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Use of protected zinc oxide in lower doses in weaned pigs in substitution for the conventional high dose zinc oxide

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ABSTRACT

This study tested the hypothesis that protected zinc oxide (ZnO) in lower doses can substitute the high dose conventional ZnO in weaned pigs for improved growth performance and alleviation of digestive disorders. A total of 150 crossbred weaning pigs (28 days old) with an average body weight (BW) of 6.48 ± 1.58 kg were blocked and stratified based on sex and randomly allotted to 1 of 6 dietary treatments [5 pigs per pen (2 barrows and 3 gilts); 5 pens per treatment] for a 6-wk trial in two phases. Treatments consisted of basal diet (NC); Basal diet without Zn in mineral premix with either 2500 ppm unprotected ZnO (PC) or 250, 500, 750 and 1000 ppm protected ZnO (PZ1, PZ2, PZ3 and PZ4 respectively). As a result of this experiment, the growth performance in pigs fed protected ZnO diets was comparable with PC diet during phase 1 and 2, except for G/F ratio in phase 1. There were cubic effects ($P < 0.05$) of protected ZnO dose on average daily gain (ADG) and average daily feed intake (ADFI) during phase 2. The coefficient of apparent total tract (CAATD) nutrient digestibility in pigs fed protected ZnO diets was comparable with PC diet. The concentration of Zn in the serum of pigs fed PC diet was higher ($P < 0.05$) than protected ZnO diets during wk 1, 3 and 6. The faecal Zn concentrations were higher ($P < 0.05$) in pigs fed PC diets compared to NC and PZ diets during wk 1, 3 and 6. A linear response of protected ZnO dose was observed on faecal Zn concentration. The *E.coli* and *Clostridium spp* counts were lower ($P < 0.05$) in the digesta from colon of pigs fed PC diet than protected ZnO diets during wk 3. Quadratic and cubic effects ($P < 0.05$) of protected ZnO dose were observed on *E.coli* counts in the digesta of ileum and colon of pigs. Linear effects ($P < 0.05$) of protected ZnO dose were observed on *Lactobacillus* and *Clostridium* counts on the digesta of ileum and colon respectively. The faecal *E.coli* counts were greater in protected ZnO than in conventional ZnO during wk 1 and 3. The faecal *Lactobacillus* counts were greater ($P < 0.05$) and *Salmonella* counts were lower ($P < 0.05$) in PZ1 and PZ2 diets than PC diets during wk 6. Linear and quadratic effects ($P < 0.05$) of protected ZnO dose on *E.coli* counts during wk 3 and linear effects on *Lactobacillus* and *Salmonella* counts during wk 6 were observed.

In conclusion, a lower dose of protected ZnO could replace the higher dose of conventional ZnO because it has comparable or better effects than conventional ZnO in a higher dose.

1. Introduction

The stress related changes in weaning pigs due to separation from sows, co-mingling with unrelated litters, alteration in food

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; CATTD, coefficient of apparent total tract digestibility; BW, body weight; DM, dry matter; N, nitrogen; ME, metabolizable energy; G:F, gain:feed; ZnO, zinc oxide

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source and housing, etc. (Campbell et al., 2013) may lead to growth deprivation, reduced feed efficiency and immune functions, changes in intestinal integrity and increased incidence of diarrhea. It has been reported that about 11% of mortality rate in post-weaning piglets is due to the incidence of diarrhea (Owusu-Asiedu et al., 2003). To alleviate the post-weaning diarrhea, nutritional intervention may play an important role. The use of in-feed pharmacological level of zinc, in the form of ZnO, has been practiced traditionally for many years in large doses up to 3000 mg/kg for the prevention and treatment of diarrhea in weaned pigs (Shen et al., 2014; Cho et al., 2015). High doses of ZnO were perceived to possess antimicrobial property and could alleviate diarrhea by reducing enterotoxigenc *E.coli* colonization and bacterial population in the gastro-intestinal tract (Fairbrother et al., 2005). However, the effects of high doses of ZnO on intestinal microbiome are not equivocal. For instance, some studies indicated that 2500–3000 ppm of dietary ZnO may increase *E. coli* population in gastro-intestinal tract and induce diarrhea (Hojberg et al., 2005; Molist et al., 2011; Pieper et al., 2012). In addition, the use of ZnO in higher doses has been criticized worldwide due to the negative effects in environment resulting from zinc-enriched slurry due to which the use of Zn in large dose has to be eventually reduced (EFSA, 2010). The reason for usage of large dose of ZnO is because most of the ZnO gets solubilized in stomach due to acidic pH and only a small amount of insoluble Zn in the form of Zn ions reaches to the intestine which is an active site of action. Thus, an attempt to reduce the usage of higher doses of ZnO in pig feed and enhance the biological effect of ZnO in reducing the incidence of diarrhea was done by some researchers. In the recent years, the modification of regular ZnO powder into porous particles or nanoparticles is a common practice. For instance, Morales et al. (2012) demonstrated that the dietary supplementation with 110 ppm of the modified form of ZnO improved the growth performance of 42–63-d-old pigs. A study by Cho et al. (2015) also reported that supplementation of modified zinc oxide that was potentiated with enhanced surface area improved ADG during phase 1 and the overall experiment period compared to control indicating, the modification of zinc using different technique would allow the use of a lower dose of ZnO. In the current study we hypothesized that if ZnO is protected by coating, it may reduce the dissociation of ZnO in the stomach and allow the delivery of protected ZnO in an undissociated form to the distal GI tract for effective absorption (Shen et al., 2014). Therefore, the objective of our study was to evaluate the use of protected zinc oxide in lower doses in weaned pigs in substitution for the conventional high dose zinc oxide.

2. Materials and methods

The experiment was conducted at the swine experimental unit of Dankook University (Anseodong, Cheonan, Choongnam, Korea). The protocol for the current experiment was approved by the Animal Care and Use Committee of Dankook University.

2.1. Source of tested product

The ZnO used in this study was provided by a commercial company (Morningbio Co., Ltd., Cheonan, Korea). As per the company's information, this product was protected using lipid matrix coating and contained 40% ZnO and 60% hydrogenated palm oil.

2.2. Experimental design, animals, housing and diets

A total of 150 crossbred weaning pigs [(Yorkshire × Landrace) × Duroc, 28 days old] with an average body weight (BW) of 6.48 ± 1.58 kg were used in a 6-wk experiment. Pigs were blocked based on BW and sex and randomly allotted to 1 of 6 dietary treatments [5 pigs per pen (2 barrows and 3 gilts); 5 pens per treatment]. Treatments consisted of basal diet, negative control (NC); Basal diet without Zn in mineral premix with either 2500 ppm unprotected ZnO; positive control (PC) or 250, 500, 750 and 1000 ppm protected ZnO (PZ1, PZ2, PZ3, and PZ4 respectively). The experiment was divided into two phases: d 1–d 21 (Phase 1) and d 22–d 42 (Phase 2). All nutrients in diets were formulated to meet or exceed the recommendation of NRC (2012) for weaning pigs fed in mash form (Table 1). Zinc oxide was supplemented into the diet by replacing the same amount of corn in different phase's basal diet. All pigs were housed in an environmentally-controlled room. An area of 0.26 × 0.53 m^2 was provided to each pig. Each pen was provided a stainless steel feeder and a nipple drinker with ad libitum access to feed and water throughout the experiment. Ventilation was provided by a mechanical system. Lighting was automatically regulated to provide 12 h of artificial light per day. Ambient temperature within the room was approximately 30 °C. It was decreased 1 °C each week of the experiment.

2.3. Sampling and measurements

The individual body weight (BW) of pigs and feed consumption from each pen were determined at the start, week 3 and week 6 of the experiment to calculate the average daily gain (ADG), average daily feed intake (ADFI), and gain:feed ratio (G:F). Chromium oxide was added to the diet as an indigestible marker at 0.20% of the diet for 7 days prior to faecal collection for digestibility estimates. Faecal samples were collected from 8 pigs per treatment (1gilt and 1 barrow from randomly selected 4 pens each per treatment) by direct rectal massage on day 42 of the experiment to determine the apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen (N) and energy (E) and a representative sample was stored in a freezer at −20 °C until analyzed. 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 Association of Official Analytical Chemists (AOAC) (2000). Chromium levels were determined via UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan). 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). Samples of diets were analyzed using standard methods of AOAC (2000) for nitrogen (N; method 968.06). Calcium (method 984.01) and Phosphorus

Table 1
Composition of the experimental weaning pig diets (as-fed basis; g/kg).

Items	Phase 1 (day 1–21)		Phase 2 (day 22–42)	
Ingredients	Basal diet	Basal diet (without Zn in mineral premix)	Basal diet	Basal diet (without Zn in mineral premix)
Corn	452.4	452.4	562.9	562.9
SBM	185.7	185.7	197.3	197.3
SBM, dehulled	79.0	79.0	70.0	70.0
Corn gluten	30	30	–	–
Soybean oil	32.9	32.9	18.2	18.2
Fish meal	34.0	34.0	15.0	15.0
Lactose	68.0	68.0	50.0	50.0
Whey	50.0	50.0	30.0	30.0
Mono Calcium Phosphate	13.2	13.2	13.4	13.4
Limestone	12.3	12.3	8.5	8.5
Sugar	30.0	30.0	20.0	20.0
Methionine(99%)	0.6	0.6	1.5	1.5
Lysine(78%)	5.0	5.0	6.0	6.0
Threonine(99%)	1.9	1.9	2.2	2.2
Vitamin premix[1]	2.0	2.0	2.0	2.0
Mineral premix (normal)[2]	2.0	0	2.0	0
Mineral premix (without Zn)[3]	0	2.0	0	2.0
Choline	1.0	1.0	1.0	1.0
Calculated composition				
ME, MJ/kg	15.06	15.06	14.86	14.86
Analysed composition				
Crude Protein	201.0	201.0	192.0	192.0
Lysine	156.0	156.0	145.0	145.0
Methionine	4.0	4.0	4.5	4.5
Calcium	9.7	9.7	7.5	7.5
Phosphorous	7.1	7.1	6.5	6.5
Lactose	101	101	69.4	69.4

[1] Provided per kilogram of complete diet: 1.3 mg vitamin A (Retinol); 0.022 mg vitamin D3 (Cholecalciferol); 45 mg vitamin E (Tocotrienol); 4.2 mg vitamin K3 (Menodione); 24.6 mg vitamin B5 (d-Ca-pantothenate); 8.6 mg vitamin B2 (Riboflavin); 0.04 mg vitamin B12 (Cobalamins).

[2] Provided per kg of complete diet: Cu (as CuSO4·5H2O), 12 mg; Zn (as ZnSO4), 85 mg; Mn (as MnO2), 8 mg; I (as KI), 0.28 mg; and Se (as Na2SeO3·5H2O), 0.15 mg.

[3] Provided per kg of complete diet: Cu (as CuSO4·5H2O), 12 mg; Mn (as MnO2), 8 mg; I (as KI), 0.28 mg; and Se (as Na2SeO3·5H2O), 0.15 mg.

(method 965.17) contents were determined according to the AOAC (1995). The individual amino acid composition was measured using an amino acid analyser (Beckman 6300, Beckman Coulter, Inc., Fullerton, CA, USA) after 24 h of 6 N HCl hydrolysis at 110 °C (AOAC 2000). To determine the methionine levels, the samples were oxidized with performic acid overnight. Nitrogen was determined using a Kjectec 2300 Nitrogen Analyser (Foss Tecator AB, Hoeganaes, Sweden). The 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 was calculated according to the method described by Fenton and Fenton (1979) using the following formula: digestibility (%) = {1 − [(Nf × Cd)/(Nd × Cf)]} × 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).

For the evaluation of minerals in blood, blood samples (5 ml) were collected from randomly chosen 10 pigs per treatment (2 pigs per pen per treatment) via jugular venipuncture using a sterile needle at wk1, wk 3 and wk 6. The blood samples were pooled on a pen basis into tubes without additive and allowed to clot at an ambient temperature for half an hour. Thereafter, serum was harvested by centrifuging the samples at 3000 × *g* for 15 min at 4 °C and serum was stored at −20 °C until further analysis. Zinc, iron and copper were determined using atomic absorption spectrophotometer (AA-6300, Shimadzu, Tokyo, Japan). Faecal samples for zinc concentration were prepared with the method described by Armstrong et al. (2004). Briefly, the faecal samples were collected via rectal massage and stored at −20 °C until further analysis. Then the faecal samples were dissolved with nitric acid and then digested using a microwave digestion system. Subsequently, the solution was diluted with deionized water and was analyzed with flame atomic absorption spectrophotometry (AA-6300, Shimadzu, Tokyo, Japan).

Faecal scores were evaluated and recorded at 08:00 and 20:00 h on days 7, 14, 21, 35 and 42. The faecal score was determined as the average value of five pigs of each pen using a 5-grade score system (Hu et al., 2012), with grade of 1 standing for hard, dry pellets in a small, hard mass, grade of 2 for hard formed stool that remains firm and soft, grade of 3 for soft formed and moist stool that retains its shape, grade of 4 for soft unformed stool that assumes the shape of the container, and grade of 5 for watery liquid stool that can be poured. Scores were recorded on a pen basis following the observations of individual pigs and signs of stool consistency in the pen.

For faecal microbial analysis, fresh faeces were collected directly from randomly chosen 10 pigs per treatment (2 pigs per pen per

treatment) via massaging the rectum at week 1, 3, and 6 and placed on ice for transportation to the laboratory. The composite faecal sample (1 g) from each pig was diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ) and then homogenized.

