Effect of nano zinc oxide or chelated zinc as alternatives to medical zinc oxide on growth performance, faecal scores, nutrient digestibility, blood profiles and faecal *Escherichia coli* and *Lactobacillus* concentrations in weaned piglets

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**ABSTRACT**

A total of 144 weaned piglets (6.80 ± 0.46 kg, [Landrace × Yorkshire] × Duroc) were used in a 4-week feeding trial to determine the effect of nano zinc oxide or chelated zinc as alternatives to medical zinc oxide (ZnO) in weaned piglets. The pigs were randomly distributed to one of 6 treatment groups (4 pigs in a pen, 6 replicate pens per treatment). The dietary treatments were negative control (NC), no additional zinc oxide in the diet; positive control (PC), NC + 2500 mg/kg ZnO; 100 mg Chelate (NC + 100 mg/kg ZnO chelate); 200 mg Chelate (NC + 200 mg/kg of zinc chelate); 100 mg Nano (NC + 100 mg/kg ZnO nanoparticles), and 200 mg Nano (NC + 200 mg/kg nanoparticles). The growth performance of pigs fed the PC and Nano diets were significantly higher than that of pigs in the Chelate treatment groups \((p<.05)\). In the overall periods, the faecal scores were significantly increased \((p<.05)\) in the PC treatment group compared to the 100 mg/kg Chelate and Nano treatment groups. The apparent total tract digestibility (ATTD) of crude protein and faecal *Lactobacillus* concentrations were significantly decreased \((p<.05)\) in the NC group compared to the other groups. The ATTD of zinc was significantly increased \((p<.05)\) in the Chelate treatment groups compared to the other treatment groups. In conclusion, 200 mg/kg Nano showed similar effects on growth performance, nutrient digestibility, and faecal *Lactobacillus* concentrations in weaning pigs as medical ZnO.

**HIGHLIGHTS**

- To evaluate the effects of nano zinc oxide or chelated zinc as an alternative to medical zinc oxide in weaned piglets
- High-dose zinc oxide and 200 mg/kg zinc oxide nanoparticle supplementation showed beneficial effects on growth performance and crude protein digestibility
- Supplementation with zinc chelated with glycine resulted in significantly higher \((p<.01)\) the ATTD of zinc than other treatments groups.
- Faecal *Lactobacillus* concentrations were significantly decreased \((p<.05)\) in the no additional zinc oxide diet group compared to the other treatment groups.
- Nano-ZnO at 100 or 200 mg/kg had similar effects on growth performance and nutrient digestibility with lower zinc excretion than that of weaned pigs given medical zinc oxide.

**Introduction**

Piglets face a variety of stress factors (separation from sows, socialisation due to group feeding, dietary changes, histological changes in the intestine, etc.) after weaning (Fairbrother et al. 2005). These stresses have negative effects on the incidence of post-weaning diarrhoea (PWD), which can lead to decreases in growth performance and the death of piglets, resulting in economic losses in the swine industry (Rhouma et al. 2017). To address this, Poulsen (1998) suggested...
that supplementation with 2500 mg/kg of zinc oxide (ZnO) could prevent PWD and improve growth performance in weaned piglets. Many researchers reported that dietary pharmacological levels (2000–3000 mg/kg) of ZnO fed to weaned piglets could prevent PWD, oxidative stress, growth retardation, and villus atrophy (Wijtten et al. 2011; Kim et al. 2012; Xia et al. 2017). However, high levels of ZnO are mostly eliminated in the faeces, causing environmental pollution, and increasing antimicrobial resistance (Grilli et al. 2015; Satessa et al. 2020). For this reason, supplementation with ZnO for pharmacological use in weaning diets is limited to 150 mg/kg and was planned to be phased out by 2022 in Europe (Commission Implementing Decision of 26.6.2017, C (2017) 4, 529 Final). In the Asian market, ZnO is limited to 1600 mg/kg in China, and the zinc concentration of compost is limited to 1200 mg/kg in South Korea (Wei et al. 2021; Ahn et al. 2021). For a sustainable swine industry, the development of alternatives that can lead to high production efficiency with lower zinc content is needed.

Organic zinc can bond to amino acids, peptides, and proteins with higher bioavailability than inorganic zinc (Oberleas and Harland 1981). Mullan and D’Souza (2005) reported that organic zinc could improve absorption through the amino acid or peptide transport system and precipitation in the small intestine. Organic zinc can alleviate heavy metal contamination in the soil through high absorption and low excretion (Oh et al. 2021). Thus, it can be an alternative (Wedekind et al. 1992). Nanoparticle-sized zinc (nano-ZnO) is inorganic zinc with a particle size of 1–100 nm, which has a large surface area, is partly dissolvable in gastric fluid, and has limited solubility in neutral environments (Donaldson et al. 2013; Liu et al. 2016; Xia et al. 2017). These properties can increase the absorption rate of zinc ions at low levels with high bioavailability through its intact particle shape (An et al. 2014). Similarly, in our previous study, pigs fed 200 mg/kg organic zinc and nano-ZnO had higher bioavailability than pigs fed 2500 mg/kg ZnO (Oh et al. 2021). The objective of this study was to evaluate the effects of nanoparticle-sized zinc oxide or zinc chelated with glycine on the growth performance, faecal scores, nutrient digestibility, zinc utilisation, blood profiles, and faecal Escherichia coli and Lactobacillus concentrations in weaned piglets and determine whether zinc could be used as an alternative to medical zinc oxide.

Materials and methods

The experimental protocols describing the management and care of animals were reviewed and approved by the Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea (approval#CNUA-1421-20-02).

Animals, facilities and dietary treatments

A total of 144 weaned piglets [(Yorkshire x Landrace) x Duroc] with initial body weight (BW) of 6.8 ± 0.46 kg were used in a 4-weeks experiment. Pigs were blocked based on initial body weight into a complete block design. There were four pigs in a pen and six replicate pens per treatment. All pigs were housed in an environmentally controlled room (30 ± 1°C). Each pen was equipped with a one-sided, stainless self-feeder and a nipple drinker. Dietary treatments were: Negative control (NC), no additional added zinc oxide in diet; Positive control (PC), NC + 2500 mg/kg zinc oxide; Chelate, NC + 100 or 200 mg/kg of zinc chelated with glycine; Nano, NC + 100 or 200 mg/kg nano-ZnO. The diets were fed during the experiment in 2 phases: days 0–14, and 14–28 (Table 1). The Zn chelated with glycine (containing 27% of Zn) was supported by Dr.Eckel Animal Nutrition GmbH & Co. KG (Anta® min; Niederzissen, Germany). The nano-ZnO was offered by Souza.

Table 1. Compositions of the basal weanling diets (as-fed basis).

| Items                      | 0–14 days | 14–28 days |
|----------------------------|-----------|------------|
| Ingredients, %             |           |            |
| Corn                       | 34.43     | 60.81      |
| Extruded corn              | 15.00     | 5.00       |
| Lactose                    | 10.00     | 3.00       |
| Dehulled soybean meal, 51% CP | 13.50     | 13.00      |
| Soy protein concentrate, 65% CP | 10.00     | 10.00      |
| Plasma powder              | 6.00      | 3.00       |
| Whey                       | 5.00      |            |
| Soy oil                    | 2.20      | 2.00       |
| Monocalcium phosphate      | 1.26      | 1.15       |
| Limestone                  | 1.40      | 0.99       |
| L-Lysine-HCl, 78%          | 0.06      | 0.11       |
| DL-Methionine, 50%         | 0.15      | 0.04       |
| Choline chloride, 25%      | 0.10      | 0.10       |
| Vitamin premixa            | 0.25      | 0.25       |
| Trace mineral premixa      | 0.25      | 0.25       |
| Salt                       | 0.40      | 0.30       |
| Total                      | 100.00    | 100.00     |
| Analysed value             |           |            |
| ME, kcal/kg                | 3462.21   | 3398.40    |
| CP, %                      | 20.70     | 18.67      |
| Lysine, %                  | 1.34      | 1.13       |
| Methionine, %              | 0.40      | 0.32       |
| Ca                         | 0.82      | 0.68       |
| P                          | 0.62      | 0.63       |

Abbreviation: CP: crude protein; ME, metabolizable energy; Ca, calcium; P, phosphorus.

a Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D₃, 1103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic, 29 mg; choline, 166 mg; and vitamin B₁₂, 33 μg.

b Provided per kg of complete diet without Zinc: Cu (as CuSO₄•5H₂O), 12 mg; Mn (as MnSO₄•5H₂O), 8 mg; I (as KI), 0.28 mg; and Se (as Na₂SeO₃•5H₂O), 0.15 mg.
Animine Co., Ltd (HiZox®, Sillingy, France). All diets, in pelleted form, were formulated to meet or exceed the nutrient requirements (National Research Council 2012) for pigs. Feed and water were available *ad libitum*.

**Performance and fecal score**

Feed intake was recorded at 08:00 h and 17:00 h every day, individual pig BW was recorded at the end of weeks 0, 2 and 4 to determine average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (G:F) ratio. The subjective faecal scores were individually recorded at 09:00 and 18:00 by the same person on days 0–28 post-weaning. The faecal score was assigned as follows: 0, diarrhoea; 1, sloppy faeces; 2 normal faeces; and 3, well-formed faeces. Scores were calculated as the average faecal score for each period (0–14 days; 14–28 days; overall period, 0–28 days) per treatment by summing the average daily faecal scores of each pen.

**Sampling and measurements**

Apparent total tract digestibility (ATTD) of gross energy (GE), dry matter (DM), and crude protein (CP) was determined using chromic oxide (0.2%) as an inert indicator by Fenton and Fenton (1979) method. Pigs were fed diets mixed with chromic oxide from days 10–14 and days 24–28. Fresh faecal grab samples collected from three pigs per pen (days 14 and 28) were mixed and pooled, and a representative sample was stored in a freezer at −20°C until analysed. Before chemical analysis, the faecal samples were thawed and dried at 70°C for 72 h, after which they were finely ground to a size that could pass through a 1 mm screen. All feed and faecal samples were then analysed for GE, DM, and CP following the procedures outlined by the AOAC (2000). Chromium was analysed via ultraviolet absorption spectrophotometry (Shimadzu, UV-1201; Shimadzu, Kyoto, Japan) following the method described by Williams et al. (1962). We analysed the GE of diets and faeces by using an adiabatic oxygen bomb calorimeter (Parr Instruments, Moline, IL). Diets, and faeces samples were wet digested using nitric-perchloric acid and then diluted with deionised-distilled water for analyses of minerals. Concentrations of zinc were analysed using UV absorption spectrophotometry (Shimadzu, UV-1201; Shimadzu, Kyoto, Japan).

For the blood profiles, two pigs per pen were sampled via an anterior vena cava puncture. Blood samples were collected into both nonheparinized tubes and vacuum tubes containing K3EDTA (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) to obtain serum and whole blood. After collection, serum samples were centrifuged (3000 g) for 20 min at 4°C. The red blood cells (RBC), and white blood cells (WBCs) levels in the whole blood were determined by using an automatic blood analyser (ADVIA 120, Bayer, Tarrytown, NY, USA). The immunoglobulin G (IgG) was determined by using an automatic biochemistry blood analyser (HITACHI 747; Hitachi, Tokyo, Japan). The zinc concentration of blood was determined according to the method described by Hill et al. (2001). The plasma samples were diluted 1:7 with deionised water, and zinc concentration were determined by flame absorption spectrophotometry (Smith-Hieftje 4000, Thermo Jarrell Ash Corp., Franklin, MA).

**Procedures of microbial shedding**

Faecal samples were collected directly via massaging the rectum of all pigs in each treatment. They were then pooled and placed on ice for transportation to the lab. One gram of the composite faecal sample from each treatment was diluted in 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) and then homogenised. Viable bacteria in the faecal samples were then counted by plating serial 10-fold dilutions (in 1% peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI, USA) and lactobacilli medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate the *E. coli* and *Lactobacillus*. The *lactobacilli* medium III agar plates were then incubated for 48 h at 39°C under anaerobic conditions. The MacConkey agar plates were incubated for 24 h at 37°C. The *E. coli* and *Lactobacillus* colonies were counted immediately after removal from the incubator. The bacterial colonies were counted in each plate and the results were expressed in colony-forming units per gram of sample using a colony counter (log10cfu/g).

**Statistical analysis**

Data of growth performance, nutrient digestibility, zinc utilisation, and blood profiles were statistically analysed as a randomised complete block design using general linear models’ procedure of SAS (Statistical Analysis System 9.1, SAS Institute, Cary, NC, USA). The faecal score and faecal *E.coli* and *Lactobacillus* concentration were compared with a chi-squared test, using the FREQ procedure of SAS. The pen was used as the experimental unit for growth performance, faecal score, nutrient digestibility, zinc
utilisation, blood profiles, and faecal $E. coli$ and $L. lactobacillus$ concentration. Orthogonal contrasts were used to compare the effect of treatments: control vs other treatments; PC vs Chelate; PC vs Nano; Chelate vs Nano. Variability in the data was expressed as the pooled standard error, and $p<.05$ was considered statistically significant.

**Results**

**Growth performance and faecal scores**

At 0–14 days, ADG and the G:F ratio were significantly increased in pigs fed the PC, and 100 and 200 mg/kg Nano diets compared to pigs fed 100 and 200 mg/kg Chelate diets ($p<.05$) (Table 2). Moreover, pigs fed 100 mg/kg Chelate diet had significantly lower G:F ratios than did pigs fed the PC and 200 mg/kg Nano diets ($p<.05$). The faecal scores were significantly decreased in pigs fed 100 and 200 mg/kg Chelate, or Nano diets compared to the PC treatment ($p<.05$; PC vs Chelate, contrast $p<.05$; PC vs Nano, contrast $p<.05$).

In the overall period, the faecal scores were significantly increased in pigs fed the PC diet compared to pigs fed 100 mg/kg Chelate or Nano diets ($p<.05$).

Moreover, pigs fed the NC diet had lower faecal scores than pigs fed other treatments (NC vs other treatments, $p<0.05$).

**Nutrient digestibility**

At 2 weeks and 4 weeks, the ATTD of DM, and CP was significantly decreased in pigs fed the NC diet compared to other treatments ($p<.05$; NC vs other treatments, $p<0.05$) (Table 3). Moreover, at 4 weeks, the ATTD of GE was significantly decreased ($p<.05$) in pigs fed the NC diet compared to other treatments.

**Zinc utilisation**

Pigs fed the PC diet had significantly higher average zinc intake and faecal zinc excretion than pigs fed Chelate and Nano diets when the NC treatment was excluded ($p<.05$). There were significant differences in the average daily zinc intake and zinc excretion between the 100 mg/kg and 200 mg/kg Chelate and Nano diet groups at 2 and 4 weeks ($p<.05$) (Table 4). Pigs fed 100 and 200 mg/kg Chelate diets had significantly higher ATTD of zinc than did pigs fed 100 and 200 mg/kg Nano diets at 2 and 4 weeks when the NC and PC treatments were excluded ($p<.05$).

**Blood profiles**

The blood concentration of zinc was significantly decreased in pigs fed the NC diet compared to other treatments ($p<.05$; NC vs other treatments, $p<0.05$) (Table 5).
Faecal microbiota

Faecal *Lactobacillus* concentrations were significantly increased in the PC, 100 mg/kg Chelate, and 100 and 200 mg/kg Nano groups compared to the NC group (p < .05; NC vs other treatments, p < .05) (Table 6). Compared to the PC treatment, faecal *Lactobacillus* concentrations were significantly decreased in pigs fed 100 and 200 mg/kg Chelate diets (PC vs Chelate, p < .05).

**Table 3.** Effects of different forms and levels of zinc as alternative to medical zinc oxide on nutrient digestibility of weaning piglets (6 replications/treatment).

| Zn, status and level, mg/kg | NC | PC | Chelate | Nano |
|-----------------------------|----|----|---------|------|
| Items | 0 | 2500 | 100 | 200 | 100 | 200 | SE | p-Value |
| 2 weeks ATTD, % | | | | | | | | |
| GE* | 76.4 | 78.7 | 77.7 | 77.6 | 78.1 | 78.1 | 0.48 | .078 |
| DM* | 77.0b | 79.1a | 79.0a | 78.2a | 78.8a | 79.3a | 0.42 | .008 |
| CP* | 76.2b | 78.8a | 77.8a | 77.5ab | 78.2a | 78.1a | 0.46 | .035 |
| 4 weeks ATTD, % | | | | | | | | |
| GE* | 80.4b | 83.1a | 82.2a | 82.4a | 82.6a | 82.5a | 0.38 | .012 |
| DM | 82.5 | 82.9 | 82.9 | 83.0 | 83.0 | 83.1 | 0.69 | .991 |
| CP | 78.2b | 79.9a | 79.5a | 79.8a | 79.8a | 79.6a | 0.33 | .014 |

Abbreviations: NC: no additional added zinc oxide in diet (negative control); PC: NC + 2500 mg/kg zinc oxide (positive control); Chelate: NC + zinc chelate; Nano: NC + nanoparticle size of zinc; SE: standard error; ATTD: apparent total tract digestibility; GE: gross energy; DM: dry matter; CP: crude protein.

**Table 4.** Effects of different forms and levels of zinc as alternative to medical zinc oxide on zinc utilisation of weaning piglets (6 replications/treatment).

| Zn, status and level, mg/kg | NC | PC | Chelate | Nano |
|-----------------------------|----|----|---------|------|
| Items | 0 | 2500 | 100 | 200 | 100 | 200 | SE | p-Value |
| 2 weeks | | | | | | | | |
| Average daily zinc intake, mg*<sup>x,y,z</sup> | 28.2d | 633.9a | 69.1c | 94.1b | 73.6c | 103.4b | 4.22 | .001 |
| Zinc excreted in faeces, mg/kg*<sup>x,y,z</sup> | 88.2d | 1931.9a | 125.7cd | 160.0bc | 167.2bc | 239.9b | 29.83 | .0001 |
| ATTD of Zinc, %*<sup>x,y,z,w</sup> | 10.7c | 11.8c | 50.4a | 60.9a | 36.2b | 34.4b | 3.18 | .0001 |
| 4 weeks | | | | | | | | |
| Average daily zinc intake, mg*<sup>x,y,z</sup> | 61.1d | 1823.4a | 200.2c | 267.3b | 179.4c | 252.4b | 22.82 | .001 |
| Zinc excreted in faeces, mg/kg*<sup>x,y,z,w</sup> | 85.9c | 1882.9a | 103.6c | 134.3bc | 139.4bc | 207.3b | 23.08 | .001 |
| ATTD of Zinc, %*<sup>x,y,z,w</sup> | 14.1c | 13.5c | 59.6a | 62.6a | 43.9b | 42.8b | 4.50 | .0001 |

Abbreviations: NC, no additional added zinc oxide in diet (negative control); PC, NC + 2500 mg/kg zinc oxide (positive control); Chelate, NC + zinc chelate; Nano, NC + nanoparticle size of zinc; SE, standard error; ATTD, apparent total tract digestibility.

**Faecal microbiota**

Faecal *Lactobacillus* concentrations were significantly increased in the PC, 100 mg/kg Chelate, and 100 and 200 mg/kg Nano groups compared to the NC group (p < .05; NC vs other treatments, p < .05) (Table 6). Compared to the PC treatment, faecal *Lactobacillus* concentrations were significantly decreased in pigs fed 100 and 200 mg/kg Chelate diets (PC vs Chelate, p < .05).

**Discussion**

Zinc is an essential mineral that must be supplied to the body to play co-enzymatic and enzymatic roles. It
is an immune-modulating nutrient, which assists with the secretion and expression of chemo-attractants such as tumour necrosis factor (TNF)-α, interleukin (IL)-6, and monocytes in immune cells, thereby regulating innate and acquired immune responses (King et al. 2005; Bonaventura et al. 2015). Dietary pharmacological doses of ZnO have been used for a long time to prevent the incidence of diarrhoea and improve growth performance in piglets (Heo et al. 2013; Grilli et al. 2015). Consistent with these results, PC treatment showed higher growth performance, faecal scores, and faecal microflora than NC treatment. However, ZnO and ZnSO4 have low bioavailability and reactivity, and for this reason, most of these compounds are excreted as manure (Oh et al. 2021). To prevent environmental pollution, we conducted a comparative experiment between nano-ZnO and zinc chelate, which have been proven to be effective, and tested which form of zinc showed good efficiency with low excretion.

Many researchers reported that supplementation with 150~450 mg/kg nano-ZnO (Hu et al. 2012; Sun et al. 2019) and 200~460 mg/kg zinc chelate (Barszcz et al. 2019; Wen-Bin et al. 2019) showed similar effects on growth performance improvement and diarrhoea prevention in piglets compared to conventional doses of ZnO. The PC treatment and 200 mg/kg Nano treatment also showed similar growth performance during the experimental periods in this study. However, the present study showed that 100~200 mg/kg zinc glycine chelate had no effect on growth performance. In agreement with this study, Li et al. (2016) demonstrated that supplementation with 120 mg/kg nano-ZnO or chelated zinc did not affect growth performance.

Weaned piglets (3~4 weeks age) do not have sufficient endogenous enzymes to digest piglet diets, which have CP concentrations of 20~23% (Vondruskova et al. 2010; O’Doherty et al. 2017). Due to undigested protein, E. coli proliferate, and intestinal damage can be caused by enterotoxigenic E. coli (Campbell et al. 2013). Although there was no significant difference in E. coli concentrations in the faecal samples in our study, diarrhoea incidence was significantly decreased in pigs in the PC group compared to the other groups at 0~14 days. However, many studies reported that supplementation with ZnO in weaned piglet diets improved the microbial composition in the intestine by increasing beneficial bacteria and reducing pathogenic bacteria such as E. coli (Yu et al. 2017; Pei et al. 2019). For this reason, more research on the interaction between diarrhoea incidence and faecal microbial is needed.

Many researchers reported that high dietary doses of ZnO could improve faecal characteristics immediately after weaning (Poulsen 1998; Wijtten et al. 2011; Grilli et al. 2015). In this study, during the overall period, 200 mg/kg nano-ZnO showed similar diarrhoea incidence to that of 2500 mg/kg ZnO. Other researchers reported that when doses higher than 300 mg/kg nano-ZnO were added to weaning diets, it improved growth performance and prevented diarrhoea incidence similar to pharmacological supplementation with ZnO (Hu et al. 2012; Sun et al. 2019; Pei et al. 2019).

Regarding nutrient digestibility, pigs fed the no additional zinc diet had lower ATTD of nutrients than pigs in the other treatment groups. Many studies have reported that dietary supplementation of diets with zinc could improve the morphology of the small intestine and nutrient digestibility (Schell & Kornegay 1996; Furbeyre et al. 2017; Lei and Kim 2018). Hu et al. (2012) demonstrated that supplementing diets with zinc could improve the activity of digestive enzymes in the intestine and pancreatic tissue, resulting in improved digestibility. In our results, it seemed that zinc chelate and nano-ZnO exhibited effects on the small intestine and pancreatic function similar to those of conventional supplementation with ZnO.

Zinc glycine chelate treatment resulted in higher absorption and lower zinc excretion than the Nano and PC treatments in the present study. Star et al. (2012) demonstrated that organic zinc had 1.64-times higher bioavailability than inorganic zinc. The bioavailability of zinc varies according to the zinc form. Organic zinc is absorbed through the transport system of amino acids and peptides, showing higher absorption than inorganic zinc (Li et al. 2016). In the present study, we tested which form of zinc showed good efficiency with low excretion.

| Item, log10 cfu/g | NC | PC | Chelate | Nano |
|------------------|----|----|--------|------|
| E. coli          | 5.48 | 5.40 | 5.47 | 5.57 |
| Lactobacillus    | 6.71c | 7.32a | 7.12ab | 7.01b |
| Abbreviations: NC: no additional added zinc oxide in diet (negative control); PC: NC + 2500 mg/kg zinc oxide (positive control); Chelate: NC + zinc chelate; Nano: NC + nanoparticle size of zinc; cfu: colony forming units; SE: standard error.

*a-c Means in the same row with different superscripts differ (p < .05).
*a Contrast: NC vs other treatments (p < .05).
*b Contrast: PC vs Chelate (p < .05).
study, nano-ZnO showed lower absorption than zinc chelate. However, it showed higher absorption and lower zinc excretion than the conventional dose of zinc oxide. Nano-ZnO has a large surface area and particle surface activity, showing a higher absorption rate than inorganic zinc, consistent with other studies (Davda and Labhasetwar 2002; Rajendran 2013; Li et al. 2016). However, we could not consider that the ATTD of Zn was accurate by comparing a low dose (200 mg/kg) of Nano-ZnO and a high dose (2500 mg/kg) of ZnO. Martinez et al. (2005) reported that feeding piglets a higher amount of zinc than the nutrient requirement artificially increased zinc excretion due to overload of the tissue transport system (metallothionein) and that zinc retention changed with increases in zinc in the diet. The excretion of zinc in swine manure has become an important topic in global livestock. Using organic and nano-ZnO forms of zinc is thought to be a way to reduce zinc excretion, but more research is needed. In the blood profiles, except for the NC treatment, there were no differences in blood zinc concentrations among the PC, Chelate, and Nano treatment groups. There were also no significant differences in blood profiles among pigs treated with different forms of zinc. Knoell and Liu (2010) reported that zinc supplementation could affect immune responses and improve immune function. Sun et al. (2019) reported that 400–600 mg/kg nano-ZnO in weaning diets could increase the levels of immunoglobulins M and G. However, in the present study, blood IgG concentrations were not affected by zinc supplementation.

Many studies have reported that pharmacological supplementation with ZnO in weaning diets could improve intestinal microflora (Starke et al. 2014; Pei et al. 2019). In the present study, the treatments did not affect faecal E. coli concentrations. However, Lactobacillus concentrations were improved by zinc supplementation compared to the no additional zinc diet group. Similarly, Cho et al. (2015) demonstrated that Lactobacillus concentrations in the faecal samples were increased at 2 and 6 weeks after feeding pharmacological doses of ZnO and 200–300 mg/kg modified ZnO. Considering zinc absorption and zinc concentrations in the blood and faecal microflora, after a certain concentration of zinc is absorbed in the body, the rest may remain in the intestine to prevent diarrhoea and improve intestinal microflora.

**Conclusion**

Dietary supplementation with 100 or 200 mg/kg of nano-ZnO had similar effects on growth performance, nutrient digestibility, blood profiles, and faecal microflora with lower zinc excretion than 2500 mg/kg ZnO supplementation. However, pigs fed 100 mg/kg of nano-ZnO in the diet had a higher incidence of diarrhoea than pigs fed 2500 mg/kg of ZnO in the diet. In conclusion, the addition of 200 mg/kg or more of nano-ZnO is recommended as an alternative to medical ZnO in piglet diets.

**Ethical approval**

The experimental protocols describing the management and care of animals were reviewed and approved by the Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea (approval#CBNUA-1421-20-02).

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**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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**Data availability statement**

Data are available from the authors upon reasonable request and with permission of corresponding authors.

**References**

Ahn T, Kim D, Lee H, Shin H, Chung E. 2021. A study on the nutrient composition and heavy metal contents in livestock manure compost-liquefied fertilizer. J Korean Soc Water Env. 37:306–314.

AOAC. 2000. Official method of analysis. 16th ed. Washington (DC): AOAC.

Barszcz M, Taciak M, Tüsnió A, Čobanová K, Grešáková L. 2019. The effect of organic and inorganic zinc source, used in combination with potato fiber, on growth, nutrient digestibility and biochemical blood profile in growing pigs. Livest Sci. 227:37–43.
Bonaventura P, Benedetti G, Albarède F, Miossec P. 2015. Zinc and its role in immunity and inflammation. Autoimmun Rev. 14(4):277–285.

Campbell JM, Crenshaw JD, Polo J. 2013. The biological stress of early weaned piglets. J Anim Sci Biotechnol. 4(1): 19.

Cho JH, Upadhyya SD, Kim IH. 2015. Effects of dietary supplementation of modified zinc oxide on growth performance, nutrient digestibility, blood profiles, fecal microbial shedding and fecal score in weaning pigs. Anim Sci J. 86(6):617–623.

Davda J, Labhasetwar V. 2002. Characterization of nanoparticle uptake by endothelial cells. Int J Pharm. 233(1–2): 51–59.

Donaldson K, Schinwald A, Murphy F, Cho WS, Duffin R, Tran NL, Poland C. 2013. The biologically effective dose in inhalation nanotoxicology. Acc Chem Res. 46(3):723–732.

Fairbrother JM, Nadeau/C19 King LE, Frentzel JW, Mann JJ, Fraker PJ. 2005. Chronic zinc deficiency in mice disrupted T cell lymphopoiesis and erythropoiesis while B cell lymphopoiesis and myelopoiesis were maintained. J Am Coll Nutr. 24(6):494–502.

Lei XJ, Kim IH. 2018. Low dose of coated zinc oxide is as effective as pharmacological zinc oxide in promoting growth performance, reducing fecal scores, and improving nutrient digestibility and intestinal morphology in weaned pigs. Anim Feed Sci Technol. 245:117–125.

Li MZ, Huang JT, Tsai YH, Mao SY, Fu CM, Lien TF. 2016. Nanosize of zinc oxide and the effects on zinc digestibility, growth performances, immune response and serum parameters of weaning piglets. Anim Sci J. 87(11): 1379–1385.

Liu J, Feng X, Wei L, Chen L, Song B, Shao L. 2016. The toxicology of ion-shedding zinc oxide nanoparticles. Crit Rev Toxicol. 46(4):348–384.

Martinez MM, Link JE, Hill GM. 2005. Dietary pharmacological or excess zinc and phytase effects on tissue mineral concentrations, metallothionein, and apparent mineral retention in the newly weaned pig. Biol Trace Elem Res. 105(1–3):97–115.

