Use of coated nano zinc oxide as an additive to improve the zinc excretion and intestinal morphology of growing pigs[1]

Miaomiao Bai, [†,‡] Hongnan Liu,[†,‖,§,2] Kang Xu,[†,‖,§] Chaoyue Wen,[†] Rong Yu,[‖] Jinping Deng,[‡] and Yulong Yin[†,‡,‖,§]

[†]Hunan Provincial Key Laboratory of Animal Nutritional Physiology and Metabolic Process; National Engineering Laboratory for Pollution Control and Waste Utilization in Livestock and Poultry Production; Key Laboratory of Agro-ecological Processes in Subtropical Region; Hunan Provincial Engineering Research Center for Healthy Livestock and Poultry Production; Scientific Observing and Experimental Station of Animal Nutrition and Feed Science in South-Central, Ministry of Agriculture, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, Hunan 410125, China; and [‡]College of Animal Science, South China Agricultural University, Guangzhou, Guangdong 510642, China; [‖] Hangzhou King Techina Technology Company Academician Expert Workstation, Hangzhou King Techina Technology Co., Ltd., Hangzhou 311107, China; [§]Hunan Co-Innovation Center of Safety Animal Production, CICSAP, Changsha, Hunan 410128, P.R. China

ABSTRACT: Two experiments were designed to explore the effects of coated zinc (**Zn**) oxide nanoparticles (**NZO**) on the diarrhea ratio, antioxidant capacity, intestinal morphology, and zinc excretion in growing pigs. In Exp.1, 270 growing pigs (21.88 ± 0.8 kg initial BW) were allocated to three treatments, each for 30 d: (i) control group (**CG**), basal diet containing Zn-free premix + 100 mg Zn/kg from $ZnSO_4$; (ii) high Zn (**HZN**), basal diet containing Zn-free premix + 2,250 mg Zn/kg from ZnO; (iii) coated nano ZnO (CNZO), basal diet containing Zn-free premix + 100 mg Zn/kg from coated NZO. In Exp.2, 21 crossbred growing pigs (17.04 ± 0.01 kg initial BW) were allocated to three treatments, each for 28 d: (i) HZN, basal diet containing Zn-free premix + 2,250 mg Zn/kg from ZnO; (ii) low concentration of nano ZnO (**LNZO**), basal diet containing Zn-free premix + 100 mg Zn/kg from 5% coated NZO material; (iii) high concentration of nano ZnO (**HNZO**), basal diet containing Zn-free premix + 100 mg Zn/kg from 10% coated NZO material. In Exp. 1, compared with the CG diet, CNZO significantly reduced the diarrhea rate ($P < 0.05$) and increased the activities of glutathione peroxidase and superoxide dismutase ($P < 0.05$). Compared with HZN, CNZO decreased the activities of serum alanine aminotransferase, and alkaline phosphatase, as well as the fecal zinc concentration ($P < 0.05$). In Exp. 2, pigs fed LNZO or HNZO had an increased final BW, average daily weigh and diarrhea rate, and a decreased level of Zn in the plasma, liver, and feces on day 14 compared with the HZN group ($P < 0.05$). The villous height and villous height/crypt depth ratio of duodenum were higher ($P < 0.05$) in the HZN group than the HNZO group, whereas the higher villous height of jejunum was observed in the LNZO group compared with that in the HNZO group ($P < 0.05$). We found that CNZO (100 mg/kg Zn) could improve the antioxidant capacity and reduce fecal Zn emission. However, the diarrhea rate was not effectively suppressed when compared with the HNZO supplementation. Furthermore, coated NZO material of 5% concentration is more effective in improving the morphology of intestinal villus.

Key words: growing pigs, intestinal morphology, zinc excretion, zinc oxide

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[2]Corresponding author: liuhn@isa.ac.cn

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INTRODUCTION

The diarrhea in growing pigs has been increased in incidence of outbreaks worldwide. Diarrhea results in a reduction of the normal growth rate by 27% to 50% in pigs (Campbell et al., 2013). An effective inhibitor for the occurrence of diarrhea is in high demand from the global swine industry.

Dietary zinc oxide (**ZnO**) at therapeutic concentrations from 2,000 to 4,000 mg/kg could effectively prevent and treat postweaning diarrhea (Shelton et al., 2011). A large dose of ZnO could increase pigs' growth performance and reduce the colonization and population of microbes in the gastrointestinal tract (Fairbrother et al., 2005; Cho et al., 2015). However, only 10% to 25% of dietary ZnO can be absorbed by pigs, which may lead to the excretion of mass heavy metals into the environment, resulting in environmental pollution. The maximum dose of Zn/kg in a pig's diet has been restricted to 150 mg in Europe (European Communities, 2003), which is far below ZnO therapeutic levels. The Ministry of Agriculture in China limited the supplementation of ZnO at 110 mg/kg for the diet of growing pigs. Thus, restricting the supplemented ZnO in piglet diets and strengthening the biological effect of ZnO have both attracted extensive attention from researchers.

Improvements in the production process, such as envelope and nanotechnology, may feature advantages for the efficiency of nutrients and additives (Bauer et al., 2004). Nanoparticles decrease the particle size (1 to 100 nm) of nutrients, which can then more readily cross gut barriers and have greater absorption and permeability rates (Florence et al., 1995; Buzea et al., 2017; Cho et al., 2013). Compared with ZnO, nano ZnO (**NZO**) has a higher bioavailability, significantly promotes animal growth and survival, and enhances immunity (Croteau et al., 2011; Sirelkhatim et al., 2015). Coating technology would be able to protect effective components from being degraded by stomach juices and released slowly into the intestine. However, scarce information is known about the effects of coated NZO on the growth performance of pigs. The present study was performed to validate the hypothesis that coated NZO, a new coating product, at a low supplementation level can substitute the high dose zinc (**Zn**) used for reducing the diarrhea rate and fecal Zn excretion and increasing the antioxidant capacity of growing pigs.

