Effects of dietary supplementation of bacteriophage with or without zinc oxide on the performance and gut development of weanling pigs

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ABSTRACT
The present study investigates the effect of zinc oxide (ZN), bacteriophage (BAC) or their combination on the growth performance and gut development in weaning pigs. A total of 200 weaned pigs were allotted to four treatments including two levels (0 and 0.34%) of ZN and two levels (0 and 0.10%) of BAC cocktail. Supplementation of both BAC and ZN in the diet improved average daily gain and gain to feed ratio in all three phases. The apparent total tract digestibility (ATTD) of dry matter was consistently increased in BAC. A higher digestibility of dry matter was observed in ZN group at phase-I and II. The ATTD of crude protein was increased in BAC group at phase-I and III. ZN increased ATTD of crude protein during phase III. In all phases, the population of total anaerobic bacteria, Bifidobacterium spp., Lactobacillus spp., Clostridium spp. and coliforms were higher in BAC and ZN groups with the exception for coliforms in ZN at the end of experiment. The duodenum (p < 0.05) and jejenum (p < 0.01) villus heights were considerably increased in BAC group but the ileal villus height was not affected by the addition of BAC in the diet. Similar increase (p < 0.05) in the duodenal (p = 0.06) and jejunal (p < 0.01) villus heights were also observed in ZN supplemented groups. The overall faecal score was reduced (p < 0.01) by BAC and tended to decrease (p = 0.07) by ZN. Thus both ZN and BAC are useful for improving the performance and gut health in weaning pigs without any interactive effects.

Introduction
Weaning is the most critical period of the pigs’ life span and weaned pigs have to face variety of nutritional, environmental and social stressors, which often leads to growth depression, post-weaning diarrheic syndrome, that sometimes even results in death (Laine et al. 2008). To overcome these issues antibiotic growth promoters were commonly introduced in the weaning pigs’ diet. However, due to their regular use, antibiotic resistance emerged as another problem and thus a ban was enforced on the use of in-feed antibiotics for livestock growth promotion (Simon 2005). In search for the better alternatives, organic acids, probiotics, antimicrobial peptides, etc. have tried to replace in-feed antibiotics with limited success (Simon 2005; Cenci-Goga et al. 2015). Zinc is an essential trace element with many known physiological functions, such as immune, growth, reproduction (Hendy et al. 2001), and is well known for enhancing the growth rates and preventing diarrhea in young pigs (Carlson et al. 1999). Zinc has been used in the pig diets for a long time and its recommended intake for growing pigs is 50–100 mg per kg feed dry matter (NRC 2012). However higher doses of Zn have been tried in the diets of nursery pigs immediately following the weaning period and was reported to have beneficial effects on growth performance, antibacterial function and diarrhoea incidence (Hill et al. 2001).

The most recent advancement, in the search for better antimicrobial, is bacteriophages (BACs). They are the viruses that multiply inside bacteria by using its biosynthetic machinery and they act as a natural predator for host (McGrath et al. 2004). In the past, BACs have been studied for their therapeutic effects in pigs and poultry challenged with disease (Toro et al. 2005; Gebru et al. 2010). Recently, a few studies on dietary supplementation of BACs have reported to improve the performance, feed efficiency and excreta microbiota in pigs (Gebru et al. 2010; Kim et al. 2014) and poultry (Wang et al. 2013) and suggested that they could be used as alternative to antibiotics growth promoters.
The present study was designed to investigate the effects of BACs, with or without ZnO on the growth performance, nutrient digestibility, gut health, microbial population and the diarrhoea incidence of weaning pigs. We hypothesised that the dietary supplementation of BAC, in combination with ZnO, may improve the growth performance and gut development of weaning pigs.

Materials and methods

The experiment was conducted at the facility of Kangwon National University farm and was approved by the Institutional Animal Care and Use Committee of Kangwon National University, Chuncheon, Republic of Korea. Proper ethical standards were followed during the experiment.

The BAC that was used in the present study was acquired from a commercial feed company (CTC Bio, Inc., Seoul, Republic of Korea). The BAC contained a cocktail of: Salmonella (Salmonella typhimurium, Salmonella enteritidis, Salmonella cholasuis and Salmonella derby), Staphylococcus aureus, Escherichia coli (k88, k99 and f41) and Clostridium perfringens types A and C with 109 plaque-forming units per gram (pfu/g) BACs. The zinc source (ZnO) for the present experiment was procured from Hanil Chemical Ind. Co. Ltd.

Animal, diets and managements

A total of 200 piglets (Landrace × Yorkshire × Duroc) of mixed sex were randomly allotted to four treatments on the basis of initial BW (average weight 7.34 ± 0.27 kg). There were five replicate pens in each treatment with 10 pigs per pen. The piglets were housed in partially slotted and concrete floor pens equipped with a self-feeder and nipple drinker to allow ad libitum access to feed and water. Two levels (0 and 0.34%) of zinc oxide (ZN) and two levels (0 and 0.10%) BAC cocktail supplemented to the diet. The experimental diets exceeded the nutrient requirements as suggested by NRC (2012) and were fed in meal form for three phases (phase-I from d 0 to 7, phase-II from d 8 to 21 and phase-III from d 22 to 35) for total of 35 days and has been presented in Table 1.

Sampling and measurements

The pigs were weighed individually at the start and at the end of the phases. The feed consumption was calculated at the end of each phase to calculate average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G/F). To determine the effect of different treatments on the apparent total tract digestibility (ATTD), chromic oxide (2.50 g/kg) was added in each diet and faecal grab samples were collected during the last 4 days of each phase of the experiment to determine the ATTD of dry matter (DM), gross energy (GE) and crude protein (CP). The faecal samples were then pooled within pen, dried in a forced air oven at 60°C for 72 h, grounded in a Wiley Mill (Thomas Model 4, Wiley Mill, Thomas Scientific, Swedesboro, NJ) using a 1-mm screen and used for chemical analysis.

To evaluate the faecal microbial content, at the final day of experiment, i.e. at d 35, fresh faecal samples from two pigs in each pen were collected and kept on ice to measure the faecal bacterial population.

Chemical and microbial analyses

Analysis for each sample was done in triplicate for DM (Method 930.15) and CP (Method 990.03), according to the methods of AOAC (2007). Gross energy of diets and excreta were measured using a bomb calorimeter (Model 1261, Parr Instrument Co., Molin, IL), while chromium concentrations were determined with an automated spectrophotometer ( Shimadzu, Japan) according

Table 1. Formula and chemical composition of experimental diets (as fed basis).

| Item               | Phase I     | Phase II    | Phase III   |
|--------------------|-------------|-------------|-------------|
| Ingredients, %     |             |             |             |
| Corn               | 36.61       | 47.29       | 63.75       |
| Whey powder        | 15.00       | 8.00        | –           |
| Fish meal          | 5.00        | 5.00        | 5.00        |
| Soybean meal dehulled | 24.20     | 29.25       | 26.31       |
| Soy protein concentrate | 5.00      | –           | –           |
| Soy oil            | 2.43        | 2.94        | 1.84        |
| Sucrose            | 3.00        | –           | –           |
| Lactose            | 6.25        | 4.80        | –           |
| L-Lysine HCl (78%) | 0.21        | 0.22        | 0.31        |
| L-Methionine (98%) | 0.14        | 0.11        | 0.09        |
| Choline chloride (50%) | 0.08      | 0.08        | 0.08        |
| Monocalcium phosphate | 0.94      | 0.99        | 1.24        |
| Lysine             | 0.44        | 0.57        | 0.63        |
| Salt               | 0.20        | 0.25        | 0.25        |
| Vitamin premixα    | 0.25        | 0.25        | 0.25        |
| Mineral premixα    | 0.25        | 0.25        | 0.25        |
| Total              | 100         | 100         | 100         |
| Chemical composition |           |             |             |
| ME, kcal/kg        | 3400        | 3400        | 3400        |
| Crude protein, %   | 23.00       | 22.00       | 23.00       |
| Calcium, %         | 0.80        | 0.80        | 0.80        |
| Available phosphorus, % | 0.50    | 0.46        | 0.50        |
| Lysine, %          | 1.53        | 1.45        | 1.53        |
| Methionine + cystine, % | 0.87   | 0.83        | 0.87        |
| Lactose, %         | 16.00       | 10.00       | –           |

aSupplied per kilogram of diet: 16,000 U vitamin A, 3000 U vitamin D3, 40 U vitamin E, 5.0 mg vitamin K3, 5.0 mg vitamin B12, 4 mg vitamin B6, 0.08 mg vitamin B12, 40 mg pantothenic acid, 75 mg niacin, 0.15 mg biotin, 0.65 mg folic acid.

bSupplied per kilogram of diet: 45 mg iron, 0.25 mg cobalt, 50 mg copper, 15 mg manganese, 25 mg zinc, 0.35 mg iodine, 0.13 mg selenium.
to the procedure described by Fenton and Fenton (1979).