Mullan B, D’Souza D. 2005. The role of organic minerals in modern pig production. In: Taylor-Pickard JA, Tucker LA, editors. Re-defining mineral nutrition. Nottingham (UK): Nottingham University Press. p. 89–106.

National Research Council. 2012. Nutrient requirements of swine. 11th rev. ed. Washington, DC: The National Academies Press.

O’Doherty JV, Bouwhuis MA, Sweeney T. 2017. Novel marine polysaccharides and maternal nutrition to stimulate gut health and performance in post-weaned pigs. Anim Prod Sci. 57(12):2376–2385.

Oberleas D, Harland BF. 1981. Phytate content of foods: effect on dietary zinc bioavailability. J Am Diet Assoc. 79(4):433–436.

Oh HJ, Park YJ, Cho JH, Song MH, Gu BH, Yun W, Lee JH, An JS, Kim YJ, Lee JS, et al. 2021. Changes in diarrhea score, nutrient digestibility, zinc utilization, intestinal immune profiles, and fecal microbiome in weaned piglets by different forms of zinc. Animals. 11(5):1356.

Pei X, Xiao Z, Liu L, Wang G, Tao W, Wang M, Zou J, Leng D. 2019. Effects of dietary zinc oxide nanoparticles supplementation on growth performance, zinc status, intestinal morphology, microflora population, and immune response in weaned pigs. J Sci Food Agric. 99(3):1366–1374.

Poulsen HD. 1998. Zinc and copper as feed additives, growth factors or unwanted environmental factors. J Anim Feed Sci. 7(1):135–142.

Rajendran D. 2013. Application of nano minerals in animal production system. Res J Biotechnol. 8:1–3.

Rhouma M, Fairbrother JM, Beaudry F, Letellier A. 2017. Post weaning diarrhea in pigs: risk factors and non-colistin-based control strategies. Acta Vet Scand. 59:1–19.

Satessa GD, Kjeldsen NJ, Mansouryar M, Hansen HH, Bache JK, Nielsen MO. 2020. Effects of alternative feed additives to medicinal zinc oxide on productivity, diarrhoea incidence and gut development in weaned piglets. Animal. 14(8):1638–1646.

Schell TC, Kornegay ET. 1996. Zinc concentration in tissues and performance of weanling pigs fed pharmacological levels of zinc from ZnO, Zn-methionine, Zn-lysine, or ZnSO4. J Animal Sci. 74(7):1584–1593.
Star L, van der Klis JD, Rapp C, Ward T L. 2012. Bioavailability of organic and inorganic zinc sources in male broilers. Poult Sci. 91:3115–20.

Starke IC, 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(2):416–427.

Sun YB, Xia T, Wu H, Zhang WJ, Zhu YH, Xue JX, He LY, Zhang LY. 2019. Effects of nano zinc oxide as an alternative to pharmacological dose of zinc oxide on growth performance, diarrhea, immune responses, and intestinal microflora profile in weaned piglets. Anim Feed Sci Technol. 258:114312.

Vondruskova H, Slamova R, Trckova M, Zraly Z, Pavlik I. 2010. Alternatives to antibiotic growth promoters in prevention of diarrhoea in weaned piglets: a review. Veterinarni Medicina. 55(5):199–224.

Wedekind KJ, Hortin AE, Baker DH. 1992. Methodology for assessing zinc bioavailability: efficacy estimates for zinc-methionine, zinc sulfate, and zinc oxide. J Anim Sci. 70(1):178–187.

Wei X, Tsai T, Howe S, Zhao J. 2021. Weaning induced gut dysfunction and nutritional interventions in nursery pigs: a partial review. Animals. 11(5):1279.

Wen-Bin C, Re-Jun F, Xin W, Cheng ZB, Yun-Bo T. 2019. The effects of zinc methionine chelate and ZnSO4 on the growth performance and immune function of the weaned piglets and on IPEC-J2 cell immune function. Kafkas Univ Vet Fak Derg. 25(2):185–192.

Wijtten PJ, van der Meulen J, Verstegen MW. 2011. Intestinal barrier function and absorption in pigs after weaning: a review. Br J Nutr. 105(7):967–981.

Williams CH, David DJ, Iismaa O. 1962. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. J Agric Sci. 59(3):381–385.

Xia T, Lai W, Han M, Han M, Ma X, Zhang L. 2017. Dietary nanoparticles alters intestinal microbiota and inflammation response in weaned piglets. Oncotarget. 8(39):64878–64891.

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. 8:825.