient digestibility. Increasing inclusion of the probiotics mixture in the diets linearly increased (p < 0.05) the digestibility of DM, N, and energy on day 42. Among diets with 0.1%, 0.2%, and 0.3% probiotics mixture, inclusion of 0.1% probiotics mixture in the diet was the sole inclusion for which each of DM, N, and energy did not differ (p > 0.05) from the diet without probiotics mixture at the end of experiment.

**Table 2.** Effect of probiotics mixture supplementation on growth performance in weaning pigs.

| Items, log10 cfu/g | CON | TRT1 | TRT2 | TRT3 | SEM | p value |
|--------------------|-----|------|------|------|-----|---------|
| **Day 0–7**        |     |      |      |      |     |         |
| ADG, g             | 200.000 | 223.000 | 266.000 & 250.000 | 13.000 | .003 | .190    |
| ADFI, g            | 258.000 | 269.000 | 333.000 & 293.000 | 9.000  | .001 | .013    |
| G:F                | 0.796  | 0.843 | 0.814 | 0.861 | 0.042 | .385    |
| **Day 8–21**       |     |      |      |      |     |         |
| ADG, g             | 439.000 | 426.000 | 452.000 & 496.000 | 13.000 | .003 | .047    |
| ADFI, g            | 594.000 | 588.000 | 609.000 & 622.000 | 13.000 | .087 | .505    |
| G:F                | 0.739  | 0.726 | 0.744 | 0.805 | 0.020 | .056    |
| **Day 22–42**      |     |      |      |      |     |         |
| ADG, g             | 523.000 | 547.000 | 530.000 & 516.000 | 16.000 | .505 | .369    |
| ADFI, g            | 836.000 | 889.000 & 881.000 | 851.000 | 16.000 | .605 | .016    |
| G:F                | 0.629  | 0.617 | 0.589 | 0.603 | 0.017 | .179    |
| **Overall**        |     |      |      |      |     |         |
| ADG, g             | 441.000 | 453.000 | 455.000 & 465.000 | 8.000  | .052 | .903    |
| ADFI, g            | 659.000 | 685.000 & 699.000 | 682.000 | 11.000 | .110 | .061    |
| G:F                | 0.672  | 0.662 | 0.651 | 0.681 | 0.011 | .708    |

**Table 3.** Effect of probiotics mixture supplementation nutrient digestibility in weaning pigs.

| Items, % | CON | TRT1 | TRT2 | TRT3 | SEM | p value |
|----------|-----|------|------|------|-----|---------|
| **Day 42** |     |      |      |      |     |         |
| Dry matter | 80.940 | 81.900 | 81.930 | 82.340 | 0.370 | .0195  |
| Nitrogen  | 81.380 | 82.190 | 82.810 & 83.290 | 0.380 | .0012 | .660    |
| Energy    | 80.260 | 80.570 | 81.700 & 83.310 | 0.450 | <0.001 | .163    |

**Table 4.** Effect of probiotics mixture supplementation on faecal microflora in weaning pigs.

| Items, log10 cfu/g | CON | TRT1 | TRT2 | TRT3 | SEM | p value |
|--------------------|-----|------|------|------|-----|---------|
| **Day 42** |     |      |      |      |     |         |
| Lactobacillus  | 7.110 | 7.100 | 7.120 | 7.220 | 0.030 | .0241   |
| E. coli        | 5.860 | 5.790 | 5.760 & 5.690 | 0.030 | .0013 | .852    |
Table 5. Effect of probiotics mixture supplementation on faecal noxious gas emission in weaning pigs.

| Items, ppm | CON | TRT1 | TRT2 | TRT3 | SEM | Linear | Quadratic |
|------------|-----|------|------|------|-----|--------|----------|
| NH₃        | 7.730 | 6.930 | 7.000 | 6.280 | .401 | .035   | .927     |
| H₂S        | 5.200 | 5.150 | 5.080 | 5.030 | .218 | .550   | 1.000    |
| Total mercaptans | 3.280 | 2.950 | 3.000 | 2.780* | .166 | .060   | .754     |

CON: Basal diet; TRT1: Basal diet + 0.1% probiotics mixture; TRT2: Basal diet + 0.2% probiotics mixture; TRT3: Basal diet + 0.3% probiotics mixture; SEM: standard error of means; NH₃: ammonia; H₂S: hydrogen sulfide.

Values of means represent two pigs per pen, nine replicate pens pooled on a pen basis (n = 9) per treatment.

*Within a row at the end of experiment for NH₃, H₂S, and total mercaptans, means with a * differ (p < .05) from CON diet.

Faecal bacterial enumeration

Table 4 shows the results on faecal microflora assay. These results showed that there was a linear improvement in Lactobacillus and decrease in E. coli concentrations with increasing inclusion of the probiotics mixture in the diets (p < .05). Among diets with 0.1%, 0.2%, and 0.3% probiotics mixture, inclusion of 0.1% probiotics mixture in the diet was the sole inclusion for which each of Lactobacillus and E. coli concentrations did not differ (p > .05) from the diet without probiotics mixture.

Faecal noxious gas emission

The data presented in Table 5 shows that total mercaptans and H₂S emission were not significantly influenced by treatment diets (p > .05). However, increasing inclusion of probiotics mixture led to a linear reduction in NH₃ (p = .035). Among diets with 0.1%, 0.2%, and 0.3% probiotics mixture, inclusion of 0.1 and 0.2 probiotics mixture in the diet was the two inclusion for which each of NH₃, H₂S, and total mercaptans concentrations did not differ (p > .05) from the diet without probiotics mixture.

Discussion

The results of this study show that increasing inclusion of the probiotics mixture levels in the diets linearly improved the ADG and ADFI for day 0–7 as well as ADG for day 8–21. Similarly, the inclusion of Bacillus-based probiotics in diet fed improved performance of young pigs (Alexopoulos et al. 2004; Gracia et al. 2004; Wang et al. 2011; Lee et al. 2014), growing pigs (Chen et al. 2005; Wang et al. 2009; Meng et al. 2010; Kim et al. 2014), and finishing pigs (Chen et al. 2006; Davis et al. 2008), as well as increased ADFI of finishing pigs (Munoz et al. 2007; Chen et al. 2013). However, the results are not always consistent. Xuan et al. (2001) indicated that no effect of a probiotic complex (2 × 10⁸ CFU kg⁻¹ of Saccharomyces cerevisiae and 1 × 10¹⁰ CFU kg⁻¹ of Bacillus spp.) on growth performance was observed in weaned pigs. In addition, the use of Bacillus-based probiotics in diet fed to finishing pigs did not have effect on ADG or feed efficiency (Munoz et al. 2007), ADFI, and G:F ratio (Chen et al. 2006). As observed, the effect of Bacillus-based probiotics on performance in practice is highly inconsistent, probably because of different diet compositions, differences in strains, dose levels (Loh et al. 2008; Khan et al. 2011). Furthermore, the age of pigs may be associated with probiotic efficacy (Lessard and Brisson 1987). The use of probiotics tended to be more effective in early age of pigs rather than the growing period (Link and Kováč 2006). In this study, addition of probiotics mixture to the diets improved the ATTD of DM, N, and energy. Our results confirm the findings of Lee et al. (2014) who reported greater the ATTD of DM and gross energy in pigs offered diets supplemented with B. subtilis fermentation biomass. Choi et al. (2011) reported that weanling pigs fed diets supplemented with multi-microbe probiotic products containing B. subtilis had improved ATTD of DM and gross energy. Balasubramanian et al. (2016) also reported that supplementation with probiotic containing B. coagulans (1 × 10⁹ cfu/g), B. licheniformis (5 × 10⁸ cfu/g), and B. subtilis (1 × 10⁹ cfu/g) to growing pig diets caused a linear effect on N and energy digestibility. Bacillus is recognised for increasing the rate of glucose transport, intestinal villous height, and crypt depth ratio (Breves et al. 2000; Rao and Wang 2011), which may have contributed to improved nutrient uptake in pigs. Moreover, probiotic products may compete with other intestinal microorganisms for nutrients or result in production of antibacterial substances (Hentges 1992), which would explain the results regarding nutrient digestibility.

The pig intestine is home to a dynamic microbial population that forms a complex ecosystem and has a symbiotic relationship with the host. The population of gut microbes, or microbiota, plays key roles in maintaining nutritional, physiological, and immunological functions of the pigs (Lee and Mazmanian 2010; Brestoff and Artis 2013). Disturbances in the gut microbial ecosystem during the rearing of pigs can dramatically increase risk of disease. In the current study, the addition of probiotics mixture significantly increased faecal Lactobacillus populations and significantly decreased E. coli counts. This is in agreement with Balasubramanian et al. (2016), who reported that growing pigs fed the diets with Bacillus-based probiotic (1 × 10⁹ CFU g⁻¹ of B. coagulans, 5 × 10⁸ CFU
g\(^{-1}\) of *B. licheniformis* and 1 × 10\(^8\) CFU g\(^{-1}\) of *B. subtilis*) increased *Lactobacillus* and decreased faecal *E. coli*. Jeong and Kim (2014) also suggested that *B. subtilis* supplementation could increase faecal *Lactobacillus* and decrease faecal *E. coli*. Number of previous studies have demonstrated that probiotics display competitive exclusion of pathogens (Giang et al. 2011, 2012). For instance, *C. butyricum* promoted the growth of *Lactobacillus* and *Bifidobacterium* (Imase et al. 2008; Kong et al. 2011), while Wu et al. (2011) suggested that during *Bacillus* spp. colonises the intestine, it consumes oxygen rapidly and reduces pH, which favours *Lactobacilli* and inhibits *E. coli* and *Salmonella*. In addition, it has been reported that *Bacillus* strain probiotic can affect the intestinal bacteria not only by a modification of pH but can also produce specific antimicrobial substances and bacteriocins that can inhibit pathogens (Dobson et al. 2012), that could be probably reasons to support our results. In the gastrointestinal tract, the improvement of *Lactobacillus* populations is expected to produce more lactic acid and reduce gut pH, and low gut pH has a beneficial effect on nutrient digestibility (Gracia et al. 2004). Moreover, reduced gut pH inhibits the development of invasive pathogens, which might be another reason for the better metabolism of DM, N, energy and ADG in this study. Therefore, our study confirmed that a probiotics mixture containing *B. coagulans*, *B. licheniformis*, *B. subtilis*, and *C. butyricum*) could be used as a good probiotic in weaning pigs.

Fecal noxious gas emission such as NH\(_3\) and H\(_2\)S has become one of the major air pollutions in modern concentative pig production. Therefore, it is of great concern to the public (Nguyen et al. 2017). High concentrations of NH\(_3\) or H\(_2\)S can cause hazardous effects to humans and animals (Zhang and Kim 2014). In this study, dietary supplementation of probiotics mixture led to decrease NH\(_3\) emission, which is in agreement with Chen et al. (2006), who suggested that supplementation with dietary *Bacillus*-based probiotic (*B. subtilis*, 1 × 10\(^8\) CFU kg\(^{-1}\); *B. coagulans*, 2 × 10\(^8\) CFU kg\(^{-1}\); *L. acidophilus*, 5 × 10\(^8\) CFU kg\(^{-1}\)) reduced NH\(_3\) emission in finishing pigs. Yan et al. (2011) reported that faecal noxious gas emission is associated with nutrient digestibility because the higher digestibility may result to lower substrate for the microbial fermentation in the large intestine, which consequently decreases faecal noxious gas emission. Therefore, the reduced faecal NH\(_3\) emissions in this study were probably due to the enhanced nutrient digestibility and *Lactobacillus* counts.

**Conclusions**

Feeding up to 0.3% probiotic mixture (1 × 10\(^{12}\) CFU kg\(^{-1}\) of *B. coagulans*, 5 × 10\(^{11}\) CFU kg\(^{-1}\) of *B. licheniformis*, 1 × 10\(^{12}\) CFU kg\(^{-1}\) of *B. subtilis* and 1 × 10\(^{11}\) CFU kg\(^{-1}\) of *C. butyricum*) to weaning pigs linearly increased growth performance for day 0–7 and day 8–21. In addition, weaning pigs fed the diets with the probiotics mixture supplementation has linearly improved digestibility of DM, N, and energy as well as *Lactobacillus* counts and decreased *E. coli* counts and faecal NH\(_3\) emissions. In conclusion, among diets with 0.1%, 0.2%, and 0.3% probiotics mixture, inclusion of 0.1% probiotics mixture in the diet was the sole inclusion for which each of growth performance, nutrient digestibility, faecal bacterial enumeration, and noxious gas emission did not differ from the diet without probiotics mixture. The largest increases in growth performance, nutrient digestibility, faecal bacterial enumeration, and noxious gas emission were obtained when probiotics mixture was supplemented at 0.3%.

**Compliance with ethical standards**

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

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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