Different concentrations of coated NZO materials may affect the biological efficiency of supplementation. We conjectured that a higher concentration of effective coated NZO might cause an increase in the adsorptive behavior of surface ions, which directly results in negative effects on intestinal morphology and growth performance of pigs. Thus, this study also investigated the effects of different concentrations of coated NZO materials on the diarrhea rate and intestinal morphology of growing pigs.

MATERIALS AND METHODS

The Chinese Academy of Science Institutional Animal Care and Use Committee reviewed and approved the design and procedures in this experiment (Kong et al., 2007). Pigs in both experiments were housed in a finishing building equipped with a controlled environment. Dry feed and clean water were freely accessible.

Experiment 1

A total of 270 crossbred growing pigs (Duroc × Landrace × Yorkshire, castrated males), initially 21.88 ± 0.8 kg BW, were blocked by weight and randomly assigned into three dietary treatments. Each treatment contained 90 piglets arranged in six replicates of 15 piglets. Dietary treatments were: (i) control group (**CG**), basal diet containing Zn-free premix + 100 mg Zn/kg from $ZnSO_4$; (ii) HZN, basal diet containing Zn-free premix + 2,250 mg Zn/kg from ZnO; (iii) CNZO, basal diet containing Zn-free premix + 100 mg Zn/kg from coated NZO. All diets were formulated to meet or exceed the NRC (2012) requirements for growing pigs (Table 1). Coated NZO, containing 5% zinc oxide, was supplied by Hangzhou King Techina Technology Co., Ltd (Hangzhou, China). The experiment lasted for 30 d.

BW and feed intake were weighed at 1 and 30 d to determine the ADG, ADFI, and F/G ratio. During the experimental process, the fecal consistency was observed and recorded twice daily beginning on the first day. Meanwhile, the anal swelling and nature of the excrement were checked at least once a day for determining the diarrhea score of

each pig. The fecal score was recorded, as described by Marquardt et al. (2013), with a score from 0 to 3 (0: normal feces, 1: moist feces, 2: mild diarrhea, and 3: severe diarrhea). The daily diarrhea rate was calculated by counting pigs with a fecal score of 2 or higher. The diarrhea rate was calculated as the number of piglets with diarrhea/(total numbers of piglets × days) × 100. The collection of fecal samples from each pen was initiated on day 28 and ended on day 30, and stored at −20 °C for zinc-level analysis using a Varian ICP-OES (Model IRIS Intrepid II, Thermal Jarrell Ash, Waltham, MA).

On day 30, a randomly selected piglet from each pen was chosen. Blood samples were drawn from a precaval vein into vacuum tubes without EDTA. Serum was collected after centrifugation at 3,500 × *g* for 15 min at 4 °C, and the supernatant was stored at −20°C until analysis for biochemical indices. Serum levels of total protein (**TP**), albumin, aspartate aminotransferase, alanine aminotransferase (**ALT**), alkaline phosphatase (**ALP**), creatine phosphokinase, and urea nitrogen were detected by an automatic biochemical analyzer (Cobas311, F. Hoffmann-La Roche Ltd, Basel, Switzerland) and commercial kits (Roche, Basel, Switzerland) according to the manufacturers' instructions.

The level of globulin (**GLB**) in the serum was assayed by a specific sandwich ELISA kit (ELISA Ready-SET-GO, eBioscience, CA). The GLB index was standardized to the protein concentration in each sample.

Additionally, the serum antioxidant enzyme activities were analyzed with commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and determined using a multimode microplate reader (Infinite M200 PRO, Tecan, Switzerland) (Ren et al., 2012). The activities of the total-antioxidant capacity, glutathione peroxidase (**GSH-Px**), superoxide dismutase (**SOD**), and catalase (**CAT**), as well as the content of malondialdehyde (**MDA**) were determined according to the manufacturer's instructions.

Experiment 2

Assessment of initial content of coated zinc oxide. The ZnO content in the NZO materials was identified by titration according to the method of Shim et al. (Hunter, 1965). Briefly, 0.2 g of coated NZO materials was transferred to 250 mL conical flasks and mixed with 5 mL of 23.4% hydrochloric acid. The mixture was heated at 80 ℃ until completely dissolved, followed by the addition of 25 mL distilled water, one drip of 0.025% methyl red ethanol solution (0.025 g methyl red in 100 mL ethanol), and 40% ammonia solution until the dissolution became microscopic yellow. Then, the mixture was added to 25 mL distilled water, 10 mL ammonia–ammonium chloride buffer (pH 10.0), and 0.1 g eriochrome black T indicator. The content of ZnO was titrated with EDTA (0.05 mol/L) until the dissolution color change from purple to pure blue. One milliliter of EDTA (1.0 mol/L) is equivalent to 81.39 mg ZnO.

The calculation formula is as follows:

$$X\% = \frac{V \times C \times 0.08139}{m} \times 100\%$$

where X is the content of zinc oxide, V is the volume of EDTA titrant consumed by sample, mL, C is the concentration of EDTA titrant, mol/L, 0.08139 is the quality of zinc oxide that equivalent to 1 mL EDTA standard solution [C (EDTA) = 1.0 mol/L], g, m is the weight of sample, g.

Animal Experimental Design

A total of 21 Duroc × Landrace × Yorkshire growing pigs (castrated males), initially 17.04 ± 0.01 kg BW, were blocked by weight and randomly assigned into three dietary treatments. Each treatment contained seven piglets, arranged in seven replicates. Pigs were housed in individual pens for 28 d. Dietary treatments were: (i) HZN, basal diet containing Zn-free premix + 2,250 mg Zn/kg from ZnO; (ii) LNZO, basal diet containing Zn-free premix + 100 mg Zn/kg from 5% coated NZO material; (iii) HNZO, basal diet containing Zn-free premix + 100 mg Zn/kg from 10% coated NZO material. All diets were formulated to meet or exceed the NRC (2012) requirements for growing pigs (Table 2). The coated NZO materials were supplied by Hangzhou King Techina Technology Co., Ltd (Hangzhou, China).

