RESEARCH ARTICLE

J Anim Sci Technol 2022;64(1):23-37
https://doi.org/10.5187/jast.2021.e120

JAST
Journal of Animal Science and Technology
pISSN 2672-0191 eISSN 2055-0391

Effects of different inorganic: organic zinc ratios or combination of low crude protein diet and mixed feed additive in weaned piglet diets

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Received: Aug 23, 2021
Revised: Oct 6, 2021
Accepted: Oct 20, 2021

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Abstract

Thirty-six weaned piglets with an initial body weight (BW) of 8.43 ± 0.40 kg (28 days of age, ([Landrace × Yorkshire] × Duroc) were randomly assigned to 6 treatments for a 2-week feeding trial to determine the effects of different inorganic zinc (IZ), organic zinc (OZ) or combination of low crude protein diet (LP) and Mixed feed additive (MFA) on diarrhea score, nutrient digestibility, zinc utilization, blood profiles, organ weight, and fecal microflora in weaned piglet diet. The pigs were individually placed in 45 × 55 × 45 cm stainless steel metabolism cages in an environmentally controlled room (30 ± 1℃). The dietary treatments included a negative control (NC), positive control (PC; zinc oxide, 1,000 mg/kg), T1 (IZ : OZ, 850 : 150), T2 (IZ : OZ 700 : 300), T3 (IZ : OZ, 500 : 500), and T4 (LP + MFA [0.1% Essential oils + 0.08% Protease + 0.02% Xylanase]). The daily feed allowance was adjusted to 2.7 times the maintenance requirement for digestible energy (2.7 × 110 kcal of DE/kg $BW^{0.75}$). This allowance was divided into two equal parts, and the piglets were fed at 08 : 30 and 17 : 30 each day. Water was provided *ad libitum* through a drinking nipple. The diarrhea score was significantly increased ($p < 0.05$) in NC treatment compared with other treatments. The apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen (N), and gross energy (GE) was significantly increased ($p < 0.05$) in the T2 treatment compared with the PC and NC treatments in week 1. In week 2, the ATTD of DM, N, and GE was significantly decreased ($p < 0.05$) in the NC treatment compared with other treatments. The T3 treatment had significantly higher ($p < 0.05$) ATTD and apparent ileal digestibility of zinc than the PC and T1 treatments. The *Escherichia coli* count in feces was significantly decreased in the T4 treatment compared with the NC and T2 treatments. The *Lactobacillus* count in feces was significantly increased in the T4 and

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Competing interests
No potential conflict of interest relevant to this article was reported.

Funding sources
This work was carried out with the support of "Cooperative Research program for Agriculture Science & Technology Development (Project No. PJ01493602)" Rural Development Administration, Korea.

Acknowledgements
Not applicable.

Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions
Conceptualization: Oh HJ, Kim MH, Kim HB, Cho JH.
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Ethics approval and consent to participate
The experimental protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea (approval #CBNUA-1530-21-01).

T1 treatment compared with the T2 and T3 treatments. In conclusion, IZ : OZ 500 : 500 levels could improve nutrient digestibility and zinc utilization in weaned piglets, Moreover, MFA in LP diets could be used as a zinc alternative.

Keywords: Zinc oxide, Alternatives, Diarrhea score, Zinc excretion, Nutrient digestibility

INTRODUCTION

Piglets frequently experience diarrhea due to various factors such as isolation from sows, dietary changes, the mixing of pigs from different pens, adaptation to a new environment, or intestinal morphologic changes after weaning [1]. Due to these stress factors, the proliferation of *Escherichia coli* in the intestine of weaned pigs is promoted through undigested proteins. The intestinal barrier is damaged by toxins from enterotoxigenic *E.coli*, causing post-weaning diarrhea (PWD) [2]. The pharmacological supplementation of weaned diet with high-dose zinc oxide (ZnO) can prevent PWD and promotes growth performance in the weaning period [1,2]. However, it has recently been restricted in many countries including the European Union (EU) due to soil heavy metalization, accumulation in livestock products, and increased antimicrobial resistance [3]. The EU limits ZnO in weaned piglet diets to 150 mg/kg, and China limits it to 1,600 mg/kg [4]. In South Korea, the Zn content in compost is limited to 1,200 mg/kg, and a penalty is imposed on swine farms if this limit is exceeded. The pharmacological level of ZnO has been allowed to be added to piglet diets for two weeks after weaning in many countries to control PWD at this time [3,4]. For this reason, studies on Zn dose control or elimination of dietary ZnO are being conducted to replace high-dose ZnO in the diet of weaned piglets during 2 weeks of post-weaning.

Studies on the effects of ZnO supplementation at doses lower than 2,500 mg/kg are limited and have shown distinctly different results [5,6]. Piglet nutrition, breeding, management, and genetics have seen tremendous growth over the period since studies suggested that 2,500 mg/kg of dietary ZnO could control PWD and improve growth performance during 2 weeks of post-weaning [3,7]. However, as described above, it is essential to investigate the effect of low-dose of dietary Zn than the pharmacological dose of Zn on incidence of diarrhea and Zn excretion in weaned piglet diets. In our previous study, we conducted to evaluate the alternative forms of Zn, such as nano-particle size and Zn chelated with glycine, with lower level to replace high dose of inorganic Zn (IZ) in weaned piglet diets [8]. In our experiment, organic Zn (OZ) showed higher utilization than other forms of Zn such as IZ and nanoparticle-sized Zn [8]. Many researchers reported that chelating Zn with an amino acid prevented precipitation and had high bioavailability through peptide or amino acid transport systems in the small intestine [9,10]. OZ has greater stability than $ZnSO_4$ or ZnO [11], so has been suggested as an alternative to IZ.

In the search for replacing the pharmacological supplementation of ZnO, low-protein diets, essential oils (EOs), and enzymes are currently in the spotlight. The National Research Council (NRC) recommends 20%–23% crude protein (CP) levels in weaned diets [12]. However, 3–4 week-old pigs cannot produce enough endogenous enzymes to digest that amount of protein, so some of the undigested protein reaches the large intestine, which can lead to PWD by proteolytic bacteria [13]. Furthermore, many researchers have reported that reducing CP levels in the weaned diet reduced the incidence of diarrhea [14,15]. EOs have powerful antimicrobial and immune-enhancing effects, improving growth performance and nutrient digestibility, intestinal morphology, and reducing PWD in weaned piglets [16]. Exogenous protease increased nutrient digestibility, particularly protein and amino acids, and increased digestive enzyme activity and growth performance in weaned pigs [17,18]. Enzymes including protease and xylanase have various

https://doi.org/10.5187/jast.2021.e120

properties such as improving intestinal health and the immune system and growth performance [19]. In particular, it was reported to show benefits in improving gut health by inhibiting the growth of pathogenic microorganisms in the intestine [19].