For digesta microbial analysis, 2 pigs each from 3 pens were randomly selected from each treatment and sacrificed at the end of the experiment. The digesta from ileum and colon was collected from selected pigs and pooled on pen basis from each treatment and placed on ice for transportation to the laboratory. The digesta sample (1 g) from the ileum and colon of each pig was diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ) and then homogenized.

Viable counts of bacteria in the faeces and digesta samples were studied by plating serial 10-fold dilutions in anaerobic diluents before inoculation on to Petri dishes of sterile agar. *Salmonella Escherichia coli, Lactobacillus* and *Clostridium spp* present in the fresh faecal and digesta samples were enumerated. The selective medium for *Salmonella* was salmonella shigella agar (Difco, USA) and for *E. coli* was Mac Conkey agar (Difco, USA). *Clostridia spp*. was grown on Blood agar (Oxoid Ltd.) and lactobacilli medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) were used to isolate *Lactobacillus*. Lactobacilli medium III and Blood agar plates were then incubated for 48 h at 39 °C and 37 °C respectively. The Shigella agar and Mac Conkey agar plates were inverted and incubated anaerobically at 37 °C for 24 h. The colony counts were then enumerated and results are presented as log10-transformed data.

After pigs were slaughtered at the end of the trial, the entire intestine of slaughtered pigs (2 pigs each from randomly selected 3 pens per treatment) was then removed and dissected free of mesenteric attachments and placed on a smooth and cold surface. The duodenum, jejunum and ileum were separated. The isolated intestinal segments were immediately opened lengthwise following the mesentery line and flushed with ice-cold saline. Approximately 2 cm segments of the duodenum at consistent locations were collected immediately, fixed in 10% formalin, then subsequently embedded, sectioned and stained with haematoxylin and eosin by routine methods. Villus height, crypt depth, and villus height to crypt depth ratio (VH: CD) of the small intestine were measured in approximately 10 microscopic fields using an image analysis system by a blinded investigator.

2.4. Statistical analyses

Data were analyzed using the GLM procedure of SAS (version 9.4; SAS Inst., Inc., Cary, NC) in a randomized complete block design. Pen served as the experimental unit. Pre-planned contrast was used to test the following: 1) the individual effect of NC versus PC diets 2) the overall effect of protected ZnO supplementation versus NC diet (NC vs PZ1, PZ2, PZ3 and PZ4) and 3) the overall effect of protected ZnO supplementation versus PC diet (PC vs PZ1, PZ2, PZ3 and PZ4). Furthermore, linear, quadratic and cubic polynomial contrasts were used to examine responses to supplemental graded levels of protected ZnO at 0 ppm, 250 ppm, 500 ppm, 750 ppm and 1000 ppm. Variability in the data was expressed as the standard error of means (SEM) and $P < 0.05$ was considered to be statistically significant and $P < 0.1$ as trends.

3. Results

3.1. Growth performance and coefficient of apparent total tract nutrient digestibility

As shown in Table 2, the BW, ADG and G:F were greater ($P < 0.05$) in pigs fed PC diet compared with NC diet during phases 1 and 2 as well as overall experiment period except G/F tended ($P = 0.07$) to be greater and ADFI was higher ($P < 0.05$) in PC versus NC diet during phase 2. The supplementation of protected ZnO led to greater ($P < 0.05$) BW and ADG and ADFI during phase 2 in weaned pigs compared to NC. The ADG was also higher ($P < 0.05$) in pigs fed protected ZnO supplemented diets compared with NC diet during the overall experiment period. The BW was higher in pigs fed PC than those fed diet supplemented with protected ZnO during phase 2. In addition, the ADG and G:F ratio were greater ($P < 0.05$) in pigs fed PC diet than protected ZnO diets during the overall experiment period. However, except for G:F ratio in phase 1, the growth performance in pigs fed protected ZnO diets were comparable with PC diet during phase 1 and 2. There were cubic effects ($P < 0.05$) of protected ZnO dose on ADG and ADFI during phase 2 in weaned pigs.

The CATTD of DM tended ($P = 0.08$) to be greater in pigs fed PC diet compared with NC. The supplementation of protected ZnO to the diet led to greater ($P < 0.05$) CATTD of DM and energy in weaned pigs compared with NC diet. The nutrient digestibility in pigs fed protected ZnO diets was comparable with PC diet. There was no significant effect ($P > 0.05$) of graded levels of protected ZnO on nutrient digestibility (Table 2).

3.2. Minerals level in serum

As shown in Table 3, the level of Zn in the serum of pigs fed PC diet was greater ($P < 0.05$) than NC and protected ZnO diets during week 1, 3 and 6. However, Fe and Cu levels in the serum of pigs fed PC diet were not affected ($P > 0.05$) compared with NC and protected ZnO diets. A linear effect ($P < 0.05$) of protected ZnO dose on serum Zn level was observed during week 3. In addition, cubic effect of protected ZnO dose on serum Fe level was also observed during week 1.

3.3. Zinc concentration in faeces

The faecal Zn concentrations were greater ($P < 0.05$) in pigs fed PC diets compared to NC and PZ diets during week 1, 3 and 6. The pigs fed NC diets had lower Zn concentration in faeces compared with protected ZnO diets during week 1 and 6. A linear response

Table 2
Effects of protected ZnO on growth performance and coefficient of apparent total tract nutrient digestibility in weaning pigs.[1]

Items								*P* -values					
											Protected ZnO (0–1000 ppm)		
	NC	PC	PZ1	PZ2	PZ3	PZ4	SEM[2]	NC vs PC	NC vs PZ	PC vs PZ	L	Q	C
Body weight, kg													
Initial	6.53	6.52	6.47	6.46	6.45	6.46	0.038	0.82	0.10	0.16	0.15	0.31	0.72
Phase1	11.96	13.12	12.14	12.80	12.48	12.41	0.316	0.02	0.17	0.07	0.20	0.21	0.80
Phase2	23.37	25.83	24.65	25.20	24.21	24.62	0.462	0.001	0.02	0.04	0.18	0.08	0.17
Phase1													
ADG, g	258.4	314.0	269.6	302.2	287.4	283.8	14.61	0.01	0.10	0.10	0.15	0.17	0.82
ADFI, g	350.2	318.4	328.0	363.6	347.6	323.2	17.48	0.21	0.63	0.27	0.36	0.22	0.09
G/F	0.74	1.01	0.82	0.83	0.83	0.90	0.065	0.01	0.15	0.04	0.11	0.87	0.49
Phase2													
ADG, g	543.6	605.0	595.8	590.8	558.4	581.0	14.49	0.01	0.03	0.16	0.44	0.14	0.03
ADFI, g	851.8	905.2	902.4	900.2	869.0	894.2	12.38	0.01	0.01	0.33	0.24	0.13	0.02
G/F	0.638	0.668	0.660	0.656	0.642	0.650	0.0109	0.07	0.26	0.21	0.87	0.36	0.19
Overall													
ADG, g	401.0	459.6	432.8	446.2	422.8	432.4	10.66	0.001	0.01	0.04	0.14	0.06	0.15
ADFI, g	601.0	611.8	615.2	632.0	608.2	608.8	10.81	0.48	0.23	0.73	0.79	0.10	0.49
G/F	0.667	0.752	0.703	0.707	0.695	0.712	0.0191	0.01	0.10	0.04	0.23	0.51	0.37
Nutrient digestibility													
Dry matter	0.801	0.819	0.826	0.820	0.819	0.818	0.0069	0.08	0.02	0.80	0.26	0.12	0.21
Nitrogen	0.802	0.807	0.819	0.810	0.811	0.813	0.0062	0.53	0.10	0.38	0.49	0.38	0.17
Energy	0.802	0.819	0.823	0.823	0.819	0.818	0.0074	0.10	0.03	0.85	0.26	0.10	0.36