The pigs' weight and feed intake were measured at 0, 14, and 28 d for calculating the ADG, ADFI, and F/G ratio. The diarrhea rate was monitored throughout the experiment. After 28 d, pigs were fasted overnight for 12 h before slaughter. Samples of plasma on 14 and 28 d, and feces on 28 d were collected as described for Exp. 1. After euthanasia, the abdominal cavity was opened quickly, and the duodenum, jejunum, and ileum were sheared into 2 cm segments, then fixed in phosphate-buffered paraformaldehyde (4%, pH 7.6) for histological measurements (Xiao et al., 2013a). The small intestinal samples were trimmed to a thickness of 5 to 6 μm. They were dehydrated in alcohol, embedded

in paraffin, and cut into 4 μm sections for staining with hematoxylin and eosin as described previously (Xiao et al., 2013b). Using light microscopy (Leica DMI3000 B, China), 10 microscopic fields were randomly selected from every animal and examined at 100× magnification for measuring the villus height and crypt depth (Tan et al., 2009). Additionally, the samples of serum (14 and 28 d), liver (28 d), and feces (28 d) were collected and analyzed for zinc levels according to the previous research (Zhang et al., 2017). Serum (5 mL), liver, and feces samples (5.00 ± 0.10 g) were weighed in triplicate and subjected to acid digestion using a mixture of nitric and perchloric acids following heating (80 °C for 60 min, 120 °C for 30 min, and 180 °C for 30 min). The samples were dried at 260 °C and redissolved in 5 mL of 1% HNO_3. Samples were then transferred to a 100-mL volumetric flask and diluted with 1% HNO_3. Subsequently, filtered solution of samples was subjected to ICP-OES for determining zinc levels.

Statistical Analysis

Each pen of pigs was the experimental unit for Exp. 1, and individual pig was the experimental unit for Exp. 2. Data were presented as means ± SEM. Results were analyzed statistically using one-way ANOVA of SPSS 23.0 (SPSS Inc., Chicago, IL). The normality of the diarrhea rate data was tested for by Kolmogorov–Smirnov's test. Differences among groups were examined using Duncan's multiple-range test, which were considered significant or a trend if the *P*-value was $P < 0.05$ or $0.05 < P \leq 0.10$.

RESULTS

Experiment 1

Growth performance and diarrhea rate. The effects of dietary coated NZO on the growth

Table 1. Diet composition for Exp. 1

	Diets[1]		
Item	CG	HZN	CNZO
Ingredient, kg/metric ton			
Corn	650.0	650.0	650.0
Soybean meal	250.0	250.0	250.0
Wheat bran	60.0	60.0	60.0
bran powder	7.83	5.03	7.995
Vitamin premix[2]	0.40	0.40	0.40
Mineral premix, Zn free[2]	1.24	1.24	1.24
Salt	3.60	3.60	3.60
Monocalcium phosphate	12.0	12.0	12.0
Phytase	0.40	0.40	0.40
Lysine	4.00	4.00	4.00
Limestone	9.60	9.60	9.60
Choline chloride	0.60	0.60	0.60
Antioxidants	0.04	0.04	0.04
$ZnSo_4$ H_2O	0.29	0.29	
ZnO		2.96	
NZO			0.125
Total	1,000	1,000	1,000
Calculated analysis			
DE, kcal/kg	3,161.45	3,152.51	3,154.80
CP, %	17.36	17.33	17.34
Ca, %	0.71	0.71	0.71
Total P, %	0.59	0.59	0.59
Available P, %	0.33	0.33	0.33
Lys, %	1.14	1.14	1.14
Met, %	0.28	0.28	0.28
Met + Cys, %	0.60	0.60	0.60
Zn, mg/kg	220	2470	220

[1]CG = control group, basal diet containing Zn-free premix + 100 mg Zn/kg from $ZnSO_4$; HZN = basal diet containing Zn-free premix + 2250 mg Zn/kg from ZnO; CNZO = basal diet containing Zn-free premix + 100 mg Zn/kg from coated nano zinc oxide.

[2]Provided per kilogram of diet: vitamin A, 1 750 IU; vitamin D_3, 200 IU; vitamin E, 11 IU; vitamin K, 0.5 mg; vitamin B_1 1.00 mg; vitamin B_2, 3.00 mg; vitamin B_6, 3.00 mg; biotin, 0.05 mg; folic acid, 0.30 mg; niacin acid, 30.00 mg; pantothenic acid, 300 mg; Cu ($CuSO_4 \cdot 5H_2O$), 5.00 mg; Fe ($FeSO_4 \cdot 7H_2O$), 100.00 mg; Mn ($MnSO_4 \cdot H_2O$), 3.00 mg; Se, 0.30 mg; I, 0.14 mg; Co, 0.12 mg.

Table 2. Diet composition for Exp. 2

Item	Diets[1]		
	HZN	LNZO	HNZO
Ingredient, kg/metric ton			
Corn	680.00	680.00	680.00
Soybean meal	280.00	280.00	280.00
bran powder	4.00	4.00	4.0625
Phytase	0.20	0.20	0.20
Vitamin premix[2]	0.30	0.30	0.30
Mineral premix, Zn free[2]	1.00	1.00	1.00
Limestone	12.00	12.00	12.00
Dicalcium phosphate	4.00	4.00	4.00
Choline	0.40	0.40	0.40
Salt	4.00	4.00	4.00
Lysine	2.00	2.00	2.00
Methionine	0.20	0.20	0.20
Others	0.98	0.98	0.98
Zeolite powder	8.67	10.8	10.8
ZnO	2.25	—	—
5% NZO	—	0.125	—
10% NZO	—	—	0.0625
Total	1,000	1,000	1,000
Calculated analysis			
DE, kcal/kg	3,213.78	3,213.78	3,214.92
CP, %	18.02	18.02	18.66
Ca, %	0.62	0.62	0.62
Total P, %	0.43	0.43	0.43
Available P, %	0.20	0.20	0.20
Lys, %	0.99	0.99	0.99
Met, %	0.32	0.32	0.32
Met + Cys, %	0.64	0.64	0.64

[1]HZN = basal diet containing Zn-free premix + 2,250 mg Zn/kg from ZnO; LNZO = basal diet containing Zn-free premix + 100 mg Zn/kg from 5% coated NZO material; HNZO = basal diet containing Zn-free premix + 100 mg Zn/kg from 10% coated NZO material.