To determine the total anaerobic bacteria (Tryptic soy agar), Lactobacillus spp. (using MRS agar + 0.200 g/l NaNO3 + 0.500 g/l l-cystine hydrochloride monohydrate), Bifidobacterium spp. (MRS-NPNL: MRS agar + nalidixic acid, paromomycin + neomycin sulphate + lithium chloride), Clostridium spp. (TSC agar) and coliforms (violet red bile agar) were used. The anaerobic conditions during the assay of total anaerobic bacteria and Clostridium spp. were created by using gas pack anaerobic system (BBL, No. 260678, Difco, Detroit, MI). The tryptic soy agar (No. 236950), MRS agar (No. 288130), violet red bile agar (No. 216695) were purchased from Oxoid (Hampshire, UK). The bacterial concentrations were transformed (log) before statistical analysis.

Small intestinal morphology

To analyse the intestinal sample, three cross-sections were prepared after staining with azure A and eosin using standard paraffin embedding procedures. A total of 10 intact, well-oriented crypt-villus units were selected in triplicate for each intestinal cross-section. The measurement of villus height was done from the tip of the villi to the villus crypt junction, while the crypt depth was defined as the depth of the invagination between adjacent villi and villus width was measured till the mid of the villus. All morphological measurements (villus height and crypt depth) were made in 10-μm increments by using an image processing and analysis system (Optimus software version 6.5, Media Cybergenetics, North Reading, MA).

Diarrhoea incidence

The incidence of diarrhoea was measured by scoring the faeces as zero (normal), 1 point (soft faeces), 2 points (mild diarrhoea) and 3 points (severe diarrhoea) in all the experiments. The overall cumulative incidence of diarrhoea was measured daily at 9:00 am for five weeks and the final diarrhoea incidence was determined as the average of the scores.

Statistical analysis

Data generated in this experiment was analyzed as a 2 × 2 factorial arrangement in a completely randomised design. Pens were considered the experimental unit for growth performance, whereas individual pig was used as experimental unit for analysis of microbial population and intestinal morphology parameters. The main effects of BACs and ZN, and their interaction were determined by mixed procedure of SAS statistical program (SAS Inst., Inc., Cary, NC). p Values ≤ 0.05 were considered statistically significant.

Results

Growth performance

Growth performance data are shown in Table 2. No BAC × ZN interactions were observed for ADG, ADFI and G/F. Supplementation of both BAC and ZN in the diet improved ADG and G/F in all three phases. The ADFI was similar in all the treatment groups and were not affected (p > 0.05) throughout the experiment.

Digestibility

There were no interactions between BAC and ZN to affect the digestibility of nutrients (p > 0.05). The ATTD of DM was consistently increased (p < 0.05) in BAC addition throughout the experiment (phase-I, II and III; Table 3). Similar increase (p < 0.05) in the ATTD of DM was also observed in ZN group at phase-I and II of the experiment. The ATTD of GE does not differ (p > 0.05) throughout the experiment. The ATTD of CP was increased (p < 0.05) in BAC group at phase-I and III of the experiment but was not affected in phase II. ZN increased ATTD of CP during phase III.

Faecal microbial population

There were no BAC × ZN interactions in all phases (Table 4). At d 7 the population of total anaerobic population...
bacteria (TAB) *Bifidobacterium spp.*, *Lactobacillus spp.*, *Clostridium spp.* and coliforms were higher (*p < 0.05*) in BAC and ZN groups (Table 4). Similar increase was also observed on d 21 and d 35 with increase (*p < 0.05*) in the count in ZN or BAC supplemented diets with the exception for coliforms in ZN at the end of experiment.

### Intestine morphology

BACs did not interact with ZN to affect the intestine morphology (*p > 0.05*). The duodenum (*p < 0.05*) and jejunum (*p < 0.01*) villus heights were considerably increased in BAC group but the ileal villus height was not affected by the addition of BAC in the diet (Table 5). Similar increase (*p < 0.05*) in the duodenal (*p = 0.06*) and jejunal (*p < 0.01*) villus heights were also observed in ZN supplemented groups. The crypt depth of duodenum, jejunum and ileum was not different in BAC or ZN groups. There was no variation in the villus height to crypt depth ratio (VH/CD) of duodenum, jejunum and ileum in any of the treatments group.

### Table 3. Effect of dietary zinc oxide, bacteriophage or their combination on apparent total tract digestibility in weaning pigs.

| Bacteriophage (BAC) | – | + | SEM* | BAC | ZN | ZN*BAC |
|---------------------|---|---|------|-----|----|--------|
| ZnO (ZN)            | – | + | +    |     |    |        |
| D 7                 |   |   |      |     |    |        |
| DM                  | 81.8 83.0 82.3 83.4 0.14 <0.01 | <0.01 0.03 |
| GE                  | 80.4 80.8 80.8 81.1 0.23 0.22 | 0.12 0.69 |
| CP                  | 75.2 75.5 75.6 75.7 0.11 0.02 | 0.06 0.47 |
| D 21                |   |   |      |     |    |        |
| DM                  | 80.0 80.7 80.6 81.2 0.2 0.02 | <0.01 0.73 |
| GE                  | 79.1 79.3 79.3 79.5 0.13 0.14 | 0.11 0.94 |
| CP                  | 73.6 73.9 73.9 74.1 0.16 0.13 | 0.16 0.63 |
| D 35                |   |   |      |     |    |        |
| DM                  | 78.7 79.3 79.4 79.7 0.21 0.02 | 0.06 0.5  |
| GE                  | 77.7 78.0 78.1 78.2 0.23 0.22 | 0.41 0.74 |
| CP                  | 72.8 73.2 73.4 73.8 0.19 <0.01 | 0.05 0.88 |

DM: dry matter; GE: gross energy; CP: crude protein.

*Standard error of means.

### Table 4. Effect of dietary zinc oxide, bacteriophage or their combination on faecal microbial populations (log10 CFU/g) in weaning pigs.

| Bacteriophage (BAC) | – | + | SEM* | BAC | ZN | ZN*BAC |
|---------------------|---|---|------|-----|----|--------|
| ZnO (ZN)            | – | + | +    |     |    |        |
| D 7                 |   |   |      |     |    |        |
| Total anaerobic bacteria | 8.37 | 8.89 | 8.93 | 9.12 | 0.17 | 0.03 0.05 0.33 |
| *Bifidobacterium spp.* | 7.94 | 8.39 | 8.31 | 8.59 | 0.08 | <0.01 <0.01 0.32 |
| *Lactobacillus spp.* | 7.96 | 8.57 | 8.6 | 9.04 | 0.11 | <0.01 <0.01 0.48 |
| *Clostridium spp.* | 8.74 | 8.25 | 8.2 | 7.88 | 0.09 | <0.01 <0.01 0.35 |
| Coliforms           | 7 | 6.49 | 6.46 | 6.2 | 0.11 | <0.01 <0.01 0.28 |
| D 21                |   |   |      |     |    |        |
| Total anaerobic bacteria | 8.92 | 9.33 | 9.34 | 9.73 | 0.05 | <0.01 <0.01 0.81 |
| *Bifidobacterium spp.* | 8.19 | 8.62 | 8.57 | 8.91 | 0.09 | <0.01 <0.01 0.65 |
| *Lactobacillus spp.* | 7.89 | 8.27 | 8.32 | 8.74 | 0.1 | <0.01 <0.01 0.79 |
| *Clostridium spp.* | 8.01 | 7.59 | 7.42 | 7.29 | 0.08 | <0.01 <0.01 0.09 |
| Coliforms           | 6.74 | 6.32 | 6.33 | 6.04 | 0.14 | 0.02 0.02 0.61 |
| D 35                |   |   |      |     |    |        |
| Total anaerobic bacteria | 9.00 | 9.31 | 9.41 | 9.88 | 0.1 | <0.01 <0.01 0.40 |
| *Bifidobacterium spp.* | 8.28 | 8.61 | 8.73 | 8.97 | 0.1 | <0.01 <0.01 0.67 |
| *Lactobacillus spp.* | 7.98 | 8.43 | 8.35 | 8.88 | 0.1 | <0.01 <0.01 0.69 |
| *Clostridium spp.* | 8.10 | 7.73 | 7.58 | 7.36 | 0.07 | <0.01 <0.01 0.31 |
| Coliforms           | 6.83 | 6.44 | 6.36 | 6.17 | 0.14 | <0.01 0.06 0.50 |

SEM: Standard error of means.

### Table 5. Effect of dietary zinc oxide, bacteriophage or their combination on small intestinal morphology in weaning pigs (D 35).

| Bacteriophage (BAC) | – | + | SEM* | BAC | ZN | ZN*BAC |
|---------------------|---|---|------|-----|----|--------|
| ZnO (ZN)            | – | + | +    |     |    |        |
| Villus height       |   |   |      |     |    |        |
| Duodenum            | 494 | 519 | 522 | 542 | 10.2 | 0.02 0.05 0.87 |
| Jejunum             | 551 | 576 | 579 | 588 | 5.7 | <0.01 <0.01 0.18 |
| Ileum               | 429 | 448 | 451 | 462 | 12.5 | 0.16 0.26 0.77 |
| Crypt depth         |   |   |      |     |    |        |
| Duodenum            | 302 | 315 | 318 | 327 | 9 | 0.16 0.3 0.92 |
| Jejunum             | 311 | 320 | 322 | 330 | 9.2 | 0.28 0.38 0.97 |
| Ileum               | 262 | 269 | 270 | 273 | 9.7 | 0.59 0.57 0.78 |
| VH/CD               |   |   |      |     |    |        |
| Duodenum            | 1.64 | 1.65 | 1.64 | 1.66 | 0.017 | 0.64 0.41 0.91 |
| Jejunum             | 1.77 | 1.80 | 1.80 | 1.78 | 0.021 | 0.71 0.71 0.31 |
| Ileum               | 1.63 | 1.66 | 1.67 | 1.69 | 0.024 | 0.28 0.36 0.90 |

SEM: Standard error of means.
Table 6. Effect of dietary zinc oxide, bacteriophage or their combination on faecal score in weaning pigs.

| Bacteriophage (BAC) | SEM BAC | ZN | ZN*BAC | p value |
|---------------------|---------|----|--------|---------|
| ZnO (ZN)            | –       | +  | +      |         |
| Phase-I (d 7)       | 2.07    | 2.15| 1.79   | 1.41    | 0.250  | 0.06  | 0.52  | 0.36   |
| Phase-II (d 21)     | 1.81    | 1.62| 1.51   | 1.38    | 0.095  | 0.01  | 0.12  | 0.72   |
| Phase-III (d 35)    | 1.41    | 1.24| 1.26   | 0.92    | 0.071  | <0.01 | <0.01 | 0.23   |
| Overall             | 1.76    | 1.67| 1.52   | 1.24    | 0.097  | <0.01 | 0.07  | 0.34   |

*0, normal; 1, soft faeces; 2, mild diarrhea; 3, severe diarrhoea.

SEM: Standard error of means.

Faecal score

There were no BAC × ZN interactions to affect faecal score in all phases (Table 6). The faecal score was not affected \((p > 0.05)\) at phase-I, but decreased at phase-II by BAC. At phase-III of the experiment, the faecal score was reduced in BAC and ZN groups. The overall faecal score was reduced \((p < 0.01)\) by BAC and tended to decrease \((p = 0.07)\) by ZN.

Discussion

Zinc has been known to improve the performance in pigs. Previously, a lot of work has been done to explore the dietary supplementation of zinc on the performance of weaning pigs (Hill et al. 2001; Han & Thacker 2009). However, the use of BAC in pig’s diets has been explored recently and found to be beneficial for improving the performance (Yan et al. 2012; Kim et al. 2014). In the present study there were considerable increases in the ADG and G/F of pigs supplemented with ZN. Similar increases were also observed after supplementing BACs in diets. This correlates the previous study of Carlson et al. (1999), as they reported that dietary supplementation of ZnO in pig’s diet had improved the growth rate in pigs. Previous studies on dietary BAC supplementation conducted in our lab also reported increased in the ADG and ADFI of pigs (Kim et al. 2014). In contrast Gebru et al. (2010) reported no variation in the performance of pigs supplemented with BAC in diets. This might be due to the use of a different type of BAC, age of pigs, health status within herds, farm hygiene and diets composition used in the study. The increase in the growth performance clearly shows the beneficial effect of dietary supplementation of BACs and ZnO. The reason behind this could be the increase in the digestibility, morphology or an increase in friendly bacteria resulting in a reduction of the pathogenic bacteria population.