[1] Abbreviation: NC, Basal diet; PC, Basal diet without Zn in mineral premix + 2500 ppm unprotected ZnO; PZ, Basal diet without Zn in mineral premix + Protected ZnO at 4 different levels (250, 500, 750 and 1000 ppm).
[2] Standard error of means.

Table 3
Effects of protected ZnO on serum mineral concentration in weaning pigs.[1]

Items, ug/dL								*P* -values					
											Protected ZnO (0–1000 ppm)		
	NC	PC	PZ1	PZ2	PZ3	PZ4	SEM[2]	NC vs PC	NC vs PZ	PC vs PZ	L	Q	C
Wk 1													
Fe	66.8	85.8	27.3	56.0	89.0	81.0	14.86	0.38	0.83	0.19	0.04	0.19	0.02
Cu	195.6	174.1	194.7	211.8	194.5	204.0	12.59	0.25	0.69	0.07	0.64	0.74	0.80
Zn	70.5	170.5	75.8	77.8	79.8	79.3	8.21	< 0.0001	0.41	< 0.0001	0.19	0.54	0.96
Wk 3													
Fe	104.0	92.8	96.8	53.8	96.3	118.3	20.33	0.70	0.58	0.94	0.63	0.05	0.79
Cu	192.3	192.1	184.8	215.9	198.1	218.5	17.09	0.10	0.54	0.53	0.29	0.92	1.00
Zn	67.7	160.9	73.0	80.0	86.8	94.0	7.32	< 0.0001	0.07	< 0.0001	0.01	0.89	0.95
Wk 6													
Fe	106.0	111.5	138.5	97.0	114.3	145.5	17.41	0.81	0.37	0.5	0.27	0.34	0.09
Cu	138.1	144.6	163.7	151.6	148.5	168.6	9.99	0.70	0.09	0.2	0.16	0.95	0.07
Zn	82.8	119.4	85.3	85.5	88.7	89.8	8.28	0.01	0.63	0.003	0.48	0.10	1.0

[1] Abbreviation: NC, Basal diet; PC, Basal diet without Zn in mineral premix + 2500 ppm unprotected ZnO; PZ, Basal diet without Zn in mineral premix + Protected ZnO at 4 different levels (250, 500, 750 and 1000 ppm).
[2] Standard error of means.

($P < 0.05$) of protected ZnO dose was observed on faecal Zn concentration. In addition, quadratic effect was seen on zinc concentration from the faeces of pigs fed graded level of protected ZnO during week 6 (Table 4).

3.4. Intestinal morphology and faecal score

The effects of ZnO on intestinal morphology are presented in Table 5. No significant effects on intestinal morphology and faecal score (data not shown) were observed among different treatments.

Table 4
Effects of protected ZnO on faeces Zn concentration in weaning pigs.[1]

Items, log_{10} cfu/g								*P* -values					
											Protected ZnO (0–1000 ppm)		
	NC	PC	PZ1	PZ2	PZ3	PZ4	SEM[2]	NC vs PC	NC vs PZ	PC vs PZ	L	Q	C
WK1	1083.1	10790.1	1398.8	1926.8	3249.6	3770.7	276.57	< 0.0001	0.001	< 0.001	< 0.001	0.07	0.07
WK3	2849.0	10795.7	2275.9	3569.0	4133.8	5468.5	540.27	< 0.001	0.11	< 0.001	0.001	0.13	0.51
WK6	1047.2	11945.2	1503.4	1863.9	3802.1	5222.2	473.60	< 0.001	0.01	< 0.001	< 0.001	0.01	0.64

[1] Abbreviation: NC, Basal diet; PC, Basal diet without Zn in mineral premix + 2500 ppm unprotected ZnO; PZ, Basal diet without Zn in mineral premix + Protected ZnO at 4 different levels (250, 500, 750 and 1000 ppm).
[2] Standard error of means.

Table 5
Effects of protected ZnO on villi length in weaning pigs.[1]

Items, μM								*P* -values					
											Protected ZnO (0–1000 ppm)		
	NC	PC	PZ1	PZ2	PZ3	PZ4	SEM[2]	NC vs PC	NC vs P Z	PC vs PZ	L	Q	C
Villus height													
Duodenum	262.9	352.6	272.5	339.0	275.9	307.4	29.36	0.06	0.30	0.13	0.26	0.37	0.64
Jejunum	293.1	352.5	301.4	348.1	325.9	348.0	28.60	0.17	0.26	0.51	0.12	0.67	0.94
Ileum	327.1	394.8	342.5	386.6	344.1	367.9	30.08	0.14	0.35	0.33	0.44	0.58	0.73
Crypt depth													
Duodenum	195.7	210.9	230.7	223.2	194.7	203.7	15.38	0.50	0.34	0.90	0.68	0.22	0.12
Jejunum	227.4	212.3	196.8	212.1	225.3	219.5	13.57	0.45	0.38	0.94	0.78	0.38	0.17
Ileum	213.6	229.7	185.4	200.0	208.5	214.5	14.23	0.44	0.48	0.11	0.62	0.30	0.37
Villus height/crypt depth													
Duodenum	1.35	1.66	1.18	1.52	1.48	1.56	0.18	0.24	0.68	0.28	0.27	0.89	0.53
Jejunum	1.29	1.70	1.54	1.69	1.45	1.66	0.23	0.23	0.27	0.67	0.35	0.55	0.43
Ileum	1.56	1.72	1.85	1.93	1.65	1.73	0.15	0.46	0.20	0.69	0.78	0.25	0.32

[1] Abbreviation: NC, Basal diet; PC, Basal diet without Zn in mineral premix + 2500 ppm unprotected ZnO; PZ, Basal diet without Zn in mineral premix + Protected ZnO at 4 different levels (250, 500, 750 and 1000 ppm).
[2] Standard error of means.

Table 6
Effects of protected ZnO on digesta microflora in weaning pigs.[1]

Items, log_{10} cfu/g								*P* -values					
											Protected ZnO (0–1000 ppm)		
	NC	PC	PZ1	PZ2	PZ3	PZ4	SEM[2]	NC vs PC	NC Vs PZ	PC Vs PZ	L	Q	C
Ileum													
E.coli	3.87	3.84	3.73	3.99	4.31	3.74	0.05	0.66	0.26	0.1	0.13	0.01	< 0.001
Lactobacillus	7.24	7.38	7.15	7.46	7.50	7.52	0.11	0.37	0.19	0.83	0.04	0.9	0.29
Clostridium	4.05	3.70	3.84	3.68	4.03	3.93	0.08	0.02	0.10	0.09	0.92	0.06	0.12
Salmonella	3.72	3.72	3.66	3.68	3.68	3.85	0.05	1.0	0.98	0.98	0.17	0.09	0.63
Colon													
E.coli	3.93	3.78	3.89	4.18	4.40	3.88	0.08	0.19	0.08	0.004	0.15	0.01	0.004
Lactobacillus	7.54	7.41	7.32	7.52	7.56	7.40	0.08	0.26	0.31	0.67	0.85	0.9	0.01
Clostridium	3.77	3.76	3.71	3.93	4.15	4.27	0.07	0.95	0.01	0.01	0.001	0.28	0.17
Salmonella	3.78	3.68	3.68	3.81	3.73	3.67	0.04	0.08	0.24	0.27	0.27	0.41	0.14

[1] Abbreviation: NC, Basal diet; PC, Basal diet without Zn in mineral premix + 2500 ppm unprotected ZnO; PZ, Basal diet without Zn in mineral premix + Protected ZnO at 4 different levels (250, 500, 750 and 1000 ppm).
[2] Standard error of means.

Table 7
Effects of protected ZnO on faecal microflora in weaning pigs.[1]

Items, log_{10} cfu/g								*P* -values					
											Protected ZnO (0–1000 ppm)		
	NC	PC	PZ1	PZ2	PZ3	PZ4	SEM[2]	NC vs PC	NC Vs PZ	PC Vs PZ	L	Q	C
wk 1													
E.coli	4.46	4.02	4.24	4.30	4.61	4.27	0.11	0.01	0.39	0.02	0.97	0.93	0.02
Lactobacillus	7.47a	7.50	7.86	7.51	7.91	7.38	0.14	0.88	0.23	0.30	0.80	0.08	0.71
Clostridium	4.12	4.62	4.89	4.70	4.48	4.37	0.26	0.19	0.11	0.96	0.92	0.10	0.24
Salmonella	4.35	4.28	4.30	3.97	4.52	4.31	0.14	0.72	0.64	0.99	0.77	0.35	0.32
wk 3													
E.coli	4.24	4.20	4.38	4.50	4.47	4.43	0.06	0.68	0.01	0.002	0.03	0.04	0.88
Lactobacillus	7.46	7.39	7.50	7.36	7.23	7.56	0.08	0.50	0.56	0.79	0.78	0.07	0.03
Clostridium	4.38	4.49	4.57	4.49	4.39	4.59	0.07	0.28	0.14	0.88	0.34	0.96	0.04
Salmonella	4.30	4.36	4.22	4.22	4.26	4.29	0.08	0.63	0.51	0.22	0.95	0.26	0.57
wk 6													
E.coli	4.64	4.50	4.42	4.51	4.51	4.51	0.07	0.19	0.07	0.83	0.45	0.18	0.17
Lactobacillus	7.37	7.50	7.53	7.49	7.73	7.64	0.04	0.04	0.001	0.04	0.001	0.22	0.38
Clostridium	4.41	4.48	4.46	4.51	4.50	4.34	0.08	0.51	0.62	0.74	0.66	0.09	0.52
Salmonella	4.15	4.53	4.16	4.24	4.54	4.62	0.06	0.001	0.004	0.05	< 0.001	0.16	0.18

[1] Abbreviation: NC, Basal diet; PC, Basal diet without Zn in mineral premix + 2500 ppm unprotected ZnO; PZ, Basal diet without Zn in mineral premix + Protected ZnO at 4 different levels (250, 500, 750 and 1000 ppm).
[2] Standard error of means.

3.5. Digesta microbiota enumeration

The microbiota counts in digesta are shown in Table 6. The *Clostridium spp* counts from the digesta of ileum of pigs fed PC diets were lower ($P < 0.05$) than NC diets. However, other microbial counts in the digesta of ileum and colon were not affected in PC diet compared to NC except for a trend ($P = 0.08$) in reduction of *Salmonella spp* counts in the colon digesta in pigs fed PC diet as compared to NC diet. The *Clostridium spp* counts from the colon digesta were slightly lower in pigs fed PZ1 diets but increased significantly ($P < 0.05$) in other PZ treatments compared to NC diet. The *E.coli* and *Clostridium spp* counts were significantly lower ($P < 0.05$) in the digesta from colon of pigs fed PC diet than protected ZnO diets during week 3. Quadratic and cubic effects ($P < 0.05$) of protected ZnO dose were observed on *E.coli* counts in the digesta of ileum and colon of pigs. Linear effects ($P < 0.05$) of protected ZnO dose on *Lactobacillus* and *Clostridium* counts on the digesta of ileum and colon respectively were also observed (Table 6).

3.6. Faecal microbiota enumeration

The faecal microbial counts are presented in Table 7. The faecal *E.coli* counts were higher ($P < 0.05$) in pigs fed NC diet than PC diet during week 1 but no difference was observed in faecal *E.coli* counts in pigs fed PC and NC diet during week 3 and week 6. The faecal *Lactobacillus* and *Salmonella* spp. counts were higher ($P < 0.05$) in PC than NC during week 6. The *E.coli* counts (week 3) and *Lactobacillus* and *Salmonella* spp counts (week 6) were higher ($P < 0.05$) in protected ZnO diets than NC diets. The *E.coli* counts were higher in protected ZnO treatments than in conventional ZnO treatment during week 1 and 3. The *Lactobacillus* counts were higher ($P < 0.05$) and *Salmonella* counts were lower ($P < 0.05$) in PZ1 and PZ2 diets than PC diets during week 6. Linear and quadratic effects ($P < 0.05$) of protected ZnO dose on *E.coli* counts during week 3 and linear effects on *Lactobacillus* and *Salmonella* counts during week 6 were observed. There were cubic effects ($P < 0.05$) of the protected ZnO dose on *E. coli* counts during week 1 and *Lactobacillus* and *Clostridium* spp counts during week 3.

4. Discussion

Zinc oxide has been used in the diet of weaning pigs to alleviate weaning stress and improve weakened immune systems and digestive disorders since 1990's. Hill et al. (2001) suggested that supplemental ZnO at 1500–2000 mg Zn/kg Zn improved post-weaning pig performance. The pharmacological dose of 3000–4000 ppm of ZnO as a replacement of in-feed antibiotics has been reported in several studies resulting in increased growth performance and nutrient digestibility (Cho et al., 2015; Milani et al., 2017). In the present study also, a significant improvement in growth performance, trend in DM digestibility was observed in piglets fed 2500 ppm ZnO supplemented diet. The proposed mechanism by some researchers for improved growth performance was due to the enhancement of intestinal morphology and function, improvement in antioxidant capacity as well as restorations of the mucosal barrier integrity in weaned piglets and alleviation of diarrhea (Ou et al., 2007; Sargeant et al., 2011; Trckova et al., 2015; Zhu et al., 2017). However the animal response to high dose ZnO is variable. In fact, some studies did not observe any improvement in growth

performance in weaned pigs fed high dose ZnO (Schell and Kornegay, 1996; Shen et al., 2014) or some studies even reported that feeding with ZnO at the dose of 2500 ppm increases the proportion of multi-resistant *Escherichia coli* in ileum and colon digesta (Pieper et al., 2012; Bednorz et al., 2013). These controversial findings have provoked the question on the use of high dose of ZnO in animal diet. Thus, different techniques are utilized including nano particle, porous particle or encapsulation/coating that are assumed to provide an opportunity to reduce the dose of ZnO and enhance the effect of ZnO for improved growth performance and health as well as to reduce the risk of heavy metal contamination.

In the current study, we tested the hypothesis that use of protected zinc oxide in lower dose in weaned pigs may substitute for the conventional high dose zinc oxide. Data from the present study indicated that supplementation of protected ZnO led to significantly higher BW and ADG during phase 2 in weaned pigs compared to pigs offered basal diet containing the nutritional level of zinc. In contrast, Shen et al. (2014) did not observe any significant effect on growth performance of weaned pigs that were offered diets containing coated zinc oxide at the dose of 250, 380, 570, 760 and 1140 mg ZnO/kg basal diet compared with non-coated ZnO at the dosage 250 mg Zn/kg feed. In addition, Oropeza-Moe et al. (2017) also indicated that ADG of piglets fed diet supplemented with commercial peat containing humic substances added with coated ZnO (257 mg Zn/l) did not differ significantly from those fed diet supplemented with commercial peat added with uncoated ZnO (2255 mg Zn mg/l). The improvement in growth performance with the supplementation of protected ZnO in the current study could be associated to the increase in bioavailability and retention of zinc due to effective delivery of protected ZnO in an undissociated form to the distal GI tract for absorption that might have enhanced gastro intestinal tract function. Shen et al. (2014) also reported that when coated ZnO was added, as a replacement of ZnO (uncoated), the percentage of the ZnO molecule reaching the gastrointestinal tract is markedly increased, which is due to the protective enteric coating of the inner ZnO. No significant linear response was seen on growth performance of pigs fed protected ZnO diets at graded levels in the current study indicating that the dose of 250 ppm protected ZnO is enough to show some positive or comparable effects on growth performance compared with NC or PC respectively.

The morphology and functions of the gastrointestinal tract are remarkably impaired in a weanling piglet (Moeser et al., 2007) leading to diarrhea. A recent study has demonstrated that high concentration of ZnO reduced the diarrhea rate of pig post weaning (Milani et al., 2017). The supplementation with coated ZnO at 380 or 570 mg Zn/kg was also reported to reduce diarrhoea index (Shen et al. 2014). In our study, the faecal score, which could represent the diarrhea status, was similar (approximately 3) among all treatments indicating no incidence of diarrhea. The supplementation of ZnO was also demonstrated to increase villus height in weaned pigs (Li et al., 2001; Castillo et al., 2008; Shen et al., 2014). However, in the present study, the supplementation of conventional or protected ZnO did not have any significant effect on villus height and crypt depth compared with control. The reason for no incidence of diarrhea could be due to better farm management and good environment where piglets were raised. The possible reason for the observation of no change in intestinal morphology with ZnO treatment may be the inappropriate time chosen for histological examination i.e. 6 week post-weaning. Thus, in future experiment, histological examination for the assessment of intestinal morphology should be considered at the early period of weaning since, weaning may impair the morphology of intestinal mucosa during the early days.

The dietary supplementation of ZnO in high doses has been reported to modulate the microbiome of ileum and colon in weaned pigs (Yu et al., 2017). However, the response of high ZnO doses on the intestinal microbiome are variable (Hojberg et al., 2005; Vahjen et al., 2011; Pieper et al., 2012; Starke et al., 2014). For instance, higher doses of ZnO was assumed to reduce the incidence of diarrhea by lowering the counts of pathogenic bacteria (Sales, 2013), while some studies have showed that ZnO increased the population of Enterobacteriales (Hojberg et al., 2005; Pieper et al., 2012). In the current study, we assessed the effect of pharmacological high dose ZnO and relatively lower levels of protected ZnO on the microbiota population including *E.coli*, *Lactobacillus, Salmonella* and *Clostridium spps* from faeces as well as from the digesta of ileum and colon. We found that the *Clostridium spps* and *E.coli* counts were reduced in the digesta obtained from animals fed pharmacological level of ZnO compared with control. The supplementation of protected ZnO at lower levels did not seem to have effect on digesta *E coli, Salmonella* and *Lactobaciilus* counts except for reduction in *Clostridium* counts with 250 ppm of protected ZnO. The bacterial communities and metabolic properties in the digesta of weaned piglets were found to be influenced by the dose of ZnO, different samples from various locations of porcine gastrointestinal tracts (stomach, small intestine, or large intestine), the different methods of culturing and methods used for analyzing the changes in the microbial community (Pieper et al., 2012; Sales, 2013; Starke et al., 2014).

The *E.coli* counts in the faeces from pigs fed 2500 ppm ZnO were reduced during the early phases of nursery periods whereas *Lactobacillus* counts were increased at week 6 in the current study. In line with our findings, other studies also indicated significant reduction in *E.coli* counts in the faeces from weaned pigs fed high dose of ZnO (Jensen-Waern et al., 1998; Slade et al., 2011; Kaevska et al., 2016). The increase in *Lactobacillus* count might have reduced the faecal *E.coli* counts during wk 6 because *E. coli* do not tolerate acidic pH condition. Increased proportions of lactic acid promote gut health by suppressing the growth of coliforms such as pathogenic *E. coli.* The dose dependent effect of protected ZnO on *E.coli* counts during wk 3 and *Lactobacillus* and *Salmonella* counts during week 6 were also observed indicating that lower doses of ZnO in protected form can have comparable or better effect on gut microbiota which may eventually enhance gastro intestinal functions.

The inclusion of ZnO in the feed not only has an impact on gut microbiome but it also has been reported to increase serum Zn concentrations (Walk et al., 2013) as well as faecal Zn concentrations. For instance, Bednorz et al. (2013) and Jang et al. (2014) reported that piglets receiving therapeutic concentration of ZnO ranging between 2000 ppm–2500 ppm have higher circulating Zn concentration than those receiving 100–200 ppm of uncoated ZnO. Our finding also showed that the concentration of Zn in serum was significantly higher in pigs fed PC diet containing 2500 ppm unprotected ZnO compared with those fed lower doses of protected ZnO diets. This may also confirm that a significant amount of unprotected zinc is dissociated in the stomach indicating the therapeutic effect of unprotected zinc is low. The increase in serum concentration with the dietary supplementation of zinc as explained

in literature is possibly due to the increase in the concentration of metallothionien in the intestinal mucosa that may further affect the transfer of the amount of zinc into systemic circulation (Carlson et al., 1999). The serum Fe and Cu concentrations in the current study were unaffected by supplementing different forms of Zn in the diet and this finding was in agreement to the results from other studies (Carlson et al., 1999; Wang et al., 2012).

The supplementation with a pharmacological dose of ZnO despite having anti-diarrhea effect could cause excessive Zn residues in animals and environment pollution, because a large amount of unabsorbed Zn is excreted in the slurry. Carlson et al. (2004) reported that faecal excretion of zinc was directly related to the amount of zinc consumed regardless of zinc source which corroborated with our finding that Zn concentrations in the faeces increased with the increasing concentrations of supplemented Zn in the diet. However, with the modification of zinc by coating technology and using low doses of Zn, the excretion of zinc from faeces could be reduced as shown from our studies as well as in the study by Shen et al. (2014). The possible explanation for reduction in excretion of Zn when protected could be due to use of low doses of zinc as compared with unprotected ones. Thus, the negative effective of heavy metal contamination in the environment from Zinc enriched slurry in farm facilities could be addressed by using lower doses of Zn in protected forms.

5. Conclusions

Taken together, our results suggest that supplementation of conventional ZnO as well as protected ZnO had beneficial effects on growth performance, nutrient digestibility and microbial counts. A lower level of protected ZnO could be used in substitution for the conventional high dose ZnO because the dietary supplementation with lower doses of protected ZnO had comparable or better result with that of conventional ZnO on growth performance, digestibility of nutrients and reduced faecal Zn concentration in weaning pig. A trend in improvement in serum Zn concentrations at week 3 was observed. The lower level of protected ZnO in weaning pig diet would be economically and environmentally beneficial. Thus, protected zinc in lower doses could be used in weaned pigs in substitution for the conventional high dose zinc oxide as a growth promoter as well as it may contribute to reduce Zn enriched slurry in pig facilities.

Conflict of interest

The authors declare that there is no conflict of interest.

References

AOAC, 1995. Official Method of Analysis, 16th ed. Association of Official Analytical Chemists, Washington, DC, USA.
AOAC, 2000. Official Methods of Analysis, 17th ed. Association of Official Analytical Chemists, Washington, DC, USA.
Armstrong, T.A., Cook, D.R., Ward, M.M., Williams, C.M., Spears, J.W., 2004. Effect of dietary copper source (cupric citrate and cupric sulfate) and concentration on growth performance and fecal copper excretion in weanling pigs. J. Anim. Sci. 82, 1234–1240.
Bednorz, C., Oelgeschläger, K., Kinnemann, B., Hartmann, S., Neumann, K., Pieper, R., Bethe, A., Semmler, T., Tedin, K., Schierack, P., Wieler, L.H., Guenther, S., 2013. The broader context of antibiotic resistance: zinc feed supplementation of piglets increases the proportion of multi-resistant Escherichia coli in vivo. Int. J. Med. Microbiol. 303, 396–403.
Campbell, J.M., Crenshaw, J.D., Polo, J., 2013. The biological stress of early weaned piglets. J. Anim. Sci. Biotechnol. 4, 19. http://dx.doi.org/10.1186/2049-1891-4-19.
Carlson, M.S., Hill, G.M., Link, J.E., 1999. Early-and traditionally weaned nursery pigs benefit from phase-feeding pharmacological concentrations of zinc oxide: effect on metallothionein and mineral concentrations. J. Anim. Sci. 77, 1199–1207.
Carlson, M.S., Boren, C.A., Wu, C., Huntington, C.E., Bollinger, D.W., Veum, T.L., 2004. Evaluation of various inclusion rates of organic zinc either as polysaccharide or proteinate complex on the growth performance, plasma and excretion of nursery pigs. J. Anim. Sci. 82, 1359–1366.
Castillo, M., Martin-Orue, S.M., Taylor-Pickard, J.A., Perez, J.F., Gasa, J., 2008. Use of mannanoligosaccharides and zinc chelate as growth promoters and diarrhea preventive in weaning pigs: effects on microbiota and gut function. J. Anim. Sci. 86, 94–101.
Cho, J.H., Upadhaya, S.D., Kim, I.H., 2015. Effects of dietary supplementation of modified zinc oxide on growth performance, nutrient digestibility, blood profiles, fecal microbials shedding and fecal score in weanling pigs. Anim. Sci. J. 86, 617–623.
EFSA, 2010. Pre-assessment of Environmental Impact of Zinc and Copper Used in Animal Nutrition. http://www.efsa.europa.eu/en/supporting/doc/74e.pdf.
Fairbrother, J.M., Nadeau, E., Gyles, C.L., 2005. *Escherichia coli* in postweaning diarrhea in pigs; an update on bacterial types, pathogenesis, and prevention strategies. Anim. Health Res. Rev. 6, 17–639.
Fenton, T.W., Fenton, M., 1979. An improvement procedure for determination of chromic oxide in feed and feces. Can. J. Anim. Sci. 59, 631–634.
Hill, G.M., Mahan, D.C., Carter, S.D., Cromwell, G.L., Ewan, R.C., Harrold, R.L., Lewis, A.J., Miller, P.S., Shurson, G.C., Veum, T.L., 2001. Effect of pharmacological concentrations of zinc oxide with or without the inclusion of an antibacterial agent on nursery pig performance. J. Anim. Sci. 79, 934–941.
Hojberg, O., Canibe, N., Poulsen, H.D., Hedemann, M.S., Jensen, B.B., 2005. Influence of dietary zinc oxide and copper sulfate on the gastrointestinal ecosystem in newly weaned piglets. Appl. Environ. Microbiol. 71, 2267–2277.
Hu, C.H., Gu, L.Y., Luan, Z.S., Song, J., Zhu, K., 2012. Effects of montmorillonite–zinc oxide hybrid on performance, diarrhea, intestinal permeability and morphology of weanling pigs. Anim. Feed Sci. Technol. 177, 103–115.
Jang, I., Kwon, C.H., Ha, D.M., Jung, D.Y., Kang, S.Y., Park, M.J., Han, J.H., Park, B.C., Lee, C.Y., 2014. Effects of a lipid-encapsulated zinc oxide supplement on growth performance and intestinal morphology and digestive enzyme activities in weanling pigs. J. Anim. Sci. Technol 56, 29.
Jensen-Waern, M., Melin, L., Lindberg, R., Johannisson, A., Petersson, L., Wallgren, P., 1998. Dietary zinc oxide in weaned pigs – effects on performance, tissue concentrations, morphology, neutrophil functions and faecal microflora. Res. Vet. Sci. 64, 225–231.
Kaevska, M., Lorencova, A., Videnska, P., Sedlar, K., Provaznik, I., Trckova, M., 2016. Effect of sodium humate and zinc oxide used in prophylaxis of post-weaning diarrhoea on faecal microbiota composition in weaned piglets. Vet. Med. 61, 328–336.
Li, B.T., Van Kessel, A.G., Caine, W.R., Huang, S.X., Kirkwood, R.N., 2001. Small intestinal morphology and bacterial populations in ileal digesta and feces of newly weaned pigs receiving a high dietary level of zinc oxide. Can. J. Anim. Sci. 81, 511–516.
Milani, N.C., Sbardella, M., Ikeda, N.Y., Arno, A., Mascarenhas, B.C., Miyada, V.S., 2017. Dietary zinc oxide nanoparticles as growth promoter for weanling pigs. Anim. Feed Sci. Technol. 227, 13–23.
Moeser, A.J., Klok, C.V., Ryan, K.A., Wooten, J.G., Little, D., Cook, V.L., Blikslager, A.T., 2007. Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig. Am. J. Physiol. Gastr. Liver Physiol. 292, G173–G181.

Molist, F., Hermes, R.G., Gomez de Segura, A., Martın-Orue, S.M., Gasa, J., Manzanilla, E.G., Perez, J.F., 2011. Effect and interaction between wheat bran and zinc oxide on productive performance and intestinal health in post-weaning piglets. Br. J. Nutr. 105, 1592–1600.

Morales, J., Cordero, G., Piñeiro, C., Durosoy, S., 2012. Zinc oxide at low supplementation level improves productive performance and health status of piglets. J. Anim. Sci. 90, 436–438.

National Research Council, 2012. Nutrient Requirement of Swine, 11th rev ed. National Academy Press, Washington, DC.

Oropeza-Moe, M., Grøntvedt, C.A., Phythian, C.J., Sørum, H., Fauske, A.K., Framstad, T., 2017. Zinc oxide enriched peat influence *Escherichia coli* infection related diarrhea, growth rates, serum and tissue zinc levels in Norwegian piglets around weaning: five case herd trials. Porcine Health Manag. 3, 14. http://dx.doi.org/10.1186/s40813-017-0060-7.

Ou, D., Li, D., Cao, Y., Li, X., Yin, J., Qiao, S., Wu, G., 2007. Dietary supplementation with zinc oxide decreases expression of the stem cell factor in the small intestine of weanling pigs. J. Nutr. Biochem. 18, 820–826.

Owusu-Asiedu, A., Nyachoti, C., Marquardt, R., 2003. Response of early-weaned pigs to an enterotoxigenic *Escherichia coli* (K88) challenge when fed diets containing spray dried porcine plasma or pea protein isolate plus egg yolk antibody, zinc oxide, fumaric acid, or antibiotic. J. Anim Sci. 81, 1790–1798.

Pieper, R., Vahjen, W., Neumann, K., Van Kessel, A.G., Zentek, J., 2012. Dose-dependent effects of dietary zinc oxide on bacterial communities and metabolic profiles in the ileum of weaned pigs. J. Anim. Physiol. Anim. Nutr. (Berl.) 96, 825–833.

Sales, J., 2013. Effects of pharmacological concentrations of dietary zinc oxide on growth of post-weaning pigs: a meta-analysis. Biol. Trace Elem. Res. 152, 343–349.

Sargeant, H.R., Miller, H.M., Shaw, M., 2011. Inflammatory response of porcine epithelial IPEC J2 cells to enterotoxigenic E. coli infection is modulated by zinc supplementation. Mol. Immunol. 48, 2113–2121.

Schell, T., Kornegay, E., 1996. Zinc concentration in tissuesand performance of weanling pigs fed pharmacological levels of zinc from ZnO, Zn-methionine, Zn-lysine, or ZnSO4. J. Anim. Sci. 74, 1584–1593.

Shen, J., Chen, Y., Wang, Z., Zhou, A., He, M., Mao, L., Zou, H., Peng, Q., Xue, B., Wang, L., Zhang, X., Wu, S., Lv, Y., 2014. Coated zinc oxide improves intestinal immunity function and regulates microbiota composition in weaned piglets. Br. J. Nutr. 111, 2123–2134.

Slade, R.D., Kyriazakis, I., Carroll, S.M., Reynolds, F.H., Wellock, I.J., Broom, L.J., Miller, H.M., 2011. Effect of rearing environment and dietary zinc oxide on the response of grouphoused weaned pigs to enterotoxigenic Escherichia coli O149 challenge. Animal 5, 1170–1178.

Starke, I.C., Pieper, R., Neumann, K., Zentek, J., Vahjen, W., 2014. The impact of high dietary zinc oxide on the development of the intestinal microbiota in weaned piglets. FEMS Microbiol. Ecol. 87, 416–427.

Trckova, M., Lorencova, A., Hazova, K., Sramkova Zajacova, Z., 2015. Prophylaxis of post-weaning diarrhoea in piglets by zinc oxide and sodium humate. Vet. Med. 60, 351–360.

Vahjen, W., Pieper, R., Zentek, J., 2011. Increased dietary zinc oxide changes the bacterial core and enterobacterial composition in the ileum of piglets. J. Anim. Sci. 89, 2430–2439.

Walk, C.L., Srinongkote, S., Wilcock, P., 2013. Influence of a microbial phytase and zinc oxide on young pig growth performance and serum minerals. J. Anim Sci. 91, 286–291.

Wang, C., Xie, P., Liu, L.L., Dong, X.Y., Lu, J.J., Zou, T.X., 2012. Use of lower level of capsulated zinc oxide as an alternative to pharmacological dose of zinc oxide for weaned piglets. Asian J. Anim. Vet. Adv. 7, 1290–1300.

Yu, T., Zhu, C., Chen, S., Gao, L., Lv, H., Feng, R., Zhu, Q., Xu, J., Chen, Z., Jiang, Z., 2017. Dietary high zinc oxide modulates the microbiome of ileum and colon in weaned piglets. Front. Microbiol. http://dx.doi.org/10.3389/fmicb.2017.00825.

Zhu, C., Lv, H., Chen, Z., Wang, L., Wu, X., Zhang, W., Liang, R., Jiang, Z., 2017. Dietary zinc oxide modulates antioxidant capacity, small intestine development, and jejunal gene expression in weaned piglets. Biol. Trace Elem. Res. 175, 331–338.