[2]Provided per kilogram of diet: vitamin A, 1 750 IU; vitamin D_3, 200 IU; vitamin E, 11 IU; vitamin K, 0.5 mg; vitamin B_1 1.00 mg; vitamin B_2, 3.00 mg; vitamin B_6, 3.00 mg; biotin, 0.05 mg; folic acid, 0.30 mg; niacin acid, 30.00 mg; pantothenic acid, 300 mg; Cu ($CuSO_4 \cdot 5H_2O$), 5.00 mg; Fe ($FeSO_4 \cdot 7H_2O$), 100.00 mg; Mn ($MnSO_4 \cdot H_2O$), 3.00 mg; Se, 0.30 mg; I, 0.14 mg; Co, 0.12 mg.

Table 3. Effects of dietary coated nano ZnO on the growth performance and diarrhea rate of growing pigs (Exp. 1)

Item	Diets[1]			*P*-value
	CG	HZN	CNZO	
Initial BW, kg	21.90 ± 1.65	21.62 ± 0.91	21.52 ± 0.60	0.971
Final BW, kg	34.83 ± 1.44	37.07 ± 1.16	33.76 ± 1.27	0.245
ADG, g/d	438.55 ± 22.91	506.91 ± 34.19	431.96 ± 17.20	0.105
ADFI, kg/d	1.12 ± 0.06	1.30 ± 0.03	1.15 ± 0.05	0.066
F/G ratio	2.29 ± 0.15	2.44 ± 0.18	2.55 ± 0.24	0.631
Diarrhea rate, %	83.39 ± 3.37[c]	39.27 ± 3.04[a]	65.65 ± 4.17[b]	<0.01

Data are expressed as means ± SEM ($n = 6$). Means within a row with different superscripts are significantly different ($P < 0.05$).

[1]CG = control group, basal diet containing Zn-free premix + 100 mg Zn/kg from $ZnSO_4$; HZN = basal diet containing Zn-free premix + 2,250 mg Zn/kg from ZnO; CNZO = basal diet containing Zn-free premix + 100 mg Zn/kg from coated nano zinc oxide.

performance and diarrhea rate of growing pigs are shown in Table 3. Compared with the CG group, HZN tended to increase the ADFI ($0.05 < P \leq 0.10$). The diarrhea rate showed a non-normal distribution (K-S test). Both HZN and CNZO treatments significantly reduced the diarrhea rate of pigs by 52.91% ($P < 0.05$) and 21.27% ($P < 0.05$), respectively, compared with the CG group.

Serum biochemical parameters and antioxidant indexes. Pigs fed the HZN diet had a higher serum ALP ($P < 0.05$) compared with the CG and CNZO diets (Table 4). Compared with CNZO, HZN had a significant trend of an increased serum ALT level ($0.05 < P \leq 0.10$).

Compared with CG, HZN showed a growing tendency for the high activity of CAT in serum ($0.05 < P \leq 0.10$). The activity of GSH-Px increased ($P < 0.05$) in HZN and CNZO-fed pigs compared with the CG group. Pigs fed CNZO had a higher activity of serum SOD ($P < 0.05$) compared with the CG and HZN groups.

Fecal zinc level. The fecal zinc level is presented in Figure 1. Compared with the CG and CNZO groups, the HZN group showed a remarkable increase in the excretion of Zn ($P < 0.05$).

Experiment 2

Coated zinc oxide content. Two different concentrations of coated NZO materials were analyzed in this study. Assay results showed that the contents of ZnO in 5% coated NZO and 10% coated NZO materials were 5.13 ± 0.000% and 10.09±0.001%, respectively. These materials were confirmed to be in accordance with the experimental requirements.

Growth performance. Table 5 shows the effects of dietary coated NZO materials from different ZnO concentrations on the growth performance and diarrhea rate. Pigs fed LNZO or HNZO had a higher ($P < 0.05$) BW on day 28 compared with those fed an HZN diet. From days 1 to 14, pigs fed LNZO or HNZO had higher ($P < 0.05$) ADG and

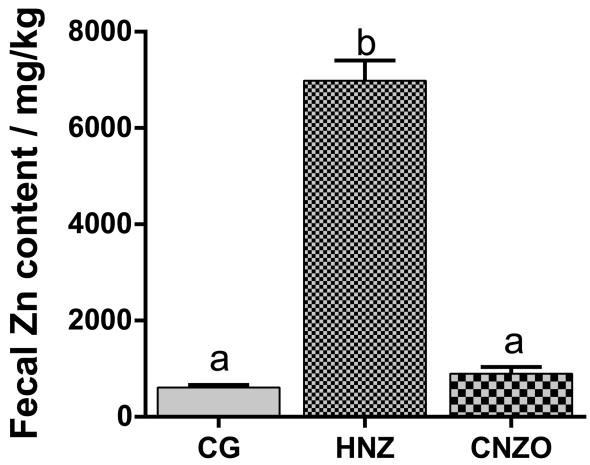

Figure 1. Effect of dietary coated nano ZnO on fecal zinc levels in growing pigs. CG = control group, basal diet containing Zn-free premix + 100 mg Zn/kg from $ZnSO_4$; HZN = basal diet containing Zn-free premix + 2,250 mg Zn/kg from ZnO; CNZO = basal diet containing Zn-free premix + 100 mg Zn/kg from coated nano zinc oxide. Data are expressed as means ± SEM ($n = 6$). Means within a row with different superscripts are significantly different ($P < 0.05$).