In the present study, the digestibility of GE was not different in ZN or BAC groups. This correlates the earlier reports where digestibility of GE was not affected in pigs supplemented with diets up to 2500 ppm zinc (Han & Thacker 2009). At d 7 the digestibility of DM was increased in either ZnO or BAC supplemented diets. In agreement with the present results, Yan et al. (2012) reported an increase in the digestibility of DM after supplementing up to 1.5 g/kg BAC cocktail in diets until the 6th week of age. The improved growth rate elicited in BAC and ZN supplementation was likely associated with the concomitant changes in nutrient digestibility and balance of gut microbiota.

The gut development in young animal is governed by many factors that include genetic, physiological and environmental conditions (Mackie et al. 1999). The digestive enzymes hydrolyze the nutrients in the small intestine, which are then fermented by the anaerobic bacteria in the large intestine to yield short chain fatty acids, which then can be resorbed and used by the host (Cummings & MacFarlane 1991). Gram-positive bacteria are most susceptible to ZnO, which is also evident from our study as the Bifidobacterium spp. and Lactobacillus spp. population increased. Using an E. coli-challenge model, Bosi et al. (2003) demonstrated that the addition of 2500 ppm ZnO can control the colonisation of total E. coli and E. coli K88 in faeces. The populations of Bifidobacterium spp. and Lactobacillus spp. consistently increased and Clostridium spp. decreased in the supplemented groups. This correlates the earlier study of Yan et al. (2012) as they reported an increase in Bifidobacterium spp. and Lactobacillus spp. population accompanied by decrease in Clostridium spp. The most interesting find was the consistent increase in the TAB, Bifidobacterium spp. and Lactobacillus spp. populations, while it can be assumed that BAC cocktail cannot directly affect their population, because the BACs in this experiment were originally active against clostridium spp. and coliforms. Therefore, it can be supposed that a decrease on the colonisation of clostridium spp. and coliforms led to an increase in the number of TAB, Bifidobacterium spp. and Lactobacillus spp. in a less competitive environment. This is evident from the present results of the growth studies and correlates the previous studies conducted on pigs and poultry, fed with BAC supplemented diets (Gebru et al. 2010; Wang et al. 2013).

The morphology of intestine was studied to evaluate the gut health. Therefore, villus height, crypt depth and VH/CD ratio of the different portions of the intestine, i.e. duodenum, jejunum and ileum were examined. In the present study, the villus height increased in supplemented groups. We utilised a BAC cocktail with known efficacy against E. coli and Clostridium spp. colonisation. Lactic acid bacteria influence the distribution and the numbers of lymphoid cells in lymphatic tissues associated with the gut, ensure the balance in the composition of the gut microflora, and through
their activity are able to maintain the integrity of the gut mucous membrane (Herich & Levkut 2002). Crypt depth did not show any difference among the groups, however, *Bifidobacterium* spp and *Lactobacilli* spp. reduces the pH of the intestinal content by stimulating the production of lactic acid bacteria in the environment and decreases the crypt depth (Salminen & Salminen 1997). This correlates with the previous studies where supplementation of dietary ZnO resulted in higher villi in pigs (Bosi et al. 2003).

Early-weaned piglets are exposed to numerous stress factors that frequently lead to post-weaning diarrhoea (Laine et al. 2008). In the present study, faecal score is mostly reduced ($p < 0.05$) in BAC and ZN. The major causative agents, for post-weaning diarrhoea are *E. coli* and *Clostridium* spp. This is evident from the present study as the population of *Clostridium* spp. and coliform is consistently decreased in either BAC or ZN. The cocktail used in the present study contains the BAC for *Escherichia coli* and *Clostridium perfringens* that may have caused the damage to the similar population. Thus the populations of coliform and *Clostridium* were decreased numerically. A similar trend was also seen in the incidence of diarrhoea as the number was decreased but was nonsignificant in BAC treatments.

**Conclusions**

The results from the present study suggested that ZnO and BACs both had beneficial impact on different aspects of weaning pig’s performance and would appear to offer as an alternative to improve the performance and gut health of weaning pigs; however, there was no interactive effects between them.

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**Disclosure statement**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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