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ORIGINAL PAPER

Dietary inclusion of *Bacillus*-based probiotic complex improved average daily weight gain and gain-to-feed ratio and reduced faecal noxious gas emission in weaned pigs

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ABSTRACT. The objective of this study was to investigate the effects of dietary supplementation with a *Bacillus*-based probiotic complex on growth performance, nutrient digestibility, blood metabolites and faecal microbial communities in weaned piglets. A total of 144 crossbred weaned pigs (Yorkshire × Landrace) × Duroc, 28 days old, were randomly allocated to one of four dietary treatments. Each treatment was replicated in six pens, with six pigs per pen (three barrows and three gilts). The treatments included a basal diet (CON), and a basal diet supplemented with graded levels (0.1%, 0.2% and 0.3%) of a probiotic complex consisting of *Bacillus subtilis* (1.0×10^9 CFU/g) and *B. licheniformis* (1.0×10^9 CFU/g). The experimental diets were administered in a mash form for six weeks. The results showed that the gain-to-feed ratio (G:F) on days 0–7, and G:F and average daily weight gain (ADG) during days 22–42 and 0–42 increased linearly with higher proportions of added probiotics ($P < 0.05$). Furthermore, the 0.3% probiotic group exhibited significantly higher ADG and G:F ratio on days 0–42 compared to the CON group ($P < 0.05$). The concentrations of NH_3 and H_2S in faeces were also significantly lower in the 0.3% probiotic group compared to the CON group ($P < 0.05$). In conclusion, the addition of the 0.3% *Bacillus* probiotic complex to the diet resulted in an increase in average daily weight gain and the feed-to-gain ratio of weaned piglets, as well as a reduction in faecal noxious gas emissions.

Introduction

Pig producers are facing increasing restrictions on the use of antibiotics as growth promoters in animal production. Significant efforts are being made to identify strategies to facilitate piglet weaning. One promising approach that has gained attention in recent years is the use of probiotics as a nutritional intervention. Probiotics are believed to act through the competitive exclusion of pathogens (Leser et al., 2008). Different bacterial strains, including *Lactobacillus acidophilus*, *Enterococci faecium*, *Bacillus species*, *Bifidobacterium bifidum*, and the yeast *Saccharomyces cerevisiae* have demonstrated probiotic properties and are currently being researched as feed additives for livestock to evaluate their efficacy in improving animal production and performance (Sureshkumar et al., 2022; Muniyappan et al., 2023).

Bacillus, as spore forming bacteria, have been reported as extremely promising microorganisms for direct feeding. Compared with non-spore-forming bacteria, such as *Lactobacilli* and *Bifidobacterium,*

Bacillus spores can withstand harsh environments, enabling long-term storage, survive at low pH of the gastric barrier, and combat pathogenic bacteria by exerting probiotic properties in the small intestine (Cutting, 2011). *Bacillus* produces extracellular enzymes in its vegetative form, which helps improve overall immune gut function, as well as enhance nutrient digestibility and absorption (Upadhaya et al., 2015). Moreover, it has been demonstrated that *Bacillus* can improve growth performance and stimulate the proliferation of beneficial bacteria in weaned pigs (Hu et al., 2014; Kim et al., 2019). The addition of a commercial probiotic containing *B. licheniformis* and *B. subtilis* spores (BioPlus 2B) to the diet of weaned, grower and finisher pigs at a rate of 0.64 to 1.28 × 106 CFU/g feed reduced morbidity and mortality (Alexopoulos et al., 2004). Guo et al. (2006) found that *B. subtilis* MA139 effectively improved feed conversion ratio (FCR) in pigs. Jørgensen et al., (2016) reported positive effects of dietary supplementation of a mixture of *B. subtilis* and *B. licheniformis* on the growth performance of pigs. In contrast, the probiotic product Micro Source S (Agtech Products Inc., Manhattan, KS, USA), containing *B. licheniformis* and *B. subtilis*, did not improve growth rate or feed intake (Davis et al., 2008) even when administered at a high dose of 1.47×10^8 CFU/g feed; however, it did increase feed conversion efficiency by 3%. (AOAC International, 2007). The varying effects of the same probiotic microorganisms may be attributed to differences in strains and dosages, husbandry practices (nutrition, housing, etc.), age of pigs and the type of feed used. Consequently, further research is necessary to clarify the effects of *Bacillus*-based probiotics. The present experiment was conducted to evaluate the role of *Bacillus* as a probiotic with the potential to improve production and performance characteristics of weaned pigs under various stressors affecting their health and performance. The study examined the effects of different doses of a *Bacillus*-based probiotic complex on growth performance, nutrient digestibility, blood metabolites, faecal microflora, and noxious gas emissions in weaned pigs.

Material 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-2-1612).

The probiotic complex used in the present study was purchased from B&B (Gyeonggi-do, Korea). According to the supplier's information, this product contained a spray dried mixture of *B. subtilis* (1.0×10^9 CFU/g) and *B. licheniformis* (1.0×10^9 CFU/g) in powder form.

Animals, diets, and experimental design

A total of 144 crossbred weaned pigs [(Yorkshire × Landrace) × Duroc, 28 days old], with an average body weight (BW) of 7.84 ± 1.75 kg, were used in a six-week experiment. The pigs were stratified by BW and sex and randomly allotted to one of four dietary treatment groups, with six pigs per pen (three barrows and three gilts) and six pens per treatment. The treatments comprised a basal diet (CON) and a basal diet supplemented with graded levels (0.1, 0.2 and 0.3%) of a probiotic complex, consisting of *B. subtilis* (1.0×10^9 CFU/g) and *B. licheniformis* (1.0×10^9 CFU/g). The experimental period was divided into two phases: the first seven days, and the second phase from day 8 to 42 of the feeding period. All nutrients in the diets were formulated to meet or exceed the recommendations of the National Research Council (NRC, 2012) for weaned pigs fed in mash form (Table 1).

Table 1. Ingredient and chemical composition of diets, %

Ingredients, %	Day 0–7	Day 7–42
Maize	62.15	66.25
Soybean meal (43%)	18.00	18.00
Fish meal	8.00	6.00
Whey powder	8.00	6.00
Soybean oil	0.35	0.35
$CaHPO_4$	0.80	0.80
Limestone	0.50	0.50
Salt	0.20	0.20
Premix[1]	0.50	0.50
L-Lys-HCl (78.8%)	0.70	0.70
DL-methionine	0.45	0.40
L-threonine	0.35	0.30
Total	100.00	100.00
Calculated nutrient level, %		
digestible energy, MJ/kg	14.06	14.11
crude protein	20.08	18.82
total Ca	0.89	0.79
total P	0.72	0.66
SID Lys	1.55	1.44
SID Met + Cys	0.89	0.83
SID Thr	0.97	0.86
Ca:P	1.24	1.19

[1] premix contained the following ingredients per kg of diets: IU: vit. A 12000, vit. D_3 2500, vit. E 30; mg: vit. K_3 3, vit. B_5 10, vit. B_{12} 27.6, niacin 30, choline chloride 400, Mn (as MnO) 40, Fe (as $FeSO_4 \cdot H_2O$) 90, Zn (as ZnO) 100, Cu (as $CuSO_4 \cdot 5H_2O$) 8.8, I (as KI) 0.35, Se (Na_2SeO_3) 0.3; SID – standard ileal digestibility; Lys – lysine, Met – methionine, Thr – threonine; maize was partially replaced with a 0.2% probiotic complex

The additive was incorporated into the diet by replacing an equivalent amount of maize. All piglets were housed in an environmentally controlled room, initially maintained at a constant temperature of 30 °C, and then gradually reduced by 1 °C each week of the experiment. Each pig was allocated a total area of 0.36 × 0.53 m^2. Each pen was equipped with a stainless-steel feeder and a nipple drinker, ensuring that the animals had *ad libitum* access to feed and water throughout the experiment. The ventilation system was mechanical, and the lighting was automatically regulated to provide a minimum of 12 h of artificial light per day.

Production performance and sampling

Initial BW and BW on days 7, 21 and 42, as well as the daily feed consumption per pen were measured to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F). Faecal scores were assessed at 08:00 and 20:00 using the following faecal scoring system: 1 – hard, dry pellets in a small, hard mass; 2 – hard, formed stool that remains firm and soft; 3 – soft, formed, and moist stool that retains its shape; 4 – soft, unformed stool that assumes the shape of the container; 5 – watery, liquid stool that can be poured. Faecal grab samples were collected from all pigs, and scores were recorded on a pen basis, following observations of individual pigs and the identification of stool consistency in the pen. Chromium oxide was incorporated into the diet at a concentration of 0.5% for a period of seven days prior to the collection of faecal samples in the sixth week. Faeces were collected by applying rectal massage to obtain fresh samples. For each treatment group, 500 g and 10 g of faeces were randomly collected from four circles to determine the faecal noxious gas emissions and faecal microbial content, respectively. This process aimed to calculate the apparent total tract digestibility (ATTD) of dry matter (DM), and nitrogen (N), and energy contents. At least two faecal samples were collected from each pen, comprising one sample from a barrow and one from a gilt. These samples were subsequently mixed and pooled, with a representative sample stored in a freezer at −20 °C until analysis. Blood samples (5 ml) were collected from a total of 24 randomly selected pigs, 12 per treatment group, via jugular venipuncture using a sterile needle on day 42. The blood samples were pooled on a pen basis into tubes without additives and allowed to clot at ambient temperature for 30 min. Subsequently, serum was collected by centrifuging the samples at 3000 *g* for 15 min at 4 °C.

Sample analysis

All feed and faecal samples were freeze-dried and finely ground to pass through a 1-mm screen. Dry matter and N digestibility were determined using methods established by the Association of Official Analytical Chemists (AOAC International, 2007). Chromium levels were determined using UV absorption spectrophotometry (UV-1201; Shimadzu, Kyoto, Japan). Prior to chemical analysis, the faecal samples were thawed and dried at 60 °C for 72 h, and then finely ground to pass through a 1-mm screen. They were subsequently analysed for nitrogen content (method 968.06; AOAC International, 2007), Ca (method 984.01; AOAC International, 2007), and P (method 965.17; AOAC International, 2007). Lysine was measured using an AA Analyser (Beckman 6300; Beckman Coulter Inc., Fullerton, CA, USA) following a 24-h hydrolysis in HCl (Spackman et al., 1958). Nitrogen content was determined using a Kjeltec 2300 Nitrogen Analyzer (Foss Tecator AB, Höganäs, Sweden), and CP was calculated as N × 6.25.

Gross energy was determined by measuring the heat of combustion in the samples using a Parr 6200 oxygen bomb calorimeter (Parr Instrument Co., Moline, IL, USA). ATTD of nutrients was calculated according to the method described by Muniyappan et al. (2023) using the following formula:

$$\text{digestibility, \%} = \{1 - [(Nf \times Cd) / (Nd \times Cf)]\} \times 100,$$

where: Nf – nutrient concentration in faeces (% DM), Nd – nutrient concentration in diet (% DM), Cd – chromium concentration in diet (% DM), and Cf – chromium concentration in faeces (% DM).

Blood samples were determined using a HITACHI 747 automatic biochemistry analyser (Tokyo, Japan).

Faecal samples were pooled on a pen and basis transported on ice to the laboratory for immediate analyses. A composite faecal sample (1 g) was collected from each pen and diluted with 9 ml of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ, USA), followed by homogenisation. The counts of viable bacteria in faecal samples were determined by plating serial 10-fold dilutions (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 Escherichia coli and Lactobacillus, respectively. Microbial plates were inoculated with three dilutions (20 µl each) in duplicate. Subsequently, Lactobacilli medium III agar plates were incubated at 39 °C for

48 h under anaerobic conditions. MacConkey agar plates were incubated at 37 °C for 24 h. The number of E. coli and Lactobacillus colonies was counted immediately upon removal of the plates from the incubator. Results were expressed as the logarithm of colony-forming units per g (log10 CFU/g).

A total of 300 g of faeces were placed in a 2.6-l plastic box with a small hole in the middle of one side of the box. The box was then sealed with adhesive tape. The samples were incubated at room temperature (25 °C) for 24 h. An air sample of 100 ml was taken from a height of approximately 2.0 cm above the faecal sample. Subsequently, the box was resealed with adhesive tape to determine the concentration of harmful substances in the faeces. Measurements were made using a gas detector (No. 3La, detector tube; Gastec Corp. Kanagawa, Japan). Prior to measurement, faecal samples were manually shaken for approximately 30 s to break up any crusts on the surface and to homogenise the samples.

Statistical analyses

Data were analysed using the MIXED procedure implemented in the statistical software SAS (SAS Institute Inc., Cary, NC, USA). Treatment diets were considered as fixed effects, while the effects of sex and weight were treated as random effects. Orthogonal polynomial contrasts were employed to identify linear effects across the data set. Significance of differences between the experimental groups was determined using an ANOVA test. Tukey's test was applied to distinguish between the least squares means of the treatments. The pen was designated as the experimental unit. Statistical significance was set at the level of $P \leq 0.05$, while P values greater than 0.05 were considered not significant.

Results

Growth performance and faecal score

As shown in Table 2, the G:F ratio during days 0–7 and the G:F ratio and ADG during days 22–42 and 0–42 increased linearly with rising proportion of probiotics ($P < 0.05$). Additionally, the 0.3% probiotic group exhibited significantly higher ADG and G:F ratio during days 0–42 compared to the CON group ($P < 0.05$). No significant differences in BW and faecal scores were observed between treatment groups ($P > 0.05$) (Table 3).

Table 2. Effect of dietary *Bacillus*-based probiotic complex supplementation on growth performance in weaned pigs

Items	Groups				SEM	*P*-value		
	CON	PRO						
		0.1 %	0.2 %	0.3 %		ANOVA	linear	quadratic
Body weight, kg								
Initial	7.85	7.84	7.84	7.84	0.744	0.988	0.989	0.998
Day 7	9.46	9.45	9.54	9.56	0.762	0.999	0.904	0.984
Day 21	14.92	15.25	15.33	15.41	0.833	0.977	0.682	0.883
Day 42	25.35	26.07	26.36	26.84	0.916	0.710	0.258	0.897
Day 0–7								
ADG, g	230	230	243	247	10.13	0.747	0.310	0.890
ADFI, g	283	279	285	287	16.32	0.987	0.801	0.848
G:F	0.813	0.826	0.852	0.86	0.014	0.076	0.012	0.867
Day 8–21								
ADG, g	390	415	413	418	16.06	0.601	0.266	0.544
ADFI, g	534	566	548	551	18.15	0.658	0.686	0.419
G:F	0.731	0.732	0.754	0.758	0.015	0.427	0.128	0.923
Day 22–42								
ADG, g	497	515	525	544	12.39	0.083	0.012	0.989
ADFI, g	825	821	821	850	10.28	0.180	0.126	0.123
G:F	0.601	0.627	0.639	0.64	0.011	0.064	0.015	0.261
Overall								
ADG, g	417^{b}	434^{ab}	441^{ab}	453^{a}	8.539	0.051	0.007	0.729
ADFI, g	638	646	641	656	8.000	0.404	0.174	0.659
G:F	0.653^{b}	0.672^{ab}	0.688^{a}	0.689^{a}	0.008	0.014	0.002	0.299

CON – control group, PRO – probiotic group receiving a probiotic complex consisting of *Bacillus subtilis* (1.0×10^9 CFU/g) and *B. licheniformis* (1.0×10^9 CFU/g); ADG – average daily gain, ADFI – average daily feed intake, G:F – gain to feed ratio, SEM – standard error of the mean; data are presented as means and SEM (n = 6); values without letters or with the same superscripts are not significantly different; means with different superscripts are significantly different at $P < 0.05$

Table 3. Effect of dietary *Bacillus*-based probiotic complex supplementation on faecal score in weaned pigs

Items	Groups				SEM	*P*-value		
	CON	PRO						
		0.1%	0.2%	0.3%		ANOVA	linear	quadratic
Day 7	3.25	3.22	3.23	3.22	0.070	0.982	0.768	0.869
Day 21	3.26	3.19	3.11	3.23	0.048	0.157	0.390	0.060
Day 42	3.15	3.14	3.12	3.15	0.025	0.724	0.835	0.357

CON – control group, PRO – probiotic group receiving a probiotic complex consisting of *Bacillus subtilis* (1.0×10^9 CFU/g) and *B. licheniformis* (1.0×10^9 CFU/g); SEM – standard error of the mean; data are presented as means and SEM (n = 6); values without letters or with the same superscripts are not significantly different; means with different superscripts are significantly different at $P < 0.05$

Table 4. Effect of dietary *Bacillus*-based probiotic complex supplementation on nutrient digestibility in weaned pigs, %

Items, %	Groups				SEM	*P*-value		
	CON	PRO						
		0.1%	0.2%	0.3%		ANOVA	linear	quadratic
Dry matter	81.02^{b}	82.14^{ab}	82.76^{a}	82.47^{ab}	0.391	0.021	0.008	0.084
Nitrogen	81.45^{b}	82.42^{ab}	83.61^{a}	83.43^{a}	0.460	0.009	0.002	0.220
Energy	80.37^{b}	80.82^{b}	82.56^{a}	83.44^{a}	0.431	<0.001	<0.001	0.624

CON – control group, PRO – probiotic group receiving a probiotic complex consisting of *Bacillus subtilis* (1.0×10^9 CFU/g) and *B. licheniformis* (1.0×10^9 CFU/g); SEM – standard error of the mean; data are presented as means and SEM (n = 6); values without letters or with the same superscripts are not significantly different; means with different superscripts are significantly different at $P < 0.05$

Nutrient digestibility and blood metabolites

Dietary inclusion of the probiotic complex improved ATTD of DM, N, and energy in weaned pigs (Table 4). Supplementation of the 0.2% probiotic complex in the diet of weaned pig increased ($P < 0.05$) ATTD of DM compared to other treatments. In addition, N and energy ATTD was also improved ($P < 0.05$) with the inclusion of both 0.2 and 0.3% probiotic complex doses compared to the control. A linear increase ($P < 0.05$) in the digestibility of DM, N, and energy was observed with increasing levels of the probiotic complex. However, supplementation with different levels of the probiotic complex did not affect blood metabolites when compared to the control (Table 5; $P > 0.05$).

Faecal microflora abundance and noxious gas emissions

Dietary inclusion of the probiotic complex increased *Lactobacillus* counts and decreased *E. coli* counts in weaned pigs (Table 6). The number of faecal *E. coli* was reduced ($P < 0.05$) in pigs fed the diet supplemented with the 0.3% probiotic complex compared to the other treatments. A linear increase ($P < 0.05$) in faecal *Lactobacillus* counts and a linear reduction ($P < 0.05$) in faecal *E. coli* counts were observed in pigs fed graded levels of the probiotic complex. Additionally, the concentrations of NH_3 and H_2S in faeces decreased linearly with increasing proportion of probiotic ($P < 0.05$) (Table 7).

Table 5. Effect of dietary *Bacillus*-based probiotic complex supplementation on blood metabolites at week 6 in weaned pigs

Items, mmol/l	Groups				SEM	*P*-value		
	CON	PRO						
		0.1%	0.2%	0.3%		ANOVA	linear	quadratic
Total cholesterol	2.73	2.97	2.96	2.98	0.231	0.874	0.487	0.634
Triglycerides	0.61	0.37	0.44	0.56	0.095	0.295	0.867	0.075
Total protein, g/l	63.4	66.1	63.9	67.4	3.185	0.799	0.496	0.904
Urea	4.63	4.50	4.98	4.32	0.408	0.709	0.818	0.525
LDL	1.66	2.16	2.05	1.82	0.231	0.435	0.739	0.127
HDL	0.45	0.44	0.46	0.66	0.155	0.759	0.446	0.770
BUN, mg/dl	9.00	9.75	9.5	9.25	0.685	0.879	0.873	0.479
Creatinine, mg/dl	0.99	0.91	0.85	0.99	0.136	0.843	0.929	0.414
Glucose, mg/dl	100.5	93.01	97.25	93.75	6.653	0.841	0.596	0.766

CON – control group, PRO – probiotic group receiving a probiotic complex consisting of *Bacillus subtilis* (1.0×10^9 CFU/g) and *B. licheniformis* (1.0×10^9 CFU/g); LDL – low-density lipoprotein, HDL – low-density lipoprotein, BUN – blood urea nitrogen, SEM – standard error of the mean; data are presented as means and SEM (n = 6); values without letters or with the same superscripts are not significantly different; means with different superscripts are significantly different at $P < 0.05$)

Table 6. Effect of dietary *Bacillus*-based probiotic complex supplementation on faecal microflora counts at week 6 in weaned pigs

Items, log_{10} CFU/g	Groups				SEM	*P*-value		
	CON	PRO						
		0.1%	0.2%	0.3%		ANOVA	linear	quadratic
lactobacillus	7.1[b]	7.09[b]	7.13[ab]	7.22[a]	0.027	0.029	0.009	0.114
Escherichia coli	5.83[a]	5.78[ab]	5.75[ab]	5.67[b]	0.024	0.006	<0.001	0.685

CON – control group, PRO – probiotic group receiving a probiotic complex consisting of *Bacillus subtilis* (1.0×10^9 CFU/g) and *B. licheniformis* (1.0×10^9 CFU/g); SEM – standard error of the mean; data are presented as means and SEM (n = 6); values without letters or with the same superscripts are not significantly different; means with different superscripts are significantly different at $P < 0.05$

Table 7. Effect of dietary *Bacillus*-based probiotic complex supplementation on faecal noxious gas emissions at week 6 in weaned pigs

Items, ppm	Groups				SEM	*P*-value		
	CON	PRO						
		0.1%	0.2%	0.3%		ANOVA	linear	quadratic
NH_3	7.63[a]	6.50[a]	6.73[a]	5.40[b]	0.530	0.074	0.019	0.854
H_2S	5.25[a]	5.23[a]	4.93[ab]	4.38[b]	0.145	0.004	<0.001	0.096
Total mercaptan	3.18	3.05	3.10	3.05	0.090	0.734	0.434	0.684

CON – control group, PRO – probiotic group receiving a probiotic complex consisting of *Bacillus subtilis* (1.0×10^9 CFU/g) and *B. licheniformis* (1.0×10^9 CFU/g); SEM – standard error of the mean; data are presented as means and SEM (n = 6); values without letters or with the same superscripts are not significantly different; means with different superscripts are significantly different at $P < 0.05$

Notably, the concentrations of NH_3 and H_2S in faeces were significantly lower in the 0.3% probiotic group compared to the CON group ($P < 0.05$).

Discussion

Probiotics based on *Bacillus* and lactic acid bacteria (mainly *Lactobacillus*, *Bifidobacterium* and *Enterococcus*) are recognised for their role in reducing pathogenic bacteria and improving growth performance in weaned pigs. Liu et al. (2015) demonstrated that L. brevis improved growth performance (ADG, ADFI, and G:F ratio) and intestinal microflora balance in weaned pigs. Similarly, Hu et al. (2014) reported that supplementation with *B. subtilis* enhanced growth performance (ADG and G:F ratio), increased *Lactobacillus* counts and decreased *E. coli* counts in the gut of weaned pigs. Consistent findings were also reported by Wang et al., (2019) in weaned pigs fed *Bacillus*. However, research on probiotics based on both *Bacillus* and lactic acid bacteria has shown inconsistent findings concerning weaned pigs (Barba-Vidal et al., 2018). Given the more pronounced beneficial effects of *Bacillus* in pigs, we were interested in evaluating the efficiency of supplementation with a *Bacillus*-based probiotic complex in weaned pigs.

The current study utilised a *Bacillus*-based probiotic complex comprising a mixture of *B. subtilis* and *B. licheniformis* to assess its efficacy in improving performance and nutrient digestibility in weaned pigs. These two species have been approved as biological supplements that have no toxic effects to livestock diets (EFSA, 2010). In addition, they are widely applied in the large-scale industrial production of extracellular enzymes and proteins (Degering et al., 2010). In the current study, dietary supplementation with the *Bacillus*-based probiotic complex at doses of 0.2 and 0.3% improved feed efficiency throughout the experimental period by 4.8 and 5.8%, respectively, compared to the control group. Moreover, higher levels of probiotic supplementation were associated with a linear increase in BW, G:F ratio, and ADG during days 22–42 and 0–42. Jørgensen et al. (2016) also indicated that the inclusion of a mixture of *B. subtilis* and *B. licheniformis* in the diet at a dose of 0.4 g/kg improved BW and tended to increase the gain-to-feed ratio during days 40 to 70. Previous studies have similarly reported that a *Bacillus*-based probiotic improved growth performance in weaned pigs (Wang et al., 2009). The positive effects of this probiotic on feed efficiency may be attributed to the presence of enzymes and other beneficial substances, as well as the adhesion and immunomodulation effects exerted by *Bacillus* spores (Leser et al., 2008).

The digestibility of DM, N, and energy in the present study improved with the inclusion of the 0.3% probiotic complex compared to the CON group. Additionally, a linear increase in the digestibility of these nutrients was also observed with the growing level of probiotic complex in the diet. In line with our study, a previous work demonstrated an improvement in ATTD of DM and CP in weaned pigs fed a diet supplemented with 3 and 4.5 g/kg fermented biomass from *B. subtilis* and citrus waste juice (Lee et al., 2014). In addition, Choi et al. (2011) reported that weaned pigs fed a *Bacillus*-based probiotic complex

exhibited enhanced ATTD of DM and energy. Moreover, Sen et al. (2012) found a linear improvement in the contents of DM, GE and CP in broilers in response to increasing dietary levels (10^7, 10^8, 10^9 CFU/kg diet) of *B. subtilis* and citrus juice waste fermentation product. The observed improved nutrient digestibility could be associated with the production of extracellular enzymes, including proteases and amylases by *B. subtilis* and *B. licheniformis* (Carlisle and Falkinham, 1989), which may have contributed to improved feed conversion.

Blood or plasma urea nitrogen concentration is an indication of protein status and nitrogen utilisation in animals (Whang et al., 2003). While blood urea nitrogen (BUN) and creatinine are not toxic per se, elevated levels of these compounds in serum correlate with the accumulation and toxicity of unidentified uremic molecules. In the current study, we aimed to evaluate the effects of the probiotic complex, on blood metabolites such as BUN, creatinine, and glucose. Previous research has demonstrated that the gut microflora can affect the concentrations of uremic toxins in animals (Takayama et al., 2003). It is well established that urea contributes to the synthesis of other toxic moieties (Stim et al., 1995). Moreover, excess urea induces the generation of free radicals and oxidative stress leading to health problems (Zhang et al., 1999). Urease-expressing bacteria may decrease blood urea, and live bacteria administration has been explored as a novel enteric approach to alleviate uraemia. For instance, feeding nephrectomised rats with *B. pasteurii* and *Lactobacillus* sporogenes reduced BUN and serum creatinine levels, indicating that probiotics can mitigate problems associated with kidney function (Ranganathan et al., 2005). In the present study, BUN, creatinine and glucose concentrations remained unaffected after dietary supplementation of the probiotic complex in weaned pigs fed a basal diet containing plasma protein, which was consistent with findings of Meng et al. (2010). The lack of effect on BUN and creatinine levels could be attributed to the absence of excess urea formation and efficient nitrogen utilization. Additionally, no effect on lipid metabolism-related blood indices was observed.

Probiotics or direct administered microbials have been reported to stimulate the development of beneficial microorganism and suppress harmful ones, thereby benefitting host health by improving microbial balance (Lescheid, 2014). In this study, the abundance of faecal *E. coli* decreased with probiotic complex supplementation, and a linear increase in *Lactobacillus* counts was recorded with increasing probiotic dose, likely due to its antimicrobial properties. In addition, no instances of diarrhoea (faecal score) were observed. In agreement with our findings, Lan et al. (2017) also suggested that *Bacillus*-based probiotic supplementation could increase the number of faecal *Lactobacillus* and reduce *E. coli* counts. *Bacillus*-based probiotics, along with other probiotics, have been reported to produce several types of thermostable bacteriocins (Cotter et al., 2005) exerting antimicrobial activity against potential pathogens. Moreover, the study revealed that the incorporation of probiotics markedly diminished the generation of faecal noxious gases in weaned pigs. The improved microbiota ecosystem might be one of the reasons for the improved feed efficiency, nutrient digestibility, and lower harmful gas emissions observed in the present study.

Conclusions

In summary, supplementing the diet of weaned pigs with a probiotic complex (a mixture of *Bacillus licheniformis* and *B. subtilis*) provided beneficial effects, improving feed efficiency, nutrient digestibility, and reducing faecal *Escherichia coli* counts and noxious gas emissions. Among the tested doses, the 0.3% *Bacillus*-based probiotic complex showed most optimal effects compared to the other doses.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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