Table 4. Effects of dietary coated nano ZnO on serum biochemical parameters and antioxidant indexes in growing pigs (Exp. 1)

	Diets[1]			
Item[2]	CG	HZN	CNZO	*P*-value
Biochemical parameters				
TP, g/L	62.50 ± 1.91	62.86 ± 1.38	61.02 ± 1.92	0.75
Glucose, mmol/L	3.21 ± 0.48	2.59 ± 0.64	2.88 ± 0.43	0.707
ALT, U/L	49.92 ± 5.22	59.52 ± 2.94	46.80 ± 1.43	0.076
GLB, g/L	35.67 ± 2.10	35.00 ± 1.56	32.33 ± 1.98	0.345
ALP, U/L	164.43 ± 12.07[a]	284.18 ± 21.01[b]	206.58 ± 30.16[a]	0.008
AST, U/L	62.53 ± 12.10	76.72 ± 9.76	78.73 ± 10.77	0.533
ALB, g/L	26.90 ± 0.83	27.82 ± 0.76	28.53 ± 1.32	0.531
CK, U/L	540.84 ± 52.62	1288.46 ± 361.43	1120.98 ± 272.15	0.151
Urea, mmol/L	3.88 ± 0.37	5.20 ± 0.51	4.75 ± 0.59	0.206
Antioxidant indexes				
T-AOC, U/mL	0.02 ± 0.001	0.03 ± 0.004	0.03 ± 0.002	0.133
CAT, U/mL	67.45 ± 1.80	123.59 ± 1.11	79.78 ± 1.57	0.076
SOD, U/mL	22.71 ± 0.34[a]	23.05 ± 0.30[a]	25.77 ± 0.37[b]	0.022
GSH-Px, U/mL	431.78 ± 9.78[a]	498.00 ± 5.76[c]	469.92 ± 2.46[b]	<0.001
MDA, nmol/mL	12.12 ± 1.42	11.16 ± 1.38	5.88 ± 0.55	0.546

Data are expressed as means ± SEM ($n = 6$). Means within a row with different superscripts are significantly different ($P < 0.05$).

[1]CG = control group, basal diet containing Zn-free premix + 100 mg Zn/kg from $ZnSO_4$; HZN = basal diet containing Zn-free premix + 2250 mg Zn/kg from ZnO; CNZO = basal diet containing Zn-free premix + 100 mg Zn/kg from coated nano zinc oxide.

[2]TP = total protein; ALB = albumin; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; CK = creatine phosphokinase; GLB = globulin; T-AOC = total antioxidant capacity; CAT = catalase; SOD = superoxide dismutase; GSH-Px = glutathione peroxidase; MDA = malondialdehyde.

Table 5. Effects of different concentrations of coated nano ZnO materials on the growth performance and diarrhea rate in growing pigs (Exp. 2)

	Diets[1]			
Item	HZN	LNZO	HNZO	*P*-value
BW, kg				
Day 1	17.05 ± 0.49	17.04 ± 0.62	17.04 ± 0.45	1
Day 14	21.13 ± 0.25	22.53 ± 0.78	22.81 ± 0.50	0.133
Day 28	25.23 ± 0.89[a]	28.86 ± 0.97[b]	28.09 ± 0.45[b]	0.015
Days 1 to 14				
ADG, g	338.46 ± 29.75[a]	437.18 ± 17.40[b]	429.49 ± 20.47[b]	0.001
ADFI, kg	1.04 ± 0.000	1.04 ± 0.000	1.04 ± 0.000	1
F/G ratio	3.11 ± 0.001[b]	2.38 ± 0.001[a]	2.44 ± 0.001[a]	0.001
Diarrhea rate, %	5.95 ± 1.94[a]	29.93 ± 2.65[b]	23.43 ± 1.89[b]	<0.01
Days 14 to 28				
ADG, g	497.22 ± 73.12	581.94 ± 73.90	439.28 ± 38.52	0.132
ADFI, kg	1.24 ± 0.06[b]	1.19 ± 0.02[ab]	1.13 ± 0.01[a]	0.037
F/G ratio	2.54 ± 0.74	2.13 ± 0.52	2.69 ± 0.22	0.192
Diarrhea rate, %	11.90 ± 0.74[a]	53.06 ± 3.24[b]	52.03 ± 2.99[b]	<0.01
Days 1 to 28				
ADG, g	354.40 ± 40.77[a]	472.57 ± 31.29[b]	441.71 ± 14.99[b]	0.032
ADFI, kg	1.08 ± 0.03	1.11 ± 0.01	1.08 ± 0.009	0.589
F/G ratio	3.18 ± 0.45[b]	2.40 ± 0.15[a]	2.45 ± 0.08[a]	0.022
Diarrhea rate, %	8.89 ± 0.98[a]	41.50 ± 2.56[b]	37.73 ± 3.33[b]	<0.01

Data are expressed as means ± SEM (n = 7). Means within a row with different superscripts are significantly different ($P < 0.05$).

[1]HZN = basal diet containing Zn-free premix + 2250 mg Zn/kg from ZnO; LNZO = basal diet containing Zn-free premix + 100 mg Zn/kg from 5% coated NZO material; HNZO = basal diet containing Zn-free premix + 100 mg Zn/kg from 10% coated NZO material.

diarrhea rates, whereas the F/G ratio was decreased ($P < 0.05$) compared with the HZN diet. From days 14 to 28, pigs fed HZN had a higher ADFI than the HNZO group ($P < 0.05$). Compared with the HZN diet, both LNZO and HNZO had significantly increased ($P < 0.05$) diarrhea rates. Overall (days 1 to 28), pigs fed LNZO and HNZO had a better ADG ($P < 0.05$), a higher diarrhea rate ($P < 0.05$), and a lower F/G ratio ($P < 0.05$) compared with the HZN diet.

Morphology of the small intestine. The villous was arranged more closely and tightly in duodenum, jejunum, and ileum in the pigs fed with the HZN diet compared with the NZO diet, as shown in Figure 2. In the HNZO group, damage to all of the small intestine (duodenum, jejunum, and ileum) structure appeared, which reduced with digestion time. However, the villous was higher and the extent of injury was relatively lower when pigs were fed an LNZO diet as opposed to the HNZO diet.

The measured data of villous height, crypt depth, and villous height/crypt depth of the small intestine are shown in Table 6. The HZN diet increased the villous height ($P < 0.05$) of the duodenum in pigs, compared with the LNZO and HNZO groups. There was a significant trend ($0.05 < P \leq 0.10$) on the villous height/crypt of the duodenum and villous heights of the jejunum depth. No significant effect on the ileum among the three treatments.

Tissues and fecal zinc levels. The content of zinc in the serum, liver, and feces of growing pigs fed dietary coated NZO materials from different ZnO concentrations is shown in Figure 3. Compared with the LNZO and HNZO treatments, dietary supplementation with HZN remarkably increased the Zn levels in the plasma on day 14 ($P < 0.05$), and improved Zn contents in liver and feces on day 28 ($P < 0.05$).

DISCUSSION

Experiment 1

Many factors, including the different growth stages of the pigs, administration periods, housing conditions, and climatic factors, may influence the effects of ZnO supplementation (Miller et al., 2009). Recently, NZO is becoming widely applicated in feed additives because of its high stability and antibacterial activity. Milani et al. (2017) proved that dietary supplementation of 60 mg/kg of zinc oxide nanoparticles could improve the immunity and growth performance of piglets. A diet with 800 mg/kg of nano ZnO increased the ADG and decreased the diarrhea rate in weaned piglets (Wang et al.,

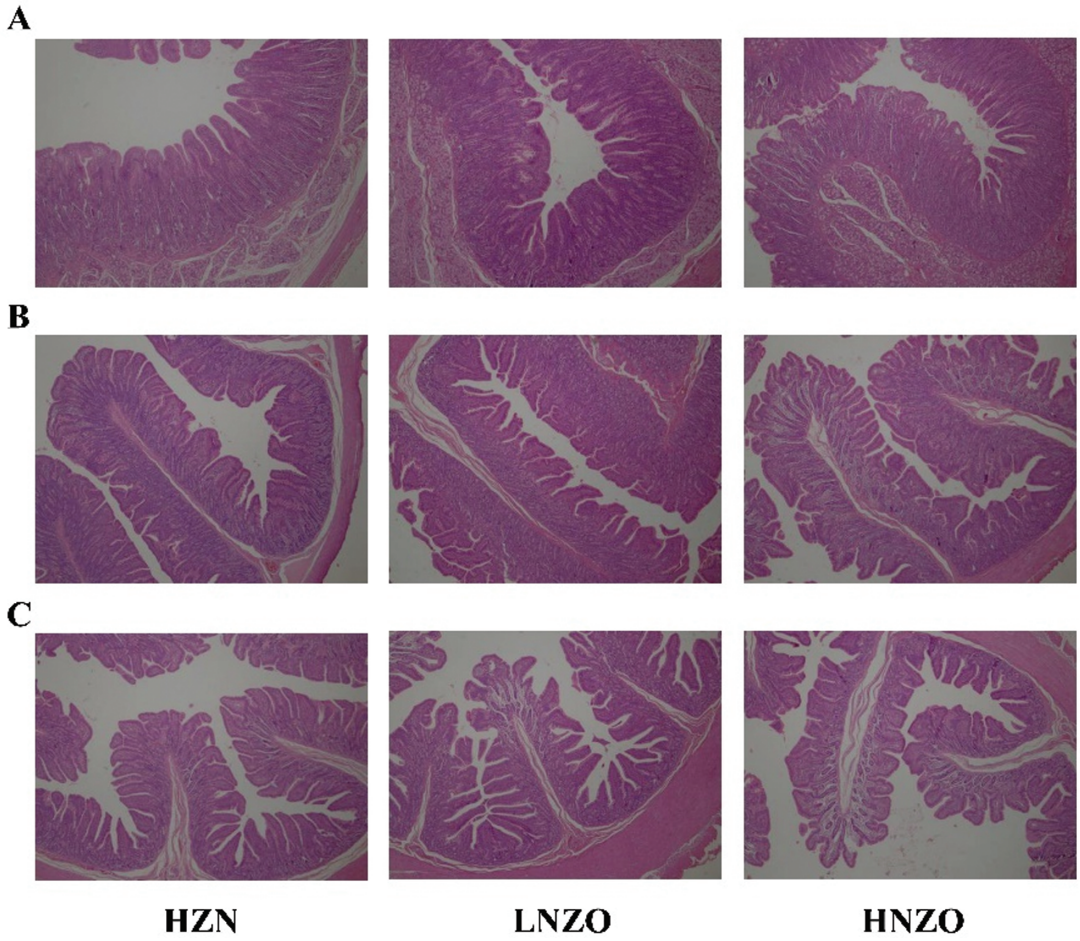

Figure 2. Hematoxylin and eosin stained sections of the intestine (200 nm). (A) Duodenum, (B) Jejunum, (C) Ileum. HZN = basal diet containing Zn-free premix + 2,250 mg Zn/kg from ZnO; LNZO = basal diet containing Zn-free premix + 100 mg Zn/kg from 5% coated NZO material; HNZO = basal diet containing Zn-free premix + 100 mg Zn/kg from 10% coated NZO material.

Table 6. Effects of different concentrations of coated nano ZnO materials on the morphology of the small intestine of growing pigs (Exp. 2)

	Diets[1]			
Item	HZN	LNZO	HNZO	*P*-value
Duodenum				
Villous height, μm	418.89 ± 15.90[b]	397.96 ± 17.60[ab]	342.53 ± 21.00[a]	0.031
Crypt depth, μm	515.00 ± 29.07	525.38 ± 44.02	509.24 ± 27.37	0.942
Villous height/Crypt depth	0.86 ± 0.04	0.80 ± 0.06	0.69 ± 0.04	0.065
Jejunum				
Villous height, μm	379.75 ± 13.84	411.58 ± 11.28	363.72 ± 16.63	0.064
Crypt depth, μm	301.05 ± 24.46	288.31 ± 8.24	290.69 ± 19.37	0.865
Villous height/Crypt depth	1.34 ± 0.12	1.50 ± 0.05	1.31 ± 0.09	0.237
Ileum				
Villous height, μm	406.56 ± 20.72	433.98 ± 16.42	380.10 ± 26.60	0.252
Crypt depth, μm	224.17 ± 16.16	241.51 ± 17.42	195.71 ± 24.73	0.293
Villous height/crypt depth	1.96 ± 0.17	1.99 ± 0.20	1.97 ± 0.11	0.994

Data are expressed as means ± SEM (n = 7). Means within a row with different superscripts are significantly different ($P < 0.05$).

[1] HZN = basal diet containing Zn-free premix + 2250 mg Zn/kg from ZnO; LNZO = basal diet containing Zn-free premix + 100 mg Zn/kg from 5% coated NZO material; HNZO = basal diet containing Zn-free premix + 100 mg Zn/kg from 10% coated NZO material.

2018). Compared with the high dose of ZnO, dietary NZO also had a good digestive physiology and strong antibacterial activity against *Escherichia coli K88* (Trckova et al., 2015). These results suggest that NZO at a low supplementation level could potentially substitute the pharmacological dose of

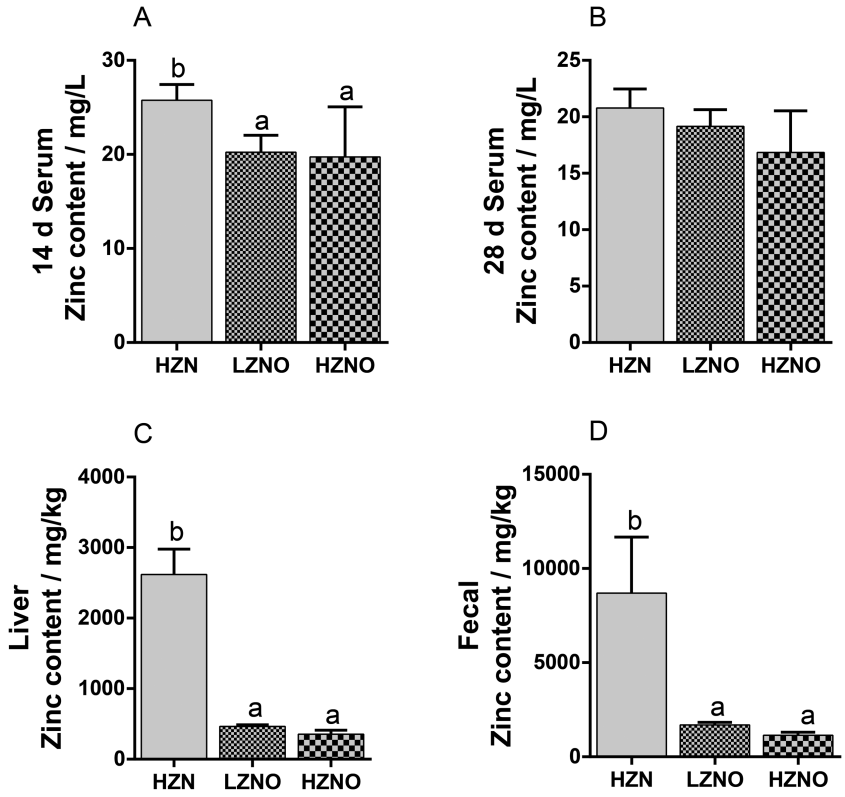

Figure 3. Effects of dietary coated nano zinc oxide materials with different concentrations on the zinc contents in growing pigs. (A) the content of zinc in the serum on day 14, (B) the content of zinc in the serum on day 28, (C) the content of zinc in the liver, (D) the content of zinc in the feces. HZN = basal diet containing Zn-free premix + 2,250 mg Zn/kg from ZnO; LNZO = basal diet containing Zn-free premix + 100 mg Zn/kg from 5% coated NZO material; HNZO = basal diet containing Zn-free premix + 100 mg Zn/kg from 10% coated NZO material. Data are expressed as means ± SEM (n = 7). Means within a row with different superscripts are significantly different ($P < 0.05$).

ZnO for improving the growth performance and decreasing the incidence of diarrhea. Compared with previous reports, we found that dietary supplementation of coated nano ZnO (100 mg/kg Zn) could decrease the diarrhea rate by 21%, but the effect was not as strong as in the high-ZnO diet. Furthermore, we sought to explore its possible mechanism in controlling diarrhea in the growing pig model.

Biochemical indicators of serum are an important index for indicating biological processes. We found that pigs fed the high dose of ZnO (HZN diet) had higher ALP and ALT activities than those fed a coated NZO diet. This finding was consistent with Cho et al. (2015), who reported that increasing the absorption of Zn could increase the ALP activity in serum. Zinc, as a cofactor, had direct stimulatory effects on ALP and bone mineralization (Sebahat et al., 2005; Érika Dantas de Medeiros et al., 2015). However, the ALT level, as a critical indictor of liver disease, escapes from injured hepatic cells into the plasma. Thus, the increase in the ALT and ALP activities in the serum may explain the damaging influence on liver cells of high-Zn-treated pigs. Additionally, the adverse effects due to the toxicity of high Zn diets have been reported by Starke et al. (2014). On the contrary, a low dietary dose of coated NZO may be more beneficial to physical health.

Furthermore, the antioxidant capacity of growing pigs is fundamental for maintaining the normal metabolic state to protect a pig's health, we hypothesized that the effects of dietary NZO could promote growth by indirectly regulating the antioxidant capacity of pigs. It has been confirmed that zinc contributes to regulating the redox status and maintaining the integrity of cell membranes (Prasad, 2008). High doses of ZnO (3000 mg/kg) supplementation reduced the serum MDA concentration and increased the T-SOD activity in piglets (Zhu et al., 2017). Our findings also show that pigs fed a high dose of ZnO had an improved antioxidant capacity (CAT and GSH-Px activities). Meanwhile, a low dose of coated NZO could increase the activities of SOD and GSH-Px in the serum. Generally, the SOD, CAT, and GSH-Px are key parameters reflecting the status of the antioxidant capacity in plasma. Zinc acts as an essential component in Cu-Zn-SOD, which was positively correlated with the dietary Zn level (Fathi et al., 2016). Here, a low

supplementation level of coated NZO had a positive effect on Cu-Zn-SOD activity, while a high dose of Zn had no effect on Cu-Zn-SOD activity. It indicates that high dose of Zn may play an antagonism role in the absorption of Cu and enzyme synthesis such as Cu-Zn-SOD. As Zhao et al. (2014) reported, only the appropriate level of NZO could increase Cu-Zn-SOD activity, thereby improving the antioxidant capacity. Dietary supplementation with lipid matrix-coated ZnO could improve the antioxidant capacity of weaning piglets (Upadhaya et al., 2018). As a result, we conclude that lower doses of coated NZO (100 mg Zn/kg) could be used in substitution for the high doses of ZnO, to enhance the antioxidant capacity of growing pigs.

Although a pharmacological dose of ZnO is widely used to decrease the incidence of diarrhea and improve the growth performance in piglets, more attention should paid to the environmental pollution caused by excessive Zn residues. Fecal excretion of Zn was positively related to the concentrations of supplemented Zn in the diet (Carlson et al., 2004). As observed in the present study, the decreased fecal Zn indicated that replacing high ZnO in the diet with coated NZO was beneficial to reduce the environmental pollution. This is consistent with Upadhaya et al.'s (2018) findings, which showed that using lower doses of lipid matrix-coated ZnO (<1,000 mg/kg) could reduce the excretion of Zn compared with the conventional high dose of Zn. The accumulation of Zn has been documented as being closely related to tissue damage (Wang et al., 2017). As suggested by the present study, compared with the high zinc diet, a low concentration of coated NZO (100 mg Zn/kg) in the diet is more suitable for alleviating diarrhea and reducing fecal zinc emissions of growing pigs.

Experiment 2

ZnO in nanosize presents strong bioactivity at low concentrations due to their high surface area to volume ratio and unique chemical and physical properties (Rai et al., 2009). The present study verified the effects of coated NZO materials with different concentrations on growing pigs. In this study, both low (5%) and high (10%) concentrations of coated NZO materials improved the final BW, ADG, and feed conversion rate for the whole trial period. However, the supplementation of coated NZO materials with different concentrations is not as good as a high concentration of Zn, in reducing the occurrence of diarrhea. This is explained due to an increase in the concentration nanoparticles, which result in the increased surface area. A lager surface area will result in a greater antimicrobial activity (Espitia et al., 2012). Padmavathy and Vijayaraghavan (2008) also suggest that the abrasive surface of ZnO nanoparticles in lager concentration could cause mechanical damage to the cell membrane. Different concentrations of coating techniques, applied in the feeding additives, might resulted in the variations of stability, absorption rates, and antimicrobial activity. Thus, more related studies are need to support this hypothesis.

The intestinal morphology plays an important role in the uptake of nutrients and the occurrence of diarrhea (Lee et al., 2013). Changes in the intestinal morphology, including villus atrophy and crypt hyperplasia, are associated with the malabsorption and growth inhibition of pigs (Xiong et al., 2016). Previous researchers have shown that a high dietary zinc treatment could improve the morphology of the small intestine (Li et al., 2006). In agreement with these studies, the present study demonstrates that pigs supplemented with a high level of ZnO had an improved intestinal morphology. Thus, the mechanism for high ZnO inhibiting diarrhea is partly due to the improvement of the damage to the intestinal morphology of pigs. The supplementation with coated NZO at 5% concentration showed a lower intestinal injury and higher villus height than that at 10%. This might be related with the coating materials used for the high concentration supplementation, which can easily cause compaction, thereby damaging the gastrointestinal tract. The mechanisms of coated NZO on the intestinal barrier and integrity need to be further explored.

Also, pigs fed on a diet with 5% NZO or 10% NZO (100 mg Zn/kg) had markedly reduced concentrations of Zn in the plasma, liver, and feces on d 14 compared with the high-Zn group. The possible explanation for the reduction in fecal excretion of Zn had no relation to the coating technology or source, which was directly affected by the low dose of Zn (Shen et al., 2014). However, the mechanisms for the intestinal transport and absorption of zinc should be further investigated.

In summary, the present research indicates that in growing pigs, dietary-coated NZO (100 mg/kg Zn) supplementation improves the antioxidant capacity and reduces the fecal Zn emission without a negative effect on the growth performance. Additionally, 5% coated NZO material caused less intestinal injury compared with 10% coated NZO material. Reducing ZnO using coated NZO improved the growth performance, but the diarrhea rate was not as effectively suppressed compared

with the high ZnO supplementation. In addition to up to 100 mg/kg of coated NZO, the control of diarrhea in piglets also requires the assistance of functional additives.

Conflict of interest statement. No conflicts of interest to this work.

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