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Comparative study of the effects of high- versus low-dose zinc oxide in the diet with or without probiotic supplementation on weaning pigs' growth performance, nutrient utilization, fecal microbes, noxious gas discharges, and fecal score

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Abstract

This study was conducted to determine the effects of high- versus low-dose (3000 vs. 300) zinc oxide (ZnO) in combination with or without a probiotic complex (0.1%) on weaned piglet production efficiency, nutrient absorption, fecal bacterial counts, noxious gas emissions, and fecal score. A 42-day experiment included 180 crossbred weaned piglets [Duroc × (Yorkshire × Landrace); 28 days old; 6.61 ± 1.29 kg] and four dietary treatments. An HZ (high ZnO) diet increased body weight at week 6, average daily gain at week 3, week 6, and overall period, and gain-to-feed ratio (G:F) at week 3 compared with an LZ (low ZnO) diet. G:F tended to increase with the LZP (LZ with probiotic) diet compared with the HZP (HZ with probiotic) diet at week 1. *Escherichia coli* count decreased by HZ diet compared with the LZ diet. In addition, *E. coli* count decreased and *Lactobacillus* count increased with the HZP diet compared with the LZP diet. There was no effect of treatment on nutrient digestibility, noxious gas emission, and fecal score. No interactive effect was seen between ZnO and probiotic. Therefore, high-dose ZnO inclusion improved growth performance and probiotic addition improved fecal microbiota, but no synergistic effect was found from ZnO and probiotic complex interaction.

Key words: zinc oxide, probiotic complex, performance, weaning pig

Introduction

Weaning is a challenging stage for pigs, comprising nutritional, psychological, ecological, microbiological, and autoimmune challenges, which can lead to financial losses due to reduced growth performance, inefficiency of feed utilization, diarrhea, and impairment of gastrointestinal function and physiology (Lalles 2008). In previous decades, antibiotic growth enhancers were used clinically on farms to improve the productive efficiency and health welfare, and perhaps to prevent infections of weaned pigs. Antibiotics have various beneficial effects on such mammals, but their use must be restricted as it leads to the expansion of susceptibility by several different strains of bacteria (Pluske 2013; Liao and Nyachoti 2017). So, numerous alternative feed additives have been explored to substitute antibiotics. As a consequence, the usage of feed additives as an immune response and gut microbial modulator has become a routine practice, enhancing pig health and productivity during the postweaning phase (Lalles et al. 2007) .

As a vital mineral ingredient, zinc (Zn) has a huge stabilizing influence on the body's improvement and evolution, immunological function, and gastrointestinal integrity. In weaned pigs, several Zn sources (e.g., montmorillonite zinc oxide (ZnO), nanoparticle ZnO, and coated ZnO) have been added to diets to promote intestinal immune function and structure and to modulate intestinal microbiota (Hu et al. 2012; Shen et al. 2014). For several years, therapeutic levels of ZnO have indeed been utilized to relieve weaning stress, enhance nutritional digestibility, and strengthen immune function as well as boost productive efficiency (Upadhaya et al. 2018). Inclusion of a modified form of ZnO in weaned pig diets improved growth performance and average daily gain (ADG) during the study period (Cho et al. 2015). In addition, ZnO pharmacological dosages (2500–3500 mg/kg) have been shown to significantly reduce diarrhea, improve pig growth responses (Hill et al. 2001), and reduce noxious gas production (Shi et al. 2019). A ZnO dose below therapeutic levels (maximum of 150 mg/kg) is identified as a growth enhancer for piglets (European Communities 2003). ZnO nanoparticles in animal diets have been demonstrated to boost body weight (BW), feed intake (FI), and feed conversion ratio (FCR) in poultry (Zhao et al. 2014), but had no effect on growth performance (Li et al. 2016) in piglets. In addition, it has been observed that an LZ diet (low ZnO; approximately 250 or 500 ppm) has no influence on the growth of newborn piglets (Hollis et al. 2005). The postulated mechanism for increased growth performance was related to improvements in gastrointestinal function and structure, antioxidant properties, mucosal immune strength, and the elimination of diarrhea in weaned piglets (Trckova et al. 2015; Zhu et al. 2017). However, the extensive use of ZnO in weanling pigs raised concerns about excretion of ZnO to the environment and its link to the development of antibiotic-resistant microorganisms (Slifierz et al. 2015; Jensen et al. 2016) .

Probiotics are single or combined populations of living microbes that provide favorable health benefits to the animals when consumed. Antimicrobial substances produced by probiotics include amino substances, H_2O_2, antimicrobials, and natural polymers, which can all hinder the activity of pathogenic bacteria (Chaucheyras-Durand and Durand 2010). Organic acids (lactic acid, acetic acid) are the most prevalent compounds formed by probiotics, and they lower the pH in the gastrointestinal tract, creating an unfavorable environment for pathogenic organisms. Probiotics also boost susceptibility to enteric pathogens by competing for attachment sites and nutrients in the intestine (Boirivant and Strober 2007). Many studies have found that adding probiotics to weaned pigs' diets can enhance growth performance (Lan et al. 2017; Dumitru et al. 2021) and nutrient digestibility (Zhao and Kim 2015), and reduce diarrhea occurrence (Giang et al. 2012; Dumitru et al. 2021). Probiotics can improve immunity (Yu et al. 2008) and reduce fecal poisonous gas discharges (Zhao and Kim 2015), resulting in fewer pollutants in the atmosphere in weaned pigs (Ferket et al. 2002). For animals (Bedford et al. 2014), probiotics have been suggested as a potential antibacterial growth booster and antibiotic substitute. To improve the effect of adding ZnO to the diet, a probiotic compound was added to either low- or high-dose ZnO. In weanling pigs, Shi et al. (2019) discovered that low-dose ZnO mixed with probiotic compounds enhanced growth efficiency and had no negative effect on nutrient absorption, immune parameters, or the occurrence of gastroenteritis. However, dietary ZnO (1200 ppm) inclusion with probiotic compounds compares favorably with the ZnO pharmacological (3000 ppm) level regarding economic growth, dietary digestion, gastrointestinal microbiota, poisonous gas discharges, and fecal index in piglets (Wang et al. 2021). So, probiotic supplementation can help to reduce the dose of ZnO applied in weaning pigs to improve the production performance and gut health. Considering the above benefits, we hypothesized that the combination of ZnO and probiotic compound will increase productive efficiency, nutrient absorption, intestinal microbiota population, noxious gas emission, and fecal score of weaned pigs by acting synergistically.

Therefore, the objective of this study was to determine the effect of high and low doses of ZnO with or without a 0.1% probiotic complex (*Bacillus coagulans*, *B. licheniformis*, *B. subtilis*, and *Clostridium butyricum*) inclusion on growth performance, fecal bacterial enumeration, noxious gas emissions, and fecal score of weaning pigs. A second objective was to estimate whether there would be any interactive effect between ZnO and probiotic.

Materials and methods

Ethical statement

All animal management and experimental procedures were approved by the Institutional Animal Care and Use Committee of Dankook University, Republic of Korea (Approval No. DK-1-1937).

Source of probiotics

The probiotic compound used in the current experiment was acquired from a commercial company (Synerbig Co. Ltd., Seoul, South Korea), named SynerZymeF10. This product is assured to comprise at least 1×10^{12} CFU/kg *B. coagulans*, 5×10^{11} CFU/kg *B. licheniformis*, 1×10^{12} CFU/kg *B. subtilis*, and 1×10^{11} CFU/kg *C. butyricum*, which is a combination of *B. coagulans*, *B. licheniformis*, *B. subtilis*, and *C. butyricum* spray-dried spores.

Experimental design and animal management

In a 42-day trial, 180 crossbred weanling piglets ([Yorkshire $\times$ Landrace] $\times$ Duroc; 6.61 ± 1.29 kg; 28 days of age) were used. According to starting BW and sex, total pigs were arbitrarily allocated to one of four nutritional treatments (nine duplicate pens, each including two gilts and three barrows). The nutritional treatment groups were TRT1, positive control: basal diet added with 3000 ppm ZnO (HZ, high ZnO); TRT2, HZ + 0.1% probiotic complex (HZP); TRT3, negative control (NC): basal diet added with 300 ppm ZnO (LZ); and TRT4, LZ + 0.1% probiotic complex (LZP). The trial period was allocated into three phases: days 0–7 of age (phase 1), days 8–21 of age (phase 2), and days 22–42 of age (phase 3). The diets (Tables 1–3) were designed to meet or surpass the National Research Council (NRC)'s nutritional requirements (NRC 2012). All pigs were kept in a biologically maintained room with a mechanical aeration system that provided a 0.26 m $\times$ 0.53 m area for each pig. All over the experiment, each pen was supplied with a self-feeder along with a nipple drinker made of stainless steel to permit the piglets ad libitum access to feed and water. Twelve hours of artificial light for each day was automatically adjusted. The room's ambient temperature was maintained at around 30 °C during the first week, and afterward the temperature was reduced by 1 °C per week.

Growth performance and nutrient absorption

On initial, 7th, 21th, and 42nd days of the trial, BWs of individual pigs were measured and feed intake was documented on a pen basis during the research to examine ADG, average daily feed intake (ADFI), and gain-to-feed ratio (G:F). Chromium oxide (Cr_2O_3, 0.20%; Samchun Pure Chemical Co., Ltd., Gyeonggi, South Korea) was included in the diet as an indigestible indicator 1 week prior to fecal assembly. On day 42, fecal specimens were collected via rectal massage from

Table 1. Experimental diet composition on a fed basis.

Item	Phase 1 (days 0–7 of age)			
	TRT1	TRT2	TRT3	TRT4
Ingredients (%)				
Corn	39.32	39.32	40.04	40.04
Soya bean meal	16.22	16.22	16.10	16.10
Fermented soya bean meal	5.00	5.00	5.00	5.00
Spray-dried plasma protein	6.00	6.00	6.00	6.00
Tallow	2.82	2.82	2.56	2.56
Lactose	12.88	12.88	12.88	12.88
Sugar	3.00	3.00	3.00	3.00
Whey protein	11.00	11.00	11.00	11.00
Monocalcium phosphate	0.88	0.88	0.88	0.88
Limestone	1.18	1.18	1.18	1.18
Salt	0.20	0.20	0.20	0.20
Methionine (99%)	0.20	0.20	0.20	0.20
Lysine	0.49	0.49	0.49	0.49
Mineral mix[a]	0.20	0.20	0.20	0.20
Vitamin mix[b]	0.20	0.20	0.20	0.20
Choline (25%)	0.03	0.03	0.03	0.03
Zinc oxide (80%)	0.38	0.38	0.04	0.04
Calculated value				
Crude protein, %	20.00	20.00	20.00	20.00
Metabolizable energy, kcal/kg	3450	3450	3450	3450
Calcium, %	0.80	0.80	0.80	0.80
Phosphorus, %	0.60	0.60	0.60	0.60
Lysine, %	1.60	1.60	1.60	1.60
Methionine, %	0.48	0.48	0.48	0.48
Fat, %	4.52	4.52	4.28	4.28
Lactose, %	20.00	20.00	20.00	20.00
Zinc oxide, ppm	3053	3053	333	333

[a]Provided per kg diet: Fe, 100 mg as ferrous sulfate; Cu, 17 mg as copper sulfate; Mn, 17 mg as manganese oxide; I, 0.5 mg as potassium iodide; Se, 0.3 mg as sodium selenite. [b]Provided per kg of diet: vitamin A, 10 800 IU; vitamin D_3, 4000 IU; vitamin E, 40 IU; vitamin K_3, 4 mg; vitamin B_1, 6 mg; vitamin B_2, 12 mg; vitamin B_6, 6 mg; vitamin B_{12}, 0.05 mg; biotin, 0.2 mg; folic acid, 2 mg; niacin, 50 mg; D-calcium pantothenate, 25 mg.

two pigs for each pen (one barrow and one gilt). On a pen basis, they were combined and pooled, and a random specimen was kept in a freezer at −20 °C until analyzed. For chemical study, fecal specimens were dried out at 60 °C for 72 h, and then finely crushed to a size that could go through a 1 mm screen. By utilizing the method developed by AOAC (2010), diet and fecal samples were examined to regulate dry matter (DM) and nitrogen (N). To determine energy (E) and excreta samples, specimens were taken and put in a calorimeter (Parr Instruments, Moline, IL, USA) to assess thermal combustion in the samples as well as chromium, which was evaluated by atomic absorption spectrophotometry (Shimadzu UV-1201; Shimadzu, Kyoto, Japan). Apparent total tract digestibility (ATTD) was then measured by the following formula:

Digestibility (%) $= [1 - (N_f \times C_d)/(N_d \times C_f)] \times 100$, where N_f represents the nutrient concentration in feces (% DM), N_d represents the nutrient concentration in diet (% DM), C_d represents the chromium concentration in diet (% DM), and C_f represents the chromium concentration in feces (% DM).

Fecal noxious gas emissions

At the end of the study, fresh fecal specimens (300 g) were amassed randomly from each pen (two pigs), mixed evenly, kept in a 2.6 L sealed plastic box with small holes on any side, fixed firmly with tape, and fermented for 7 days to determine ammonia (NH_3) at 25 °C, hydrogen sulfide (H_2S), methyl mercaptans, CO_2, and acetic acid, utilizing a GV-100 gas sampling pump (model GV100S; Gastec Corp., Tokyo, Japan). Various measurement tubes (No. 3L, No. 4LT, and No. 70L; Gastec) were used to detect total mercaptans. On the 8th day, a 100 mL sample was grabbed from the headspace (2 cm) for air passage, and it was sealed again. To understand the creation of a hard crust on the top, the specimen box was shaken manually for 30 s. Finally, the concentrations of NH_3, H_2S, methyl mercaptan, CO_2, and acetic acid were evaluated.

Fecal microbiota

Fecal specimens were taken from two pigs from each pen by massaging the rectums and kept on ice for transfer to the research laboratory, where they were instantly tested to de-

Table 2. Experimental diet composition on a fed basis.

	Phase 2 (days 8–21 of age)			
Item	TRT1	TRT2	TRT3	TRT4
Ingredients (%)				
Corn	51.67	51.67	52.39	52.39
Soya bean meal	16.74	16.74	16.62	16.62
Fermented soya bean meal	4.00	4.00	4.00	4.00
Spray-dried plasma protein	3.00	3.00	3.00	3.00
Tallow	2.82	2.82	2.56	2.56
Lactose	7.78	7.78	7.78	7.78
Sugar	3.00	3.00	3.00	3.00
Whey protein	7.00	7.00	7.00	7.00
Monocalcium phosphate	1.08	1.08	1.08	1.08
Limestone	1.20	1.20	1.20	1.20
Salt	0.10	0.10	0.10	0.10
Methionine (99%)	0.15	0.15	0.15	0.15
Lysine	0.65	0.65	0.65	0.65
Mineral mix[a]	0.20	0.20	0.20	0.20
Vitamin mix[b]	0.20	0.20	0.20	0.20
Choline (25%)	0.03	0.03	0.03	0.03
Zinc oxide (80%)	0.38	0.38	0.04	0.04
Calculated value				
Crude protein, %	18.00	18.00	18.00	18.00
Metabolizable energy, kcal/kg	3400	3400	3400	3400
Calcium, %	0.80	0.80	0.80	0.80
Phosphorus, %	0.60	0.60	0.60	0.60
Lysine, %	1.50	1.50	1.50	1.50
Methionine, %	0.40	0.40	0.40	0.40
Fat, %	4.91	4.91	4.67	4.67
Lactose, %	12.00	12.00	12.00	12.00
Zinc oxide, ppm	3054	3054	334	334

[a]Provided per kg diet: Fe, 100 mg as ferrous sulfate; Cu, 17 mg as copper sulfate; Mn, 17 mg as manganese oxide; I, 0.5 mg as potassium iodide; Se, 0.3 mg as sodium selenite. [b]Provided per kg of diet: vitamin A, 10 800 IU; vitamin D_3, 4000 IU; vitamin E, 40 IU; vitamin K_3, 4 mg; vitamin B_1, 6 mg; vitamin B_2, 12 mg; vitamin B_6, 6 mg; vitamin B_{12}, 0.05 mg; biotin, 0.2 mg; folic acid, 2 mg; niacin, 50 mg; D-calcium pantothenate, 25 mg.

termine the gastrointestinal microbial flora. Fecal samples were obtained to study the microbiota in the feces. Every sample had 1 g of fecal droppings combined with 9 mL of 1% peptone broth mixed using a vortex stirrer for 1 min. Samples were serially blended from 10^{-1} to 10^{-6}, and were injected by 50 μL in two selective agar media, MacConkey agar plates (Difco Laboratories, Detroit, MI, USA) and *Lactobacillus* medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany), for the purpose of isolating *Escherichia coli* and *Lactobacillus*. For the *Lactobacillus* bacterial count, the agar plates in the media were incubated in anaerobic environments for 48 h at 39 °C, and incubated anaerobically for 24 h at 37 °C for the *E. coli* counts. Colony counts were then totaled and the outcomes were displayed as $\log_{10}$ transformed data.

Fecal score

Fecal scores were estimated and documented on a pen source at 08:00 and 20:00 h on days 7, 14, 21, 28, 35, and 42. Using the above fecal scoring scheme, the fecal score was calculated as the average of the five pigs in each pen: 1, hard, dry pellet; 2, firm, formed stools; 3, soft, moist stools that maintain shape; 4, soft, unformed stools that adapt to the form of the vessel; and 5, liquids that are watery and pourable.

Statistical analysis

The data were analyzed with the generalized model with a 2 × 2 factorial arrangement of SAS (SAS Institute Inc., Cary, NC, USA), using the pen as a trial unit. Data were log transformed prior to statistical analysis of microbial counts. The data variability was expressed as pooled SE, with $P < 0.05$ being significant and $P < 0.10$ indicating a trend.

Results

Growth performance

Table 4 summarizes the outcomes of the growth performance. When HZ was added to the piglets' diets, BW increased significantly ($P < 0.05$) at week 6 compared with

Table 3. Experimental diet composition on a fed basis.

Item	Phase 3 (days 22–42 of age) TRT1	TRT2	TRT3	TRT4
Ingredients (%)				
Corn	58.48	58.48	59.18	59.18
Soya bean meal	22.60	22.60	22.48	22.48
Fermented soya bean meal	3.00	3.00	3.00	3.00
Tallow	2.77	2.77	2.53	2.53
Lactose	3.18	3.18	3.18	3.18
Sugar	3.00	3.00	3.00	3.00
Whey protein	3.00	3.00	3.00	3.00
Monocalcium phosphate	1.15	1.15	1.15	1.15
Limestone	1.22	1.22	1.22	1.22
Salt	0.10	0.10	0.10	0.10
Methionine (99%)	0.08	0.08	0.08	0.08
Lysine	0.61	0.61	0.61	0.61
Mineral mix[a]	0.20	0.20	0.20	0.20
Vitamin mix[b]	0.20	0.20	0.20	0.20
Choline (25%)	0.03	0.03	0.03	0.03
Zinc oxide (80%)	0.38	0.38	0.04	0.04
Calculated value				
Crude protein, %	18.00	18.00	18.00	18.00
Metabolizable energy, kcal/kg	3350	3350	3350	3350
Calcium, %	0.80	0.80	0.80	0.80
Phosphorus, %	0.60	0.60	0.60	0.60
Lysine, %	1.40	1.40	1.40	1.40
Methionine, %	0.35	0.35	0.35	0.35
Fat, %	5.14	5.14	4.93	4.93
Lactose, %	5.00	5.00	5.00	5.00
Zinc oxide, ppm	3057	3057	337	337

[a]Provided per kg diet: Fe, 100 mg as ferrous sulfate; Cu, 17 mg as copper sulfate; Mn, 17 mg as manganese oxide; I, 0.5 mg as potassium iodide; Se, 0.3 mg as sodium selenite. [b]Provided per kg of diet: vitamin A, 10 800 IU; vitamin D_3, 4000 IU; vitamin E, 40 IU; vitamin K_3, 4 mg; vitamin B_1, 6 mg; vitamin B_2, 12 mg; vitamin B_6, 6 mg; vitamin B_{12}, 0.05 mg; biotin, 0.2 mg; folic acid, 2 mg; niacin, 50 mg; D-calcium pantothenate, 25 mg.

when LZ was added to the diet. Similarly, in week 3, week 6, and overall period, the ADG in pigs fed HZ diets increased ($P < 0.05$) compared with those fed LZ diets. Pigs fed HZP diets had a trend of higher G:F ($P < 0.05$) than pigs fed LZP diets. Furthermore, G:F was greater ($P < 0.05$) for the HZ diets at week 3 compared with the LZ diets. Throughout the experiment, the addition of ZnO (HZ vs. LZ) and the probiotic to the diet had no effect on ADFI ($P > 0.05$). In the growth performance measures, no interacting effects between ZnO and probiotic were found.

Nutrient digestibility

The ATTD values for DM, *E*, and N are summarized in Table 5. When HZ was added to the diets of weaning pigs, there was no significant change in nutritional digestibility ($P > 0.05$) when compared with the LZ diet. Furthermore, there was no significant improvement in nutritional digestibility when probiotics were added to the diet. In terms of nutrient digestibility, no interaction between ZnO and probiotic was discovered.

Fecal noxious gas emissions

As shown in Table 6, the supplementation of HZ into diet had no significant effect ($P > 0.05$) on fecal noxious gas emissions (NH_3, H_2S, and total mercaptans) when compared with LZ diet. Furthermore, the piglet administered a probiotic complex in its diet showed no influence on gas emission ($P > 0.05$). No interaction between ZnO and the probiotic was observed.

Fecal microbiota

Table 7 shows the findings of the fecal microbiota analysis. HZ-supplemented diets had significantly lower ($P < 0.05$) *E. coli* counts as compared with LZ, while failed ($P > 0.05$) to affect *Lactobacillus* enumeration. However, piglets fed the HZP diet showed decreased *E. coli* ($P < 0.05$) and increased ($P < 0.05$) *Lactobacillus* counts compared with the LZP diet. For fecal microbiota, no interactions between probiotic and ZnO were observed.

Table 4. The effect of dietary supplementation of probiotic complex additive on growth performance in weaning pigs.

	High ZnO (3000 ppm)		Low ZnO (300 ppm)			*P* value		
Items	Probiotic− (HZ)	Probiotic+ (HZP)	Probiotic− (LZ)	Probiotic+ (LZP)	SEM	Probiotic effect	ZnO effect	ZnO × probiotic
Body weight, kg								
Initial	6.55	6.55	6.55	6.55	0.002	0.998	0.998	0.998
Week 1	7.94	8.00	8.02	7.95	0.03	0.970	0.887	0.954
Week 3	12.77	12.95	12.87	12.86	0.10	0.893	0.254	0.773
Week 6	23.53ab	24.23a	23.51b	23.95ab	0.24	0.411	0.034	0.853
1 week								
ADG	166	162	166	169	4	0.860	0.355	0.651
ADFI	200	202	203	205	6	0.493	0.942	0.890
G/F	0.911	0.891	0.893	0.903	0.011	0.062	0.185	0.624
3 weeks								
ADG	407ab	412a	396b	398ab	8	0.859	0.045	0.483
ADFI	473	490	484	479	8	0.955	0.455	0.174
G/F	0.841ab	0.842a	0.831b	0.838ab	0.007	0.689	0.015	0.432
6 weeks								
ADG	604ab	624a	592b	612ab	10	0.241	0.025	1.000
ADFI	840	849	843	854	9	0.653	0.229	0.910
G/F	0.719	0.735	0.703	0.717	0.010	0.218	0.184	0.939
Overall								
ADG	461ab	474a	459b	464ab	6	0.3 587	0.019	0.825
ADFI	611	619	616	621	6	0.6 499	0.222	0.649
G/F	0.756	0.763	0.745	0.748	0.008	0.1 886	0.071	0.911

Abbreviations: TRT1, positive control: basal diet added with 3000 ppm ZnO (HZ); TRT2, HZ + 0.1% probiotic complex (HZP); TRT3, negative control: basal diet added with 300 ppm ZnO (LZ); TRT4, LZ + 0.1% probiotic complex (LZP); ADG, average daily gain; ADFI, average daily feed intake; G/F, gain-to-feed ratio; SEM, standard error of means.

Table 5. The effect of dietary supplementation of probiotic complex additive on nutrient digestibility in weaning pigs.

	High ZnO (3000 ppm)		Low ZnO (300 ppm)			*P* value		
Items	Probiotic− (HZ)	Probiotic+ (HZP)	Probiotic− (LZ)	Probiotic+ (LZP)	SEM	Probiotic effect	ZnO effect	ZnO × probiotic
Dry matter	81.47	82.40	80.67	81.14	0.81	0.777	0.213	0.397
Nitrogen	78.87	79.74	78.56	78.74	0.66	0.500	0.403	0.654
Energy	79.69	80.66	79.27	79.43	0.65	0.396	0.220	0.542

Abbreviations: TRT1, positive control: basal diet added with 3000 ppm ZnO (HZ); TRT2, HZ + 0.1% probiotic complex (HZP); TRT3, negative control: basal diet added with 300 ppm ZnO (LZ); TRT4, LZ + 0.1% probiotic complex (LZP); SEM, standard error of means.

Table 6. The effect of dietary supplementation of probiotic complex additive on gas emission in weaning pigs.

	High ZnO (3000 ppm)		Low ZnO (300 ppm)			*P* value		
Items	Probiotic− (HZ)	Probiotic+ (HZP)	Probiotic− (LZ)	Probiotic+ (LZP)	SEM	Probiotic effect	ZnO effect	ZnO × probiotic
NH_3	1.4	1.0	1.9	1.6	0.7	0.649	0.472	0.932
H_2S	1.9	1.6	2.3	3.0	0.7	0.779	0.221	0.445
Methyl mercaptans	2.3	1.6	4.9	3.5	1.1	0.351	0.058	0.757
CO_2	700	450	875	725	258	0.424	0.370	0.839
Acetic acid	0.5	0.2	0.9	0.7	0.2	0.258	0.071	0.955

Abbreviations: TRT1, positive control: basal diet added with 3000 ppm ZnO (HZ); TRT2, HZ + 0.1% probiotic complex (HZP); TRT3, negative control: basal diet added with 300 ppm ZnO (LZ); TRT4, LZ + 0.1% probiotic complex (LZP); SEM, standard error of means.

Table 7. The effect of dietary supplementation of probiotic complex additive on fecal microbiota in weaning pigs.

	High ZnO (3000 ppm)		Low ZnO (300 ppm)			*P* value		
Items	Probiotic− (HZ)	Probiotic+ (HZP)	Probiotic− (LZ)	Probiotic+ (LZP)	SEM	Probiotic effect	ZnO effect	ZnO × probiotic
E. coli	6.18[b]	6.09[b]	6.26[a]	6.20[a]	0.08	0.007	0.002	0.495
Lactobacillus	9.15[b]	9.23[a]	9.16[b]	9.22[a]	0.05	0.012	0.902	0.638

Abbreviations: TRT1, positive control: basal diet added with 3000 ppm ZnO (HZ); TRT2, HZ + 0.1% probiotic complex (HZP); TRT3, negative control: basal diet added with 300 ppm ZnO (LZ); TRT4, LZ + 0.1% probiotic complex (LZP); SEM, standard error of means.

Fecal score

The results of the piglets' fecal scores are summarized in Table 8. In comparison to LZ, adding HZ to the diet had no significant influence on the fecal score of weaned pigs. Similarly, the addition of the probiotic complex to the piglets' diet had no effect on the piglets' fecal score ($P > 0.05$), and no interacting effects were detected.

Discussion

Feed rejection, malnutrition, lower growth, and poor health condition in piglets are all symptoms of weaning stress. In an intensified farming industry, animal producers employ a variety of ways to alleviate weaning distress in piglets. ZnO and probiotics have been used in animal farming for a long period of time, providing good results (Lan et al. 2017; Upadhaya et al. 2018).

Effect of high- versus low-dose ZnO diet

In the present research, pigs provided therapeutic levels of ZnO had significantly greater BW, ADG, and G:F than those provided 300 ppm ZnO. Weaning pigs fed therapeutic doses of ZnO increased ADG, ADFI, and G:F (Hill et al. 2001; Sales 2013). Moreover, pigs fed ZnO as a positive control (ZH) diet increased ADG and BW compared with ZnO as an NC diet (ZL) (Shi et al. 2019). Supplementing with 3000 ppm standard ZnO and 200 ppm customized ZnO enhanced BW (Cho et al. 2015), and 2500 mg/kg of Zn from ZnO increased ADG (Hollis et al. 2005), but had no effect on ADFI or G:F. In agreement with our study, the addition of protected ZnO resulted in significantly greater BW, ADG, and ADFI in weaning pigs (Upadhaya et al. 2018) compared with pigs fed a basal diet with the nutritional content of zinc. In contrast, research found no change in growth performance (BW, ADG, ADFI, and G:F) in weanling pigs fed HZ compared with LZ (Shen et al. 2014). The enhancements in BWG, ADG, and G:F found in this trial may be due to the antibacterial properties of ZnO administration and improved gut health.

Several studies have indicated enhanced growth rate and nutrient digestibility using a therapeutic level (3000–4000 ppm) of ZnO as a substitute for in-feed antibiotics (Cho et al. 2015; Milani et al. 2017). In line with present research, nutrient digestibility was not influenced by supplementing ZH into diet (Shi et al. 2019) compared with ZL. In contrast, adding protected ZnO resulted in higher ATTD of DM and *E* when compared with the NC diet in piglets (Upadhaya et al. 2018). As the concentration of ZnO reduces, the nutritional digestibility also reduces quantitatively, where the ATTD of DM and N were found to be identical (Wang et al. 2021). Fecal noxious gas emissions such as NH_3 and H_2S have become one of the leading air pollutants in recent intensive pig production. There are few reports on the effects of ZnO feeding on noxious gas emissions in weaned piglets. In line with our finding, incorporating a probiotic complex into a diet with LZ and HZ did not affect fecal gas emissions in weaned piglets (Shi et al. 2019; Wang et al. 2021). Our findings found no differences in nutrient consumption; therefore, it was reasonable to presume that there would be no differences in noxious gas emissions as well, consistent with Hoque et al. (2021).

Escherichia coli concentrations in the feces of weaned pigs fed diets containing HZ were found to be significantly lower, and *Lactobacillus* was higher (Kaevska et al. 2016; Upadhaya et al. 2018), coherent with current research. In contradiction to this study, consuming ZnO at a level of 2500 mg/kg enhances the level of *E. coli* (Bednorz et al. 2013) in the intestine. With high dosages of ZnO, Hojberg et al. (2005) found that *Lactobacillus* was reduced, but there was no impact on *E. coli* (Broom et al. 2006). Carlson et al. (2008) and Shen et al. (2014) revealed that a greater level of ZnO lowered the diarrhea incidence of weaning pigs. The fecal score, which could indicate diarrhea status, was lower in piglets fed the customized ZnO (Cho et al. 2015) and ZnO nanoparticles (Milani et al. 2017) compared with control diet. The fecal count, which can indicate diarrhea severity, was similar across all treatments, confirming no diarrhea (Shen et al. 2014; Upadhaya et al. 2018), coherent with current research. Higher ZnO doses were thought to minimize the incidence of diarrhea by reducing harmful bacteria counts (Sales 2013). These findings indicated that the impact of ZnO in decreasing diarrhea could be unrelated to the direct inhibition of pathogenic *E. coli*, but rather is linked to an enhancement in gut microbiome, which promotes competition among enterobacteria (Amezcua et al. 2008; Wang et al. 2021). In the current investigation, diarrhea was not observed in pigs given ZnO diet, which could be related to the decreased *E. coli* count and pigs being raised in a hygienic method.

Effect of probiotics

Probiotics have a favorable influence on the host's health and improve the functioning of the intestinal tract. Decades of research has indicated that the use of probiotics in farm animals is beneficial as it improves feed efficiency, weight gain, and immune response (Al-Shawi et al. 2020). In this study, probiotic inclusion showed a tendency to decrease G:F. Previous research from our laboratory revealed that includ-

Table 8. The effect of dietary supplementation of probiotic complex additive on fecal score in weaning pigs.

	High ZnO (3000 ppm)		Low ZnO (300 ppm)			*P* value		
Items	Probiotic− (HZ)	Probiotic+ (HZP)	Probiotic− (LZ)	Probiotic+ (LZP)	SEM	Probiotic effect	ZnO effect	ZnO × probiotic
Fecal score								
Week 1	3.61	3.59	3.66	3.61	0.06	0.543	0.515	0.760
Week 2	3.45	3.52	3.54	3.55	0.07	0.524	0.380	0.722
Week 3	3.43	3.39	3.45	3.46	0.11	0.922	0.704	0.819
Week 4	3.27	3.32	3.30	3.32	0.07	0.575	0.785	0.813
Week 5	3.29	3.29	3.34	3.29	0.07	0.765	0.743	0.765
Week 6	3.21	3.21	3.25	3.27	0.07	0.907	0.542	0.907

Abbreviations: TRT1, positive control: basal diet added with 3000 ppm ZnO (HZ); TRT2, HZ + 0.1% probiotic complex (HZP); TRT3, negative control: basal diet added with 300 ppm ZnO (LZ); TRT4, LZ + 0.1% probiotic complex (LZP); SEM, standard error of means.

ing multistrain probiotics (0.1%, *B. licheniformis* and *B. subtilis*) in the diets had a positive impact on ADG, ADFI, and G:F all through the experiment (Lan et al. 2016; Zhang et al. 2020). Indeed, adding a probiotic complex into diet increased growth performance in weaned pigs and broilers in our prior study (Balamuralikrishnan et al. 2017; Nguyen et al. 2019). Moreover, the diet inclusion of ZnO with probiotic complex decreased ADG, but had no effect on ADFI and G:F (Wang et al. 2021). The administration of *Bacillus*-based probiotics in the diet of finishing pigs had no effect on ADFI and G:F (Chen et al. 2006), ADG, or feed efficiency (Munoz et al. 2007), which is slightly in line with our research. Some recent studies found that feeding *B. subtilis* and *B. licheniformis* to basal diet improved BW and FCR for grower–finisher pigs (Alexopoulos et al. 2004), and enhanced ADG and ADFI for fattening goats (Lu et al. 2021). Probiotics would support stabilizing the microbiota by encouraging the beneficial bacteria, increasing gut health, decreasing the occurrence of diarrhea, and boosting growth performance (Hu et al. 2014).

Weaning pigs' feeds enriched with multimicrobe probiotic-containing products such as *B. subtilis* enhanced ATTD of DM, N, and *E*, according to Nguyen et al. (2019), which correlates with Lee et al. (2014). Multistrain probiotic (0.1%) administration can improve ATTD of DM, N, and *E* (Upadhaya et al. 2015; Lan et al. 2016), which is supported by Hu et al. (2021). However, diverse effects were accompanied by Chen et al. (2006), who showed that addition of *Bacillus*-based probiotics into diet had no effect on DM and N digestibility, in agreement with current study. In contrary, probiotics (*B. subtilis* and *C. butyricum* endospore) administration in growing–finishing pig diet enhanced ATTD of N and E, whereas interactive effects of probiotics were detected on the digestibility of N at week 10 and *E* at week 5 (Meng et al. 2010). These variable probiotic actions were linked to different types of probiotics and the level of feeding and management (Yu et al. 2020). Nutritive probiotic addition is assumed to have optimistic influence on the gut microbiome ecosystem, causing a shift in the intestinal flora that leads to increased nitrogen consumption and, in turn, reduced noxious gas emissions from the feces (Jeong et al. 2015). In comparison to control diet, Chen et al. (2006) and Hu et al. (2021) discovered that dietary addition with a *Bacillus*-based complex (0.2%) lowered NH_3 and amounts of total mercaptan. As observed, probiotic treatment with *B. subtilis* and *B. licheniformis* considerably reduces slurry noxious NH_3 emission, but had no effect on H_2S and mercaptan discharge in growing pigs (Wang et al. 2009). In the present investigation, dietary inclusion of probiotic had no effect on noxious gas emission. If there is no change in digestibility, there will be no change in noxious gas discharges.

While the beneficial bacterium *Lactobacillus* is responsible for maintaining intestinal health and can help to prevent diarrhea, *E. coli* is one of the most common intestinal pathogens, and some strains are serious enough to cause diarrhea (Hu et al. 2014). In contrast with current research, *E. coli* increased, but *Lactobacillus* decreased in the intestinal flora of weaned piglets (Konstantinov et al. 2006). Lan et al. (2016) and Upadhaya et al. (2017) found that probiotic treatment resulted in higher intestinal *Lactobacillus* counts and decreased *E. coli* counts in weanling piglets, which is in agreement with our findings. Probiotics benefit the host animal by maintaining intestinal barrier and producing gut microecological environments that suppress infectious organisms while increasing helpful bacteria (Chen et al. 2013). In weaned piglets, Giang et al. (2012) discovered that *Bacillus*-based microbiota lowered diarrhea incidence and reduced fecal score. Furthermore, Alexopoulos et al. (2004) discovered that feeding probiotic spores significantly reduced occurrence of diarrhea in postweaning period. In this study, addition of dietary probiotic to the diet increased fecal *Lactobacillus* and reduced *E. coli* counts in piglets, resulting in no diarrhea incidence. Probiotics in the feed had a significant impact on intestinal bacterial populations, thus decreasing diarrhea scores (Dowarah et al. 2017).

Interactive effects between ZnO and probiotics

In all of the analyzed parameters (growth performance, nutrient utilization, fecal microbiome, noxious gas production, and fecal index), there were no interaction effects between ZnO and probiotics.

Conclusion

In conclusion, high-dose ZnO proved its beneficial effect on growth performance and gut condition of weaning pigs.

However, there was no individual or synergistic effect between probiotic addition and ZnO on weaning pigs' performance and nutrient digestibility. This study suggests to apply a higher dose or different combinations of probiotics to select a suitable lower dose of ZnO to facilitate weaning pigs' overall performance.

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Data availability

The data that aid this investigation will be shared upon reasonable request to the corresponding author.

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There are no recognized conflicting interests for the authors.

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