Therefore, we hypothesized that different ratios of IZ and OZ at 1,000 mg/kg or a low-protein diet with commercial feed additives containing either EOs, protease and xylanase (MFA) could replace high-dose ZnO by preventing diarrhea and improving nutrient digestibility and gut health. Thus, we conducted this study to evaluate (1) the effects of different IZ : OZ ratios on diarrhea scores, nutrient digestibility, Zn utilization, blood profiles, organ weight, and fecal microflora toward replacing high-dose Zn oxide in weaned piglet diets and (2) whether 10% reduced protein diet with EOs, protease, and xylanase could replace high-dose Zn oxide by showing similar effects.

MATERIALS AND METHODS

The experimental protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea (approval #CBNUA-1530-21-01).

The OZ was chelated with glycine (containing 27% of Zn) from Dr. Eckel Animal Nutrition GmbH & Co. KG (Anta® min; Niederzissen, Germany). The EOs (Avi® power, containing thymol 1.4% and carvacrol 1.4%; VetAgro SpA, Reggio Emilia, Italy), xylanase (Signis®, AB Vista, Marlborough, UK), and protease (PT125TM®, an alkaline serine endopeptidase produced by *Streptomyces* spp.; Eugene-Bio, Suwon, Korea) were mixed feed additives supported by a Eugene-Bio.

Animals, facilities and dietary treatments

A total of 36 weaned piglets [Landrace × Yorkshire] × Duroc; 28 day of old) were allotted to a completely randomized block design. The pigs (average initial body weight [BW] of 8.43 ± 0.40 kg) were individually placed in 45 cm × 55 cm × 45 cm stainless steel metabolism cages in an environmentally controlled room (30 ± 1℃). There were one pig treatment in a cage and six replicate cage per treatments. The dietary treatments consisted of negative control (NC; no additional added ZnO in diet), positive control (PC; NC + 1,000 mg/kg ZnO), T1 (NC + IZ : OZ 850 : 150 mg/kg), and T2 (NC + IZ : OZ 700 : 300 mg/kg), T3 (IZ : OZ 500 : 500 mg/kg), and T4 (10% reduced protein diet [LP] + mixed additives [0.1% essential oil + 0.08% protease + 0.02% xylanase, MFA]). All diets were formulated to meet or exceed the NRC (Table 1). The daily feed allowance was adjusted to 2.7 times the maintenance requirement for digestible energy (DE; 2.7 × 110 kcal of DE/kg $BW^{0.75}$). This allowance was divided into two equal parts, and the piglets were fed at 08 : 30 h and 17 : 30 h each day. The diets were mixed with water in a 1 : 1 ratio (Wt/Wt) before feeding. Water was provided *ad libitum* through a drinking nipple. We individually weighed the pigs at the beginning of each period and recorded the amount of feed supplied and any residual feed quantity for each period. The subjective diarrhea scores were individually recorded at 09 : 00 h and 18 : 00 h from the same pigs on days 0 to 14 post weaning. The diarrhea score was assigned as follows: 0, diarrhea; 1, sloppy feces; 2 normal feces; and 3, well-formed feces. Scores were calculated as the average diarrhea score for each period (0 to 7 days; 7 to 14 days; overall period, 0 to 14 days) per group by summing the average daily diarrhea scores of each pig. The first experimental period consisted of a 4-day adaptation period, followed by a 3-day collection period to collect feces. The feed was the same during the second experimental period as that in the first experimental period. We set a 4-day feces collection period and alternated the feeding time between the day of slaughter and the previous 2 days so that pigs could be slaughtered within the designated time. The entire

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

Items	Basal diet	10% reduced CP diet
Ingredient (%)	100.00	100.00
Corn	34.43	38.34
Extruded corn	15.00	15.00
Lactose	10.00	10.00
Dehulled soybean meal (51% CP)	13.50	10.00
Soy protein concentrate (65% CP)	10.00	10.00
Plasma powder	6.00	4.50
Whey	5.00	6.00
Soy oil	2.20	2.20
Monocalcium phosphate	1.26	1.26
Limestone	1.40	1.40
L-Lysine-HCl (78%)	0.06	0.12
DL-Methionine (50%)	0.15	0.18
Choline chloride (25%)	0.10	0.10
Vitamin premix[1)]	0.25	0.25
Trace mineral premix[2)]	0.25	0.25
Salt	0.40	0.40
Calculated value		
ME (kcal/kg)	3,508	3,503
CP (%)	20.78	18.70
Lysine (%)	1.35	1.34
Metionine (%)	0.39	0.40
Ca (%)	0.82	0.82
P (%)	0.65	0.65
Zn (%)	0.01	0.01

[1)]Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D_3, 1,103 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 B_{12}, 33 μg.

[2)]Provided per kg of complete diet without Zinc: Cu (as $CuSO_4 \cdot 5H_2O$), 12 mg; Mn (as MnO_2), 8 mg; I (as KI), 0.28 mg; and Se (as $Na_2SeO_3 \cdot 5H_2O$), 0.15 mg.

CP, crude protein; ME, metabolizable energy; Ca, calcium; P, phosphorus; Zn, zinc.

liver and spleen were weighed. The fecal collected by total collection method. The intestinal tract was incised along the abdominal gland to remove 20 cm from the end of the ileum. Then the contents were frozen in a plastic bag. The ileal digesta was freeze dried. Samples were finely crushed and stored at −20℃ to measure Zn content. Feces were immediately collected as they appeared in the metabolism cages. They were stored in a freezer at −20℃ until analyzed. Fecal samples were dried at 70℃ for 72 hours in a forced-air oven and ground through a 1-mm screen. They were thoroughly mixed before a subsample was collected for chemical analysis.

Chemical analysis for diet and feces

Diets and feces were analyzed for dry matter (DM), nitrogen (N), and gross energy (GE) using AOAC methods (2007). For N of the diets and feces, we added 10 % concentrated sulfuric acid for nitrogen fixation. We analyzed the GE of the diets and feces using an adiabatic oxygen bomb calorimeter (Parr Instruments, Moline, IL, USA). Diets, feces, and ileal digesta samples were wet digested using nitric-perchloric acid and then diluted with deionized distilled water for mineral analysis. The concentration of Zn was analyzed using UV absorption spectrophotometry (UV-1201;

https://doi.org/10.5187/jast.2021.e120

Shimadzu, Tokyo, Japan). We calculated the apparent total tract digestibility (ATTD) of DM, N, GE, and Zn, as well as the average daily mineral intake, using the following equations: ATTD lrb% = [(DI × NID – OF × NIF] / (DI × NID)] × 100; Average daily mineral intake = ADFI × MD; DI is the DM intake (g), NID is the nutrient content (DM, N, GE, and Zn) of diet on a DM basis; OF is the output of feces (g); and NIF is the nutrient content of the feces on a DM basis. MD is the Zn content in the diet.

For the blood profiles, all pigs were sampled via an anterior vena cava puncture before the slaughter. Blood samples were collected into both nonheparinized tubes and vacuum tubes containing K_3EDTA (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) to obtain serum and whole blood. After collection, serum samples were centrifuged (3,000 g) for 20 min at 4℃. The red blood cells (RBC), white blood cells (WBC), lymphocyte, monocyte, eosinophil, basophil, glucose, cholesterol and blood urea nitrogen (BUN) levels in the whole blood were determined by using an automatic blood analyzer (ADVIA 120, Bayer, Tarrytown, NY, USA). The immunoglobulin G (IgG) and immunoglobulin M (IgM) were determined by using commercial enzyme-linked immunosorbent assay (ELISA, Bethyl Laboratories, Montgomery, TX, USA) kits. The Zn concentration of blood was determined according to the method described by Hill et al. [6]. The blood samples were diluted 1 : 7 with deionized water, and Zn concentration were determined by flame absorption spectrophotometry (Smith-Hieftje 4000, Thermo Jarrell Ash, Franklin, MA, USA).

Procedures of microbial shedding

Fecal 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 fecal sample from each treatment was diluted in 9 mL of 1% peptone broth (Becton, Dickinson and Co.) and then homogenized. Viable bacteria in the fecal samples were then counted by placing 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 *Escherichia coli* and *Lactobacillus*. The *Lactobacilli* medium III agar plates were then incubated for 48 hours at 39℃ under anaerobic conditions. The MacConkey agar plates were incubated for 24 hours at 37℃. The *E. coli* and *Lactobacillus* colonies were counted immediately after removal from the incubator.

Statistical analysis

Data of growth performance, nutrient digestibility, Zn excretion, ATTD of Zn, apparent ileal digestibility (AID) of Zn, blood profiles, and organ weight were statistically analysed as a randomized complete block design using general linear models procedure of SAS (Statistical Analysis System 9.1, SAS Institute, Cary, NC, USA). The diarrhea score and fecal microflora were compared with a chi-squared test, using the FREQ procedure of SAS. The individual pig was used as the experimental unit. Orthogonal contrasts were used to compare the possible relationship about the effect of treatments: NC vs. other treatments; PC vs. T1, T2, T3; T4 vs. T1, T2, T3. Variability in the data was expressed as the pooled standard error, and $p < 0.05$ was considered statistically significant.

RESULTS

Diarrhea score

At 8 to 14 days, pigs fed the NC diet had higher ($p < 0.05$; contrast $p < 0.01$) diarrhea score than pigs fed the T1 and T3 diets (Table 2).

Table 2. Effects of different inorganic : organic zinc ratios or combination of low crude protein diet and feed additives on diarrhea scores in weaned piglet diets

Treatment	NC	PC	T1	T2	T3	T4	SE	*p*-value
Diarrhea score[1)]								
0 to 7 days	1.929	1.701	1.781	1.622	1.741	1.595	0.252	0.947
8 to 14 days[2)]	1.164[a]	0.778[ab]	0.440[b]	0.692[ab]	0.464[b]	0.833[ab]	0.165	0.045
Overall period (0 to 14 days)	1.278	0.982	0.919	0.997	0.908	1.086	0.166	0.635

NC, no additional added zinc oxide in diet (negative control); PC, NC+1,000 mg/kg zinc oxide (positive control); T1, NC + inorganic : organic zinc 850 : 150 mg/kg ; T2, NC + inorganic : organic zinc 700 : 300 mg/kg; T3, NC + inorganic : organic zinc 500 : 500 mg/kg; T4, 10% reduced CP diet + 0.1% essential oil + 0.08% protease + 0.02% xylanase.

[1)]Diarrhea score was determined as follow: 0, well-formed feces; 1, normal feces; 2, sloppy feces; and 3, diarrhea.

[2)]Contrast: NC vs other treatments ($p < 0.05$).

[a,b]Means in the same row with different superscripts differ ($p < 0.05$).

Nutrient digestibility and zinc utilization

The ATTD of DM, N, and GE were significantly ($p < 0.001$; contrast $p < 0.05$) decreased in the NC treatment compared with other treatments in weeks 1 and 2 (Table 3). In week 1, pigs fed the T2 diet had higher ($p < 0.05$) the ATTD of DM, N, and GE than the pigs fed the PC diet.

Table 3. Effects of different inorganic : organic zinc ratios or combination of low crude protein diet and mixed feed additives on nutrient digestibility and zinc utilization in weaned piglets

Treatment	NC	PC	T1	T2	T3	T4	SE	*p*-value
Nutrient digestibility								
One week ATTD (%)								
Dry matter[1)2)]	86.7[c]	88.2[b]	88.9[ab]	89.8[a]	89.1[ab]	89.2[ab]	0.5	0.001
Nitrogen[1)]	77.1[c]	81.8[b]	81.8[b]	84.4[a]	83.0[ab]	81.6[b]	0.8	0.001
Gross energy[1)2)]	82.8[c]	85.1[b]	86.7[ab]	87.4[a]	85.5[b]	86.3[ab]	0.6	0.001
Two week ATTD (%)								
Dry matter[1)]	87.6[b]	89.8[a]	89.7[a]	89.9[a]	90.5[a]	90.3[a]	0.4	0.001
Nitrogen[1)]	77.8[b]	81.0[a]	80.4[a]	81.3[a]	82.2[a]	81.7[a]	0.8	0.017
Gross energy[1)]	83.3[b]	86.4[a]	86.4[a]	86.9[a]	87.7[a]	87.3[a]	0.6	0.001
Zinc utilization								
One week								
Feed intake (g)	340.0	340.0	340.0	340.0	340.0	340.0	0.0	1.000
Zinc intake (mg)[1)2)3)]	34.0[c]	382.5[a]	374.0[a]	340.0[b]	340.0[b]	34.0[c]	3.1	0.001
Zinc excretion (mg)[1)2)3)]	32.3[d]	344.8[a]	299.2[b]	264.5[c]	253.7[c]	29.2[d]	7.5	0.001
ATTD of zinc[1)2)3)]	5.1[d]	9.6[c]	19.9[ab]	22.3[ab]	25.3[a]	14.2[bc]	2.7	0.001
Two week								
Feed intake (g)[1)2)3)]	350.0[c]	380.0[a]	380.0[a]	370.0[b]	380.0[a]	350.0[c]	0.0	0.001
Zinc intake (g)[1)2)3)]	35.0[c]	427.5[a]	418.0[a]	370.0[b]	380.0[b]	35.0[c]	2.4	0.001
Zinc excretion (mg)[1)2)3)]	31.4[c]	381.1[a]	349.3[a]	298.6[b]	291.8[b]	29.5[c]	10.6	0.001
ATTD of zinc[1)2)]	10.4[b]	10.9[b]	16.4[ab]	19.3[ab]	23.2[a]	15.8[ab]	3.2	0.045
AID of zinc (%)[1)2)]	8.9[c]	9.3[c]	14.1[b]	18.1[ab]	21.1[a]	14.1[b]	1.5	0.001

NC, no additional added zinc oxide in diet (negative control); PC, NC+1,000 mg/kg zinc oxide (positive control); T1, NC + inorganic : organic zinc 850 : 150 mg/kg; T2, NC + inorganic : organic zinc 700 : 300 mg/kg; T3, NC + inorganic : organic zinc 500 : 500 mg/kg; T4, 10% reduced crude protein diet + 0.1% essential oil + 0.08% protease + 0.02% xylanase.

[1)]Contrast: NC vs other treatments ($p < 0.05$).

[2)]Contrast: PC vs T1, T2, and T3 ($p < 0.05$).

[3)]Contrast: T4 vs T1, T2, and T3 ($p < 0.05$).

[a–d]Means in the same row with different superscripts differ ($p < 0.05$).

ATTD, apparent total tract digestibility; AID, apparent ileal digestibility.

https://doi.org/10.5187/jast.2021.e120

The N intake and excretion were significantly decreased ($p < 0.001$; contrast $p < 0.05$) in the T4 treatment compared with other treatments in weeks 1 and 2. Pigs fed with the PC and T1 diets had significantly higher ($p < 0.05$) Zn intake than did pigs fed with the T2 and T3 diets in weeks 1 and 2. Pigs fed with the T1, T2 and T3 diets had significantly lower ($p < 0.05$; contrast $p < 0.05$) Zn excretion in feces and higher the ATTD of Zn than pigs fed the PC treatment in week 1. Pigs fed with the T2 and T3 diets had significantly lower ($p < 0.05$; contrast $p < 0.05$) Zn excretion in feces compared with pigs fed with the PC and T1 diets in week 2. The ATTD of Zn was significantly increased ($p < 0.05$; contrast $p < 0.05$) in the T3 treatment compared with the PC treatment in the same period. The AID of Zn was significantly decreased ($p < 0.05$; contrast $p < 0.05$) in the PC treatment compared with the T1, T2 and T3 treatments, moreover, pigs fed with the T3 diet had significantly higher ($p < 0.05$; contrast $p < 0.05$) AID of Zn than pigs fed with the T1 diet.

Blood profiles

There was a high tendency for the blood concentration of lymphocyte in the T4 treatment compared with the NC, PC, T1 and T2 treatments (Table 4). The blood concentration of BUN was significantly decreased ($p < 0.05$; contrast $p < 0.05$) in the T4 treatment compared with the NC, PC, and T1 treatments (Table 5).

Organ weight

No significant differences were observed ($p > 0.05$) in the liver and spleen weight (Table 5).

Fecal microflora

The *E.coli* count in feces was significantly decreased ($p < 0.05$; contrast $p < 0.05$) in the T4 treatment compared with the NC and T2 treatments (Table 6). The *Lactobacillus* count in feces was significantly increased ($p < 0.05$; contrast $p < 0.05$) in the T4 and T1 treatments compared with the T2 and T3 treatments.

Table 4. Effects of different inorganic : organic zinc ratios or combination of low crude protein diet and mixed feed additives on blood profiles in weaned piglets

Treatment	NC	PC	T1	T2	T3	T4	SE	*p*-value
Red blood cell (10^6/μL)	7.32	7.37	7.14	7.52	7.64	7.59	0.40	0.949
White blood cell (10^3/μL)	17.43	17.72	17.85	19.68	18.11	17.76	2.79	0.994
Lymphocyte (%)[1)]	49.88	49.35	49.18	50.48	57.68	66.58	4.58	0.065
Monocyte (%)	3.48	4.63	2.47	4.33	3.88	4.77	0.73	0.252
Eosinophil (%)	0.41	0.55	0.43	0.42	0.40	0.52	0.14	0.958
Basophil (%)	0.40	0.35	0.33	0.40	0.55	0.43	0.09	0.548
Immunoglobulin G (mg/dL)	174.0	160.3	185.2	148.5	185.0	162.3	23.2	0.816
Immunoglobulin M (mg/dL)	47.3	43	47.7	50.7	48.0	44.0	3.7	0.774
Cholesterol (mg/dL)	66.2	71.2	76.5	72.7	82.5	68.0	5.4	0.313
Glucose (mg/dL)	109.7	107.3	108.2	111.2	106.0	109.7	9.6	0.999
Blood urea nitrogen (mg/dL)[1)]	6.83[a]	6.83[a]	6.83[a]	6.33[ab]	5.50[ab]	5.00[b]	0.48	0.049
Zinc (ug/dL)	94.9	102.4	104.0	107.3	99.4	97.2	5.0	0.654

NC, no additional added zinc oxide in diet (negative control); PC, NC + 1,000 mg/kg zinc oxide (positive control); T1, NC + inorganic : organic zinc 850 : 150 mg/kg; T2, NC + inorganic : organic zinc 700 : 300 mg/kg; T3, NC + inorganic:organic zinc 500 : 500 mg/kg; T4, 10% reduced crude protein diet + 0.1% essential oil + 0.08% protease + 0.02% xylanase.

[1)]Contrast: T4 vs T1, T2, and T3 ($p < 0.05$).

[a,b]Means in the same row with different superscripts differ ($p < 0.05$).

Table 5. Effects of different inorganic : organic zinc ratios or combination of low crude protein diet and mixed feed additives on organ weight in weaned piglets

Treatment	NC	PC	T1	T2	T3	T4	SE	*p*-value
Body weight (kg)	10.0	10.1	10.3	10.6	10.7	10.0	0.2	0.399
Relative organ weight (%)								
Liver	3.046	2.922	2.844	2.709	2.837	2.760	0.211	0.892
Spleen	0.205	0.282	0.231	0.208	0.268	0.214	0.026	0.257

NC, no additional added zinc oxide in diet (negative control); PC, NC+1,000 mg/kg zinc oxide (positive control); T1, NC + inorganic : organic zinc 850 : 150 mg/kg; T2, NC + inorganic : organic zinc 700 : 300 mg/kg; T3, NC + inorganic : organic zinc 500 : 500 mg/kg; T4, 10% reduced crude protein diet + 0.1% essential oil + 0.08% protease + 0.02% xylanase.

Table 6. Effects of different inorganic : organic zinc ratios or combination of low crude protein diet and feed additives on fecal microflora in weaned piglets

Treatment	NC	PC	T1	T2	T3	T4	SE	*p*-value
Escherichia coli (Log CFU/g)[1)]	5.241^{a}	4.986^{ab}	4.897^{ab}	5.263^{a}	5.110^{ab}	4.742^{b}	0.139	0.087
Lactobacillus (Log CFU/g)[1)]	6.969^{ab}	6.804^{b}	7.254^{a}	6.814^{b}	6.701^{b}	7.256^{a}	0.132	0.017

NC, no additional added zinc oxide in diet (negative control); PC, NC+1,000 mg/kg zinc oxide (positive control); T1, NC + inorganic : organic zinc 850 : 150 mg/kg; T2, NC + inorganic : organic zinc 700 : 300 mg/kg; T3, NC + inorganic : organic zinc 500 : 500 mg/kg; T4, 10% reduced crude protein diet + 0.1% essential oil + 0.08% protease + 0.02% xylanase.

[1)]Contrast: T4 vs T1, T2, and T3 ($p < 0.05$).

[a,b]Means in the same row with different superscripts differ ($p < 0.05$).

DISCUSSION

Zn is an essential mineral that has various enzymatic and co-enzymatic roles. It improves immunity and the composition of the body structure. It helps in developing the gastrointestinal tract, preventing diarrhea, and affecting the growth of pigs [10]. The Zn content in feedstuff is insufficient for pigs, and Zn is mainly added in an inorganic form, such as ZnO or $ZnSO_4$ [20]. The ZnO form has low reactivity and bioavailability, and the $ZnSO_4$ form is hygroscopic and reacts with rapid ions to form free radicals to accelerate the breakdown of fatty acids, vitamins, and other nutrients in the feed [21]. In addition, to prevent diarrhea in the weaning phase, Zn that cannot be absorbed by adding 2,000 to 3,000 mg/kg of ZnO to the weaning diets, is discharged in the feces, which is a major environmental problem [22]. The hypothesis of the present experiment was that there would be an additive effect of replacing IZ with OZ and LP diet with MFA, leading to reduced diarrhea, and improved nutrient digestibility, Zn utilization, and blood profiles. This would result in positive effects similar to pharmacologic levels of ZnO.

In the present study, IZ : OZ at ratios of 850 : 150 mg/kg and 500 : 500 mg/kg (T1, T3) decreased diarrhea scores, which means it reduced diarrhea compared to non- Zn diets (NC) but had no significant difference compared to 1,000 mg/kg ZnO (PC) at 8 to 14 days. Also, the diarrhea scores of pigs in the low-protein diet with MFA were similar to those of pigs treated with Zn. The supplementation of ZnO has usually led to better fecal scores and lower incidence of PWD and mortality [23]. Effective Zn sources can also be OZ forms [24]. Different Zn forms like Zn-methionine or Zn-lysine can increase Zn concentrations in the plasma more than ZnO or other IZ forms [25]. Reductions in diarrhea with increasing OZ levels can be explained by the increased bioavailability of OZ compared to IZ in the intestine. EOs have gained attention as ZnO alternatives for reducing PWD in animal diets [26]. They demonstrated many properties such as strong antimicrobial, antioxidant, and anti-inflammatory activity [16]. *E. coli*, known as the main etiological agent of PWD, proved to be susceptible to several EOs, including cinnamon, clove, and thyme oils, thereby leading to reduced fecal scores and incidence of diarrhea [27]. Also, supplementation with dietary enzymes including protease and xylanase could reduce diarrhea in

https://doi.org/10.5187/jast.2021.e120

pigs. These beneficial effects were attributed to the development of the digestive tract, an increase in enzymatic activity in the digestive system, and improvement in nutrient digestibility derived from the enzymes [28,29]. The decrease in diarrhea from the addition of enzymes and EOs can be explained by the abovementioned mechanism.

In nutrient digestibility, pigs fed diets with different IZ : OZ ratios (PC, T1–T3) or LP diet with MFA had a higher ATTD of DM, N, and GE compared to the one and two-week NC treatments. These results were consistent with the results of Lei and Kim [30] who reported that the addition of Zn to the diet increased DM and N digestibility. Hu et al. [31] reported that dietary supplementation with ZnO could improve the activation of the digestive enzymes in the small intestine and pancreatic tissue, thereby improving the digestibility of nutrients. Other studies have reported that small intestine morphology was improved from pharmacological supplementation with ZnO [32]. Schlegel et al. [33] reported that the bioavailability of organic and IZ forms ranged from 85% to 117%. Unlike our hypothesis that improvements would be seen from increasing OZ ratios, the replacement of IZ with OZ did not make a dramatic difference among the treatments except for NC, but the IZ : OZ ratios of 500 : 500 mg/kg showed high digestibility. However, there was no significant difference in nutrient digestibility among the different dietary Zn levels [34]. This may have been due to the dosage or type of Zn. Additionally, environmental conditions, dietary ingredients, phosphorous levels, and nutritional composition may have caused these results. The LP diet with enzymes and EOs contributed to improving nutrient digestibility similar to the Zn treatments. At weaning, the high buffering capacity of hard diets and the low HCl production in the piglet stomach cause LP digestion [35]. The use of LP in this study led to improved digestibility due to the abovementioned mechanisms. Additionally, these improvements were attributed to feed additives like enzymes and EOs. Previously published studies reported the improved digestibility of energy and nutrients by supplementation with EOs [36–38]. Although studies on how EOs affect digestibility are handicapped by the complexity of EOs, we confirmed the results of the studies by Platel and Srinivasan [39], and Zhai et al. [40]. They reported that these improvements could be explained by the enhanced secretion of bile and enzymes and altered gut peristalsis. The use of xylanase and protease in the swine diet improved nutrient digestibility [17].

Many researchers reported that the bioavailability of Zn was increased by OZ compared to the inorganic form of Zn sulfate owing to the amino acid or the peptide transport systems [41,42]. These results were also observed in our study. We found that the ATTD and AID of Zn gradually increased as the ratio of OZ in the diets increased. The reasons for the improvements in Zn digestibility were considered to result from reduced fecal excretion and improved efficiency of the organic form. It was possible to confirm the effect of reducing diarrhea incidience, improving nutrient digestibility and Zn utilization when feeding OZ in a certain ratio rather than adding IZ alone. Also, piglets fed LP diet with MFA had higher ATTD and AID of Zn than piglets fed the NC diet. Diet acidification with formic, benzoic butyric, lactic, fumaric, and citric acids increased the ATTD of minerals with Ca and P in pigs [43]. Interestingly, dietary citric acid improved P utilization in growing pigs [22], and 1.5% citric acid improved the availability of other minerals in young pigs [44]. Sauer et al. [45] reported that the digestibility of minerals increased as benzoic acid levels in the diet increased. According to a recent study, the actions and mechanisms of EOs overlapped with those of benzoic acid, and some benzoic acid could be spared by the addition of EOs. Additionally, EOs increased the utilization rate of Zn and reduced the discharge of Zn [46].

Zn plays a critical role in the immune system of the host, and it affects various immune responses in different parts of the body, from innate immune functions to the skin barrier [47,48]. Sun et al. [49] reported that when 400–600 mg/kg of nano-ZnO was supplied, IgM and IgG levels were increased. However, Ma et al. [50] showed that dietary supplementation with $ZnSO_4$,

chitosan + $ZnSO_4$, and Zn chitosan chelate did not affect serum IgG levels in weaned piglets. Also, IgG levels remained unaffected by Zn-ASP supplementation to growing pigs [51]. Previous studies indicated that plasma Zn concentrations increased linearly with supplemental Zn [52]. Our results showed that there was no significant difference in blood profiles except for lymphocytes and BUN among the treatments. This discrepancy may have been due to the dosage or type of Zn, nutritional composition, or experimental period. BUN can be used to determine protein digestibility and be a parameter of protein utilization [53]. In the current study, BUN was decreased in the non- Zn pigs receiving a low CP diet with MFA, consistent with previous studies reporting that decreased protein levels resulted in lower BUN levels [14,54,55]. The lower BUN levels indicated improved protein utilization. The organ weight of pigs is used as an indicator to determine good health, disease-free status, and a resting state [56]. In the present study, there was no significant difference between the treatments. These results may have been because the dosage of Zn and the multiple feed additives was a safe dose for organ development.

Many researchers have shown that the addition of dietary ZnO improved the microbial composition in the intestine, thereby reducing pathogenic microorganisms and increasing beneficial bacteria [57,58] However, similar results were not seen in our study. In the present study, the effect of Zn treatment was not different compared to the NC treatment. These results are consistent with the results of Li et al. [59] who reported that ZnO did not affect the *Enterobacteriaceae*, *Lactobacilli*, and *Clostridia* counts in the ileal digesta and feces in piglets. Additionally, supplementation with Zn, regardless of the form, had no effect on coliform bacteria and lactic acid bacteria counts in the small intestine or cecum [30]. The inconsistent intestinal microflora results may have been due to several reasons. First of all, the doses, forms, and duration of ZnO supplementation may have caused these differences. Also, different sampling areas in the intestine or feces and different analysis methods could have led to the differences and changes in the microbial communities [60,61]. Interestingly, the LP diet with MFA resulted in increases in *Lactobacillus* count and decreases in *E.coli* counts in the feces compared to the NC and Zn treatments. These improvements in intestinal bacterial composition could have been caused by several factors. The high-protein diets caused a higher acid-binding environment and increased the pH of the gastrointestinal tract to nearly neutral conditions, which provided a favorable environment for the proliferation of pathogenic bacteria, whereas the LP diet alleviated the negative effects of high protein and significantly lowered the number of *E.coli* in the ileum and colon [62]. EOs have strong antimicrobial action against pathogenic bacteria while not harming beneficial bacteria such as *bifidobacteria* and *lactobacilli*. Moreover, the increased number of *lactobacilli* and reductions in *E.coli* in the intestinal microbiota resulted in a decreased incidence of diarrhea in piglets [37]. In the present study, lower diarrhea in the T4 group was caused by the abovementioned mechanism. The *E. coli* and total anaerobe counts in the rectum were significantly reduced in pigs fed EOs, whereas the number of *lactobacilli* was slightly increased in the colon and rectum of pigs fed EOs. The effect of enzymes on the intestinal microbiota is related to changes in the physicochemical properties of the substrate in the intestine and the release of prebiotics and bioactive compounds [63]. Commercial xylanase may also contain feruloyl esterase produced by the microorganisms producing xylanase [64] that release phenolic compounds cross-linked to xylan [65,66]. Studies have shown that phenolic compounds could modulate the intestinal microbiota by reducing enterotoxigenic *E. coli* (ETEC) K88 and F18+ growth in porcine feces [67]. Kim et al. [68] reported that the addition of multiple enzymes including xylanase, amylase, *β*-mannanase, protease, and phytase increased the *Lactobacillus* spp. count and decreased *E. coli* and *Clostridium* spp. counts in the digesta of the ileum and cecum.

https://doi.org/10.5187/jast.2021.e120

CONCLUSION

Pigs in the LP + MFA group showed similar PWD, ATTD of nutrients, and fecal microbiota as organic and IZ-supplemented treatments. During 1 week of post-weaning, 700 : 300 mg/kg of IZ : OZ ratio could improve nutrient digestibility, and zinc utilization compared with 1,000 mg/kg ZnO. Likewise, in overall periods, a 500 : 500 mg/kg IZ : OZ ratio showed improvements in ATTD/AID of Zn, and reductions in Zn excretion compared to 1,000 mg/kg ZnO. By partially replacing IZ with OZ, it showed the possibility of being presented as an alternative to high dose of ZnO in weaned piglet diets. In conclusion, reducing protein with EOs, protease, and xylanase and a 700 : 300 or 500 : 500 mg/kg inorganic and OZ ratio were reduce Zn excretion and effective alternatives of high-dose of ZnO in weaned piglet diets.

REFERENCES

1. Fairbrother JM, Nadeau É, Gyles CL. Escherichia coli in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. Anim Health Res Rev. 2005;6:17-39. https://doi.org/10.1079/AHR2005105
2. Lallès JP, Bosi P, Smidt H, Stokes CR. Weaning — a challenge to gut physiologists. Livest Sci. 2007;108:82-93. https://doi.org/10.1016/j.livsci.2007.01.091
3. Satessa GD, Kjeldsen NJ, Mansouryar M, Hansen HH, Bache JK, Nielsen MO. Effects of alternative feed additives to medicinal zinc oxide on productivity, diarrhoea incidence and gut development in weaned piglets. Animal. 2020;14:1638-46. https://doi.org/10.1017/S1751731120000154
4. Wei X, Tsai T, Howe S, Zhao J. Weaning induced gut dysfunction and nutritional interventions in nursery pigs: a partial review. Animals. 2021;11:1279. https://doi.org/10.3390/ani11051279
5. Mavromichalis I, Webel DM, Parr EN, Baker DH. Growth-promoting efficacy of pharmacological doses of tetrabasic zinc chloride in diets for nursery pigs. Can J Anim Sci. 2001;81:387-91. https://doi.org/10.4141/A01-005
6. Hill GM, Mahan DC, Carter SD, Cromwell GL, Ewan RC, Harrold RL, et al. Effect of pharmacological concentrations of zinc oxide with or without the inclusion of an antibacterial agent on nursery pig performance. J Anim Sci. 2001;79:934-41. https://doi.org/10.2527/2001.794934x
7. Poulsen HD. Zinc and copper as feed additives, growth factors or unwanted environmental factors. J Anim Feed Sci. 1998;7:135-42. https://doi.org/10.22358/jafs/69961/1998
8. Oh HJ, Park YJ, Cho JH, Song MH, Gu BH, Yun W, et al. Changes in diarrhea score, nutrient digestibility, zinc utilization, intestinal immune profiles, and fecal microbiome in weaned piglets by different forms of zinc. Animals. 2021;11:1356. https://doi.org/10.3390/ani11051356
9. Ward TL, Asche GL, Louis GF, Pollmann DS. Zinc-methionine improves growth performance of starter pigs. J Anim Sci. 1996;74:182.
10. Case CL, Carlson MS. Effect of feeding organic and inorganic sources of additional zinc on growth performance and zinc balance in nursery pigs. J Anim Sci. 2002;80:1917-24. https://doi.org/10.2527/2002.8071917x
11. Schlegel P, Nys Y, Jondreville C. Zinc availability and digestive zinc solubility in piglets and broilers fed diets varying in their phytate contents, phytase activity and supplemented zinc source. Animal. 2010;4:200-9. https://doi.org/10.1017/S1751731109990978
12. National Research Council. Nutrient requirements of swine. 11th rev. ed. Washington, DC: The National Academies Press; 2012.

13. O'Doherty JV, Bouwhuis MA, Sweeney T. Novel marine polysaccharides and maternal nutrition to stimulate gut health and performance in post-weaned pigs. Anim Prod Sci. 2017;57:2376-85. https://doi.org/10.1071/AN17272
14. Heo JM, Kim JC, Hansen CF, Mullan BP, Hampson DJ, Pluske JR. Effects of feeding low protein diets to piglets on plasma urea nitrogen, faecal ammonia nitrogen, the incidence of diarrhoea and performance after weaning. Arch Anim Nutr. 2008;62:343-58. https://doi.org/10.1080/17450390802327811
15. Heo JM, Kim JC, Hansen CF, Mullan BP, Hampson DJ, Pluske JR. Feeding a diet with decreased protein content reduces indices of protein fermentation and the incidence of postweaning diarrhea in weaned pigs challenged with an enterotoxigenic strain of Escherichia coli. J Anim Sci. 2009;87:2833-43. https://doi.org/10.2527/jas.2008-1274
16. Rossi B, Toschi A, Piva A, Grilli E. Single components of botanicals and nature-identical compounds as a non-antibiotic strategy to ameliorate health status and improve performance in poultry and pigs. Nutr Res Rev. 2020;33:218-34. https://doi.org/10.1017/S0954422420000013
17. Zuo J, Ling B, Long L, Li T, Lahaye L, Yang C, et al. Effect of dietary supplementation with protease on growth performance, nutrient digestibility, intestinal morphology, digestive enzymes and gene expression of weaned piglets. Anim Nutr. 2015;1:276-82. https://doi.org/10.1016/j.aninu.2015.10.003
18. Kim YJ, Lee JH, Kim TH, Song MH, Yun W, Oh HJ, et al. Effect of low protein diets added with protease on growth performance, nutrient digestibility of weaned piglets and growing-finishing pigs. J Anim Sci Technol. 2021;63:491-500. https://doi.org/10.5187/jast.2021.e49
19. He X, Yu B, He J, Huang Z, Mao X, Zheng P, et al. Effects of xylanase on growth performance, nutrients digestibility and intestinal health in weaned piglets. Livest Sci. 2020;233:103940. https://doi.org/10.1016/j.livsci.2020.103940
20. Holodova M, Cobanova K, Sefcikova Z, Barszcz M, Tuśnio A, Taciak M, et al. Dietary zinc and fibre source can influence the mineral and antioxidant status of piglets. Animals. 2019;9:497. https://doi.org/10.3390/ani9080497
21. Zhang B, Guo Y. Beneficial effects of tetrabasic zinc chloride for weanling piglets and the bioavailability of zinc in tetrabasic form relative to ZnO. Anim Feed Sci Technol. 2007;135:75-85. https://doi.org/10.1016/j.anifeedsci.2006.06.006
22. Han YM, Roneker KR, Pond WG, Lei XG. Adding wheat middlings, microbial phytase, and citric acid to corn-soybean meal diets for growing pigs may replace inorganic phosphorus supplementation. J Anim Sci. 1998;76:2649-56. https://doi.org/10.2527/1998.76102649x
23. Johansen M, Jørgensen L, Schultz MS. Effect of zinc and organic acids on diarrhoea in the weaner period. Copenhagen: SEGES; 2007.
24. Nitrayova S, Windisch W, von Heimendahl E, Müller A, Bartelt J. Bioavailability of zinc from different sources in pigs. J Anim Sci. 2012;90:185-7. https://doi.org/10.2527/jas.53895
25. Zhang Y, Ward TL, Ji F, Peng C, Zhu L, Gong L, et al. Effects of zinc sources and levels of zinc amino acid complex on growth performance, hematological and biochemical parameters in weanling pigs. Asian-Australas J Anim Sci. 2018;31:1267-74. https://doi.org/10.5713/ajas.17.0739
26. Bonetti A, Tugnoli B, Piva A, Grilli E. Towards zero zinc oxide: feeding strategies to manage post-weaning diarrhea in piglets. Animals. 2021;11:642. https://doi.org/10.3390/ani11030642
27. Omonijo FA, Ni L, Gong J, Wang Q, Lahaye L, Yang C. Essential oils as alternatives to antibiotics in swine production. Anim Nutr. 2018;4:126-36. https://doi.org/10.1016/j.aninu.2017.09.001
28. Heo JM, Opapeju FO, Pluske JR, Kim JC, Hampson DJ, Nyachoti CM. Gastrointestinal

https://doi.org/10.5187/jast.2021.e120

health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhoea without using in-feed antimicrobial compounds. J Anim Physiol Anim Nutr. 2013;97:207-37. https://doi.org/10.1111/j.1439-0396.2012.01284.x
29. Park S, Lee JJ, Yang BM, Cho JH, Kim S, Kang J, et al. Dietary protease improves growth performance, nutrient digestibility, and intestinal morphology of weaned pigs. J Anim Sci Technol. 2020;62:21-30. https://doi.org/10.5187/jast.2020.62.1.21
30. Lei XJ, Kim IH. 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. 2018;245:117-25. https://doi.org/10.1016/j.anifeedsci.2018.06.011
31. Hu CH, Gu LY, Luan ZS, Song J, Zhu K. Effects of montmorillonite–zinc oxide hybrid on performance, diarrhea, intestinal permeability and morphology of weanling pigs. Anim Feed Sci Technol. 2012;177:108-15. https://doi.org/10.1016/j.anifeedsci.2012.07.028
32. Furbeyre H, van Milgen J, Mener T, Gloaguen M, Labussière E. Effects of dietary supplementation with freshwater microalgae on growth performance, nutrient digestibility and gut health in weaned piglets. Animal. 2017;11:183-92. https://doi.org/10.1017/S1751731116001543
33. Schlegel P, Sauvant D, Jondreville C. Bioavailability of zinc sources and their interaction with phytates in broilers and piglets. Animal. 2013;7:47-59. https://doi.org/10.1017/S1751731112001000
34. Wang H, Kim KP, Kim IH. Evaluation of the combined effects of different dose levels of zinc oxide with probiotics complex supplementation on the growth performance, nutrient digestibility, faecal microbiota, noxious gas emissions and faecal score of weaning pigs. J Anim Physiol Anim Nutr. 2021;105:286-93. https://doi.org/10.1111/jpn.13493
35. Halas D, Heo JM, Hansen CF, Kim JC, Hampson DJ, Mullan BP, et al. Organic acids, prebiotics and protein level as dietary tools to control the weaning transition and reduce post-weaning diarrhoea in piglets. CAB Rev Perspect Agric Vet Sci Nutr Nat Resour. 2007;2:079. https://doi.org/10.1079/PAVSNNR20072079
36. Yan L, Wang JP, Kim HJ, Meng QW, Ao X, Hong SM, et al. Influence of essential oil supplementation and diets with different nutrient densities on growth performance, nutrient digestibility, blood characteristics, meat quality and fecal noxious gas content in grower–finisher pigs. Livest Sci. 2010;128:115-22. https://doi.org/10.1016/j.livsci.2009.11.008
37. Li P, Piao X, Ru Y, Han X, Xue L, Zhang H. Effects of adding essential oil to the diet of weaned pigs on performance, nutrient utilization, immune response and intestinal health. Asian-Australas J Anim Sci. 2012;25:1617-26. https://doi.org/10.5713/ajas.2012.12292
38. Zeng Z, Xu X, Zhang Q, Li P, Zhao P, Li Q, et al. Effects of essential oil supplementation of a low-energy diet on performance, intestinal morphology and microflora, immune properties and antioxidant activities in weaned pigs. Anim Sci J. 2015;86:279-85. https://doi.org/10.1111/asj.12277
39. Platel K, Srinivasan K. Digestive stimulant action of spices: a myth or reality? Indian J Med Res. 2004;119:167-79.
40. Zhai H, Liu H, Wang S, Wu J, Kluenter AM. Potential of essential oils for poultry and pigs. Anim Nutr. 2018;4:179-86. https://doi.org/10.1016/j.aninu.2018.01.005
41. Star L, van der Klis JD, Rapp C, Ward TL. Bioavailability of organic and inorganic zinc sources in male broilers. Poult Sci. 2012;91:3115-20. https://doi.org/10.3382/ps.2012-02314
42. Li MZ, Huang JT, Tsai YH, Mao SY, Fu CM, Lien TF. Nanosize of zinc oxide and the effects on zinc digestibility, growth performances, immune response and serum parameters of weanling

piglets. Anim Sci J. 2016;87:1379-85. https://doi.org/10.1111/asj.12579
43. Jongbloed AW, Mroz Z, van der Weij-Jongbloed R, Kemme PA. The effects of microbial phytase, organic acids and their interaction in diets for growing pigs. Livest Prod Sci. 2000;67:113-22. https://doi.org/10.1016/S0301-6226(00)00179-2
44. Höhler D, Pallauf J. Untersuchungen zum einfluß von Citronensäure auf die mineralstoffverwertung beim ferkel anhand einer mais-soja-Diät mit und ohne Zn-ergänzung. J Anim Physiol Anim Nutr. 1993;69:133-42. https://doi.org/10.1111/j.1439-0396.1993.tb00797.x
45. Sauer W, Cervantes M, Yanez J, Araiza B, Murdoch G, Morales A, et al. Effect of dietary inclusion of benzoic acid on mineral balance in growing pigs. Livest Sci. 2009;122:162-8. https://doi.org/10.1016/j.livsci.2008.08.008
46. Cho JH, Liu SD, Yun W, Kim KS, Kim IH. Effect of supplemented microencapsulated zinc oxide and organic acids and pure botanicals on growth performance, nutrient digestibility, blood profiles, feces microflora, and zinc level of feces in weanling pigs. Can J Anim Sci. 2018;99:66-73. https://doi.org/10.1139/cjas-2017-0114
47. Haase H, Rink L. The immune system and the impact of zinc during aging. Immun Ageing. 2009;6:9. https://doi.org/10.1186/1742-4933-6-9
48. Knoell DL, Liu MJ. Impact of zinc metabolism on innate immune function in the setting of sepsis. Int J Vitam Nutr Res. 2010;80:271-7. https://doi.org/10.1024/0300-9831/a000034
49. Sun YB, Xia T, Wu H, Zhang WJ, Zhu YH, Xue JX, et al. 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. 2019;258:114312. https://doi.org/10.1016/j.anifeedsci.2019.114312
50. Ma Y, Huang Q, Lv M, Wu Z, Xie Z, Han X, et al. Chitosan-Zn chelate increases antioxidant enzyme activity and improves immune function in weaned piglets. Biol Trace Elem Res. 2014;158:45-50. https://doi.org/10.1007/s12011-014-9910-1
51. Jiao Y, Li X, Kim IH. Changes in growth performance, nutrient digestibility, immune blood profiles, fecal microbial and fecal gas emission of growing pigs in response to zinc aspartic acid chelate. Asian-Australas J Anim Sci. 2020;33:597-604. https://doi.org/10.5713/ajas.19.0057
52. Borah S, Sarmah BC, Chakravarty P, Naskar S, Dutta DJ, Kalita D. Effect of zinc supplementation on serum biochemicals in grower pig. J Appl Anim Res. 2014;42:244-8. https://doi.org/10.1080/09712119.2013.824888
53. Fang LH, Jin YH, Do SH, Hong JS, Kim BO, Han TH, et al. Effects of dietary energy and crude protein levels on growth performance, blood profiles, and carcass traits in growing-finishing pigs. J Anim Sci Technol. 2019;61:204-15. https://doi.org/10.5187/jast.2019.61.4.204
54. Lee JH, Kim HB, Yun W, Kwak WG, Lee CH, Oh HJ, et al. Effects of reducing dietary crude protein and metabolic energy in weaned piglets. S Afr J Anim Sci. 2017;47:574-81. https://doi.org/10.4314/sajas.v47i4.16
55. Zhao Y, Tian G, Chen D, Zheng P, Yu J, He J, et al. Effects of varying levels of dietary protein and net energy on growth performance, nitrogen balance and faecal characteristics of growing-finishing pigs. Rev Bras Zootec. 2019;48:e20180021. https://doi.org/10.1590/rbz4820180021
56. Upton RN. Organ weights and blood flows of sheep and pig for physiological pharmacokinetic modelling. J Pharmacol Toxicol Methods. 2008;58:198-205. https://doi.org/10.1016/j.vascn.2008.08.001
57. Yu T, Zhu C, Chen S, Gao L, Lv H, Feng R, et al. Dietary high zinc oxide modulates the microbiome of ileum and colon in weaned piglets. Front Microbiol. 2017;8:825. https://doi.org/10.3389/fmicb.2017.00825

https://doi.org/10.5187/jast.2021.e120

58. Pei X, Xiao Z, Liu L, Wang G, Tao W, Wang M, et al. 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. 2019;99:1366-74. https://doi.org/10.1002/jsfa.9312
59. Li BT, Van Kessel AG, Caine WR, Huang SX, Kirkwood RN. 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. 2001;81:511-6. https://doi.org/10.4141/A01-043
60. Vahjen W, Pieper R, Zentek J. Increased dietary zinc oxide changes the bacterial core and enterobacterial composition in the ileum of piglets. J Anim Sci. 2011;89:2430-9. https://doi.org/10.2527/jas.2010-3270
61. Sales J. Effects of pharmacological concentrations of dietary zinc oxide on growth of post-weaning pigs: a meta-analysis. Biol Trace Elem Res. 2013;152:343-9. https://doi.org/10.1007/s12011-013-9638-3
62. Opapeju FO, Krause DO, Payne RL, Rademacher M, Nyachoti CM. Effect of dietary protein level on growth performance, indicators of enteric health, and gastrointestinal microbial ecology of weaned pigs induced with postweaning colibacillosis. J Anim Sci. 2009;87:2635-43. https://doi.org/10.2527/jas.2008-1310
63. Petry AL, Patience JF, Koester LR, Huntley NF, Bedford MR, Schmitