Effects of probiotics complex supplementation in low nutrient density diet on growth performance, nutrient digestibility, faecal microbial, and faecal noxious gas emission in growing pigs

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ABSTRACT

The objective of the present study was to evaluate the effect of supplementing low nutrient density diet of growing pigs with probiotics complex on growth performance, nutrient digestibility, faecal microbial, and faecal noxious gas emission. A total of 140 crossbred female and castrated male healthy growing pigs [(Yorkshire × Landrace) × Duroc, 54 days of age] with an average initial body weight (BW) of 24.39 ± 2.58 kg were used in a 42-day trial. All pigs were randomly allotted to one of four treatment diets based on initial BW and sex (seven replicate pens/treatment; two gilts, and three barrows/pen). Dietary treatments were: (i) HD, high nutrient density diet, (ii) LD, low nutrient density diet, (iii) T1, LD + 0.05% probiotics. (iv) T2, LD + 0.10% probiotics. At the end of the experiment BW, average daily gain (ADG), and gain: feed ratio (G:F) tended to be higher (p < .1) in HD diet compared with LD treatment. However, supplementation of probiotics complex to LD diet showed a comparable effect as that of HD diet. There were no differences (p > .05) in average daily feed intake (ADFI), backfat thickness, lean meat percentage, and apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen (N), and energy among the treatments. The supplementation of probiotics complex to LD diet increased (p < .05) faecal Lactobacillus counts and reduced NH3 gas emission compared with the LD diet. In conclusion, the results of this study demonstrated the beneficial effects of the probiotics complex supplementation on growth performance, faecal Lactobacillus concentration, and faecal NH3 emission in LD diet suggesting.

HIGHLIGHTS

- To evaluate the effects of supplementing low nutrient density diet with 2 levels (0.05%, 0.1%) of probiotics complex (Bacillus subtilis ms1, Bacillus licheniformis SF5-1, and Saccharomyces cerevisiae) in growing pigs.
- Probiotics complex supplementation has shown the beneficial effects on growth performance.
- The supplementation of probiotics complex to low nutrient density diets increased (p < .05) faecal Lactobacillus counts and reduced NH3 gas emission.
- The probiotics of B. subtilis ms1, B. licheniformis SF5-1, and S. cerevisiae complex may be a helpful alternative to antibiotics.

Introduction

In the recent years, it has become necessary to find effective antibiotic alternatives due to growing concerns about the transfer of resistance to drugs against human pathogens in food animal products and ban on in-feed antibiotics around the world (Meng 2003). The probiotics and prebiotics supplementation to the diet of animals have been widely used as an in-feed antibiotics growth promote and medication alternative due to their antagonistic effects on various microorganisms and growth-promoting effects (Pérez et al. 2016; Guo et al. 2020).

Probiotics are referred to a group of non-pathogenic organisms that provide health benefits to the host when ingested in adequate amounts (FAO/WHO 2001). The role of probiotics in the gut of animals is particularly prominent, which major mechanisms of action include the enhancement of the epithelial barrier, production of anti-microbial substances and modulation of the immune system (Vanderhoof and...
Young 2004; Bermudez-Brito et al. 2012). Currently, the most commonly used probiotics in livestock include *Bacillus* (*B. cereus, B. coagulans, B. licheniformis,* and *B. subtilis*), yeast, and lactic acid bacteria (LAB). *Bacillus* and *Saccharomyces cerevisiae* have good large-scale production performance and high stability and viability during feed preparation and storage (Ohashi and Ushida 2009), and *B. subtilis, B. licheniformis,* and *S. cerevisiae* have been listed to be added as non-toxic, biological supplements to livestock diets (EFSA 2013). Some studies have suggested that *Bacillus* or yeast could improve the growth performance (Balasubramanian et al. 2016), nutrient digestibility (Liu et al. 2018) and intestinal microbial balance (Lan et al. 2017) in pigs. Jørgensen et al. (2016) have demonstrated that the *Bacillus*-based probiotics significantly improved growth rate by 11.2% and F:G by 8.6% during the grower period stage (70–120 days) in pigs. In weanling pigs, Lee et al. (2014) reported that *B. subtilis* LS 1–2 showed beneficial effects on growth performance, coefficient of total tract apparent digestibility of nutrients, and intestinal morphology. Balasubramanian et al. (2018) also demonstrated that multi-species probiotic supplementation in growing-finishing pigs had played a beneficial role in pigs such as growth performances, faecal microbiota, and meat quality. Although previous studies have been adequately reported in pigs, the effectiveness of probiotics is actually highly inconsistent (Chen et al. 2006).

The health benefit attributed to one strain may not be applicable to another strain even within species mostly because the role of probiotics is often strain-specific (Williams 2010). In addition, to the best of our knowledge, since most of the previous related studies have been performed in weaned piglets or supplemented with single strain, and no studies have been performed to assess the supplementary role of *B. subtilis* ms1, *B. licheniformis* SF5-1, and *S. cerevisiae* in the diet of growing pig, especially when supplemented to low nutrient density diet. Moreover, High nutrient density diet is costly. To allow an opportunity to formulate a low-cost diet, probiotics complex supplementation to a low nutrient diet was evaluated in this study. Therefore, the objective of this study was to evaluate the effects of a probiotics complex in low nutrient density diet on growth performance, nutrient digestibility, faecal microbial, and faecal noxious gas emission in growing pigs.

**Materials and methods**

The experimental protocols describing the management and care of animals were reviewed and approved by the Animal Care and Use Committee of Dankook University (DK-2-1915).

**Source of probiotics**

The probiotics complex used in this study was obtained from a commercial company (Microsolution, Gwangju, Korea). This product is a mixture of spray-dried spores of *Bacillus subtilis* ms1, *Bacillus licheniformis* SF5-1, and *Saccharomyces cerevisiae* and is guaranteed to contain at least $1.5 \times 10^9$ CFU g$^{-1}$ of *B. subtilis* ms1, $1.5 \times 10^9$ CFU g$^{-1}$ of *B. licheniformis* SF5-1, and $1.5 \times 10^9$ CFU g$^{-1}$ of *S. cerevisiae*.

**Experimental design, animals and housing**

A total of 140 crossbred female and castrated male healthy growing pigs [(Yorkshire × Landrace) × Duroc, 54 days of age] with an average initial body weight (BW) of 24.39 ± 2.58 kg (mean ± SE) were used in a 42-day trial. All pigs were randomly allotted to 1 of 4 treatment diets based on initial BW and sex (7 replicates/pens/treatment; 2 gilts and 3 barrows/pen). Dietary treatments were: (i) HD, high nutrient density diet, (ii) LD, low nutrient density diet, (iii) T1, LD + 0.05% probiotics. (iv) T2, LD + 0.10% probiotics. The diets (Table 1) were formulated to meet or exceed the nutrient requirements recommended by NRC (2012) for growing-finishing pigs. The high-nutrient-density diet was analysed to contain 17.5% crude protein, 3.8% crude fibre, 0.99% total lysine, 0.75% calcium, and 0.42% phosphorus, whereas the low-nutrient-density diet was analysed to contain 16% crude protein, 3.9% crude fibre, 0.88% total lysine, 0.65% calcium, and 0.39% phosphorus. Pigs were housed in an environmentally controlled room with a slatted plastic floor. A stainless steel feeder and a nipple drinker were provided in each pen to allow pigs’ *ad libitum* access to feed and water throughout the experimental period. The ambient temperature within the room was kept between 20 °C and 25 °C.

**Growth performance and nutrient digestibility**

Pigs were weighed on a pen basis at the beginning and on d 42 of the experimental period, and feed consumption was recorded throughout the experiment to calculate the average daily gain (ADG), average daily feed intake (ADFI) and gain: feed ratio (G: F). Before the commencement and at the end of the experiment, the backfat thickness and lean percentage of all pigs (n = 35 per treatment) were measured 5 cm from the...
samples were thawed and dried for 72 h at 60°C. Prior to chemical analysis, all feed and faecal DM and N following the procedures outlined by the AOAC (2005). The feed and faecal samples were analysed for which they were ground to pass through a 1 mm screen. The feed samples were stored at –20°C until analysed. Prior to chemical analysis, all feed and faecal samples were thawed and dried for 72 h at 60°C, after which they were ground to pass through a 1 mm screen. The feed and faecal samples were analysed for DM and N following the procedures outlined by the AOAC (2005). Chromium concentrations were analysed by via UV absorption spectrophotometry (Shimadzu, UV-1201, Kyoto, Japan) and nitrogen was determined using a Kjeltec 2300 Analyser (Foss Tecator AB, Hoeganaes, Sweden). Gross energy was determined by measuring the heat of combustion in the samples using a Parr 6100 oxygen bomb calorimeter (Parr Instrument Co., Moline, IL, USA). The apparent total tract digestibility (ATTD) was then calculated using the following formula: Digestibility (%) = [1 - [(Nd × Cd)/(Nd × Cf)]] 100 where Nd = nutrient concentration in faeces (% DM), Cd = nutrient concentration in diet (% DM), Cf = chromium concentration in diet (% DM), and Cf = chromium concentration in faeces (% DM).

### Faecal microbial

Fresh faecal samples were collected directly by massaging the rectum of two pigs (one barrow and one gilt) were randomly selected) in each pen at the end of the experiment, and pooled on a pen basis and placed on ice for transport to the laboratory, where the microbial analysis was immediately carried out. On the same day, we collected samples of faecal from the same pig for analysis of gas emissions.

One gram faecal sample from each pen for faecal microbial flora was diluted with 9 ml of 1% peptone broth (Becton, Dickinson) and it was homogenised. Viable count of bacteria in the faecal samples was then conducted by plating serial 10-fold dilution (in 1% peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI, USA) and lactobacilli medium III agar plates (Medium 638; DSMZ, Braunschweig, Germany) to isolate the *Escherichia coli* (*E. 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 removing from the incubator.

### Faecal noxious gas emission

The stock faeces (300 g) were placed in 2.6-L plastic boxes with a small hole in the middle of one side that was sealed with adhesive plaster. The samples were fermented for 24 h at room temperature (25°C), and 100 ml of the headspace air was sampled from approximately 2.0 cm above the faecal sample. After the collection, boxes were re-sealed with adhesive plaster to measure the faecal noxious content. The faecal samples were manually shaken for approximately 30 s before measurement to disrupt any crust

| Items                  | High nutrient density | Low nutrient density |
|------------------------|-----------------------|---------------------|
| Ingredients, %          |                       |                     |
| Corn                    | 37.57                 | 37.98               |
| Wheat                   | 19                    | 24                  |
| Rice bran               | 2                     | 2                   |
| Wheat bran              | 2                     | –                   |
| Parm kernal meal        | 2                     | 3                   |
| Soybean meal            | 3                     | 3                   |
| Dehulled soybean meal   | 15.11                 | 11.34               |
| Rape seed meal          | 4                     | 4                   |
| Sesame meal             | 2                     | 2                   |
| Brown rice              | 5                     | 5                   |
| Animal fat              | 3.79                  | 3.26                |
| Molasses                | 2                     | 2                   |
| Limestone               | 1.05                  | 1.08                |
| Monocalcium phosphate   | 0.16                  | 0.1                 |
| Salt                    | 0.3                   | 0.3                 |
| Methionine 98%          | 0.01                  | –                   |
| Threonine 98%           | 0.02                  | 0.01                |
| Lysine 25%              | 0.5                   | 0.49                |
| Choline Chloride 50%    | 0.09                  | 0.09                |
| Vitamin/Mineral mixture | 0.4                   | 0.35                |
| Chemical composition    |                       |                     |
| Digestible energy (kcal/kg) | 3560                | 3540               |
| Metabolic energy (kcal/kg) | 3280                | 3260               |
| Crude protein (%)       | 17.50                 | 16.00               |
| Crude fat (%)           | 6.70                  | 5.90                |
| Crude fibre (%)         | 3.80                  | 3.90                |
| Total lysine (%)        | 0.99                  | 0.88                |
| Calcium (%)             | 0.75                  | 0.65                |
| Phosphorus (%)          | 0.42                  | 0.39                |

*Abbreviation: HD: high nutrient density diet; LD: low nutrient density diet; aProvided per kilogram of complete diet: vitamin A, 10,000 IU; vitamin D₃, 2000 IU; vitamin E, 48 IU; vitamin K₃, 1.5 mg; riboflavin, 6 mg; niacin, 40 mg; d-pantothenic, 17 mg; biotin, 0.2 mg; folic acid, 2 mg; choline, 166 mg; vitamin B₆, 2 mg; and vitamin B₁₂, 28 mg, Fe (as FeSO₄·7H₂O), 50 mg; Cu (as CuSO₄·5H₂O), 15 mg; Zn (as ZnSO₄·H₂O), 50 mg; Mn (as MnSO₄·5H₂O), 34 mg; I (as KI), 0.99 mg; and Se (as Na₂SeO₃·3H₂O), 0.25 mg.*
formation on the surface of the faecal sample and to homogenise the samples. Two samples from each pen were measured and then the average was calculated. Concentrations of NH₃, H₂S, mercaptan, and acetic acid were measured within the scopes of 5.0–100.0 ppm (No. 3La, detector tube; Gastec Corp. Kanagawa, Japan) and 2.0–20.0 ppm (4LK, detector tube; Gastec Corp.).

Statistical analyses

All data were subjected to statistical analyses as a randomised block based on sex and BW, design using the contrasts model procedure of the SAS software (SAS Inst. Inc., Cary, NC). Pen was used as an experimental unit. For microbial counts, data were log-transformed prior to statistical analysis. Orthogonal contrasts used to separate treatment means were: HD vs LD, HD vs T1, 2 and LD vs T1, 2. A probability level of $p < .05$ was considered to be statistically significant, and the probability level of less than 0.1 was considered a tendency.

Results

Growth performance and nutrient digestibility

The results of the supplementation of probiotics complex to low nutrient density diets on growth performance and nutrient digestibility are shown in Table 2. At the end of the experiment, BW, ADG, and G: F tended to be higher ($p < .1$) for pigs fed with the high nutrient density diet compared with low nutrient density diet, however, a comparable effect ($p > .05$) on growth performance was observed between high nutrient density diets and probiotics complex which was supplemented to low nutrient density diets. There were no differences ($p > .05$) in backfat thickness, and lean meat percentage among the treatments. Furthermore, no differences ($p > .05$) were observed in ATTD of DM, N, and energy among the treatments.

Faecal microbial

The supplementation of probiotics complex to the low nutrient density diets showed higher ($p < .05$) Lactobacillus counts compared with low nutrient density diet without probiotics complex supplementation. The E. coli count was not affected ($p > .05$) by the dietary treatments (Table 3).

Faecal noxious gas emission

At the end of the experiment, the faecal NH₃ emission for pigs receiving probiotics complex in low nutrient density diet was lower ($p < .05$) than those fed low nutrient density diets without probiotics complex supplementation. The faecal noxious gas of H₂S, methyl mercaptans, acetic acid, and CO₂ were unaffected ($p > .05$) across the treatments (Table 4).

Discussion

In the current study, BW, ADG, and G: F tended to decrease in low nutrient density diet compared with high nutrient density diet treatment. The findings of the current study showed that feeding the pigs with the low nutrient density diets supplemented with probiotics complex resulted in a comparable effect on growth performance compared to the pigs fed high nutrient density diets, which indicated that probiotics

Table 2. Effect of dietary supplementation of probiotics additive on growth performance and nutrient digestibility in growing pigs.  

| Items               | HD       | LD       | T1       | T2       | SEMb   | p-Value          |
|---------------------|----------|----------|----------|----------|--------|------------------|
|                     |          |          |          |          |        | HD vs LD | HD vs T1,2 | LD vs T1,2 |
| Final BW, kg        | 52.90    | 51.58    | 51.77    | 53.08    | 0.50   | .079     | .443       | .189       |
| ADG, g              | 679      | 647      | 652      | 683      | 12     | .083     | .456       | .192       |
| ADFI, g             | 1650     | 1633     | 1640     | 1653     | 15     | .410     | .852       | .449       |
| G:F                 | 0.41     | 0.39     | 0.39     | 0.41     | 0.01   | .091     | .430       | .226       |
| Backfat thickness, mm |        |          |          |          |        |          |            |            |
| Initial             | 5.5      | 5.4      | 5.5      | 5.5      | 0.23   | .794     | .880       | .651       |
| Final               | 8.5      | 8.3      | 8.5      | 8.7      | 0.22   | .592     | .836       | .412       |
| Lean meat percentage, % |        |          |          |          |        |          |            |            |
| Initial             | 75.3     | 75.0     | 75.2     | 75.3     | 0.28   | .378     | .837       | .415       |
| Final               | 66.3     | 66.6     | 66.4     | 66.2     | 0.31   | .525     | .971       | .486       |
| Nutrient digestibility, % |        |          |          |          |        |          |            |            |
| Dry matter          | 77.38    | 77.05    | 77.13    | 77.45    | 0.58   | .703     | .905       | .748       |
| Nitrogen            | 76.00    | 75.98    | 75.98    | 76.08    | 0.67   | .939     | .0977      | .906       |
| Energy              | 76.70    | 76.58    | 76.63    | 76.75    | 0.69   | .900     | .989       | .896       |

*aAbbreviation: HD: high nutrient density diet; LD: low nutrient density diet; T1: LD diet + 0.05% probiotics; T2: LD diet + 0.10% probiotics; ADG: average daily gain; ADFI: average daily feed intake; G:F: gain to feed ratio.
bStandard error of means.
had beneficial effects on growth performance in growing pigs. Probiotics can make beneficial regulation to various microorganisms through the several mechanisms related to competitive adhesion to mucous membranes and epithelium, secretion of antibacterial substances, enhancement of intestinal epithelial barrier, and regulation of the immune system (Collado et al. 2010). In previous studies, Lan et al. (2017) demonstrated that probiotic (B. coagulans, B. licheniformis, B. subtilis, and C. butyricum) complex supplementation in low nutrient density diets has shown improved ADFI more dramatically in weaning pigs. At weaning, piglets are stressed due to feed changes, social and nutritional factors and probiotics can decrease weaning pressure by improving intestinal microbial balance (O’Loughlin et al. 2011). Dong et al. (2014) suggested that weanling pig diet supplemented with probiotics complex (L. plantarum GF103 and B. subtilis B27) showed a significant beneficial effect on growth performance. From this, we can speculate that one cause of the lack of significant differences may be the different growth stages, in which the digestive system and immune enhancement from weaning to growing pigs.

On the other hand, Dowarah et al. (2017) reported that probiotics supplementation has improved the growth performance in the basal diet in grower-finisher pigs. Meng et al. (2010) reported that probiotics (B. subtilis and C. butyricum endospores) supplementation improved the growth performance of the high- and low-energy and high- and low nutrient density diets. Furthermore, Wang et al. (2009) reported that the supplementation of 0.05% or 0.1% of probiotics had no effects on the growth performance of growing pigs, while the supplementation of 0.2% of probiotics increased ADG and ADFI significantly. The results of these studies indicated that another reason for the lack of significant differences in pig growth performance may be due to the different probiotic species and their inclusion levels. Furthermore, the success of probiotic microorganisms in providing beneficial effects to the host depends on their ability to withstand heat, osmotic pressure, and oxygen stress in the storage, processing, and adverse environment in the gastrointestinal tract of the animal (Ross et al. 2005). Contrary to our results, Kritas and Morrison (2004) reported that supplementation of the feed with probiotics complex (B. subtilis and B. licheniformis) does not improve growth performance in pigs, Lan and Kim (2019) also suggested that probiotic (B. licheniformis and B. subtilis) supplementation has not shown a significant effect on growth performance in growing-finishing pig.

We did not observe any significant effects on the retention of nutrient when the pigs were fed with the diets supplemented with or without probiotic in our study. Consistent with our study, Balasubramanian et al. (2016) suggested that probiotics complex containing B. coagulance, B. licheniformis, and B. subtilis supplementation had no effect on nutrient digestibility in grower pigs. However, Liu et al. (2018) reported that probiotic (B. subtilis and S. cerevisiae) supplementation improved nutrient digestibility in growing pigs, similar effect also was founded by Lan et al. (2017) and Jørgensen et al. (2016). The different results may be associated with different types and doses of probiotics, and feeding strategy.

In the current study, supplementing the diets with probiotics complex showed a higher effect on

| Table 3. Effect of dietary supplementation of probiotics additive on faecal microbial in growing pigsa. |
|---------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| Items                          | HD  | LD  | T1  | T2  | SEMb | p-Value |
| Final, log10 CFU/g             | 7.52| 7.46| 7.56| 7.57| 0.04 | .398  |
| Lactobacillus                  | 6.11| 6.13| 6.05| 6.07| 0.03 | .705  |
| E coli                         |     |     |     |     |      | .234  |

aAbbreviation: HD: high nutrient density diet; LD: low nutrient density diet; T1: LD diet + 0.05% probiotics; T2: LD diet + 0.10% probiotics.
bStandard error of means.

| Table 4. Effect of dietary supplementation of probiotics additive on faecal noxious gas emission in growing pigsa. |
|---------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| Items                          | HD  | LD  | T1  | T2  | SEMb | p-Value |
| Gases, ppm                     |     |     |     |     |      |        |
| NH3                            | 1.6 | 1.9 | 1.4 | 1.2 | 0.19 | .287  |
| H2S                            | 4.8 | 5.1 | 4.8 | 4.6 | 0.30 | .565  |
| Total mercaptans               | 3.3 | 2.7 | 2.8 | 2.9 | 0.25 | .162  |
| Acetic acid                    | 6.8 | 6.6 | 6.6 | 6.8 | 0.34 | .647  |

aAbbreviation: HD: high nutrient density diet; LD: low nutrient density diet; T1: LD diet + 0.05% probiotics; T2: LD diet + 0.10% probiotics.
bStandard error of means.
Lactobacillus count but had no effect on E. coli count suggesting that probiotics complex (B. subtilis ms1, B. licheniformis SFS-1, and S. cerevisiae) supplementation was beneficial for the growth of Lactobacillus in the gut of the host animal. Anyway, we assumed that probiotics have a beneficial effect on the gut microflora in growing pigs. When animals are administered with probiotics, they usually attach and adhere to intestinal mucus and epithelial cells, and attachment is believed to be the key to their immunomodulatory benefits effects (Negretti 1997). It is reported that Bacillus can produce some useful enzymes (such as cellulase, α-amylase, β-glucanase, α-galactosidase, β-mannanase) to improve nutrient absorption in the intestinal, while S. cerevisiae can produce antibacterial substances and reduce the level of potential pathogens in the intestinal lumen (Czerucka and Rampal 2002; Latorre et al. 2016). Our results are in consistent with many published studies that have shown increased faecal Lactobacillus count through probiotic supplementation (Giang et al. 2012; Hu et al. 2015; Dowarah et al. 2017). Lan and Kim (2019) also reported that dietary supplementation with B. licheniformis and B. subtilis complex resulted in increased faecal Lactobacillus counts, but there was no effects on E. coli population in growing pigs. In addition, Giang et al. (2011) suggested that B. subtilis and LAB supplementation increased faecal LAB concentration and decreased E. coli counts in growing pigs. Moreover, Balasubramanian et al. (2018), suggested that probiotics complex containing B. coagulans (1 × 109 CFU g⁻¹), B. licheniformis (5 × 10⁵ CFU g⁻¹), B. subtilis (1 × 10⁹ CFU g⁻¹), and C. butyricum (1 × 10⁸ CFU g⁻¹) supplementation increased faecal Lactobacillus and reduced E. coli counts at week 6 and 16 in growing-finishing pigs. These different findings suggest that inconsistent results may be related to the dose level and different probiotic species.

In the current study, probiotic supplements also showed to be effective on NH₃ concentration but did not have any significant effects on the faecal gas emission of H₂S, total mercaptans, and acetic acid. It showed that probiotic supplementation can reduce the amount of harmful gases emission from the faeces. Similarly, Liu et al. (2018) reported that the NH₃ content in the faeces of pigs fed with probiotic supplements (including B. subtilis and S. cerevisiae) was lower than that of non-probiotic growing pigs. Chen et al. (2006) also demonstrated that supplementing the diets with B. subtilis, B. coagulans, and L. acidophilus complex resulted in a lower faecal NH₃ emission in finishing pigs. In previous study reports, Ferket et al. (2002) indicated that probiotics can indirectly reduce environmental pollutants in animal manure by increasing the digestibility of nutrient and improving intestinal flora ecosystems. The increased digestibility may reduce the substrate for microbial fermentation in the large intestine, thereby reducing the emission of harmful gases in the faeces (Yan et al. 2011). It should be noted that, in our study, the supplementation of B. subtilis ms1, B. licheniformis SFS-1, and S. cerevisiae did not affect the digestibility of nutrients significantly, while faecal Lactobacillus concentration was reduced by probiotics supplementation. Therefore, we believe that the decrease in the concentration of the ammonia in the faeces may not be the result of the increased digestibility, but the mechanism by which probiotics indirectly affect harmful gas emissions from faeces is unclear, and further research is needed. On the other hand, lowering the pH of the gut will lead to inhibition of the growth of various pathogenic bacteria and re-establishing the balance of the intestinal flora (Doron and Gorbach 2006). We speculate that the cause of decreased ammonia emission from faeces may be related to intestinal pH. It has been previously reported that a decrease in intestinal pH (for four days) can also suppress ammonia emission in pig manure (Varel and Miller 2004), and the significant increase in Lactobacillus population in our experiment might have lowered the intestinal pH thereby suppressing ammonia emission.

Conclusions

The results of the current study provided positive effects or trend of the use of probiotics complex in low nutrient density diet, suggesting that a probiotic of B. subtilis ms1, B. licheniformis SFS-1, and S. cerevisiae complex may be a potential alternative to antibiotics growth promoter.

Disclosure statement

We confirm that there are no known conflicts of interest associated with this publication.

Animal welfare statement

The authors confirm that the ethical policies of the journal, as noted on the journal’s author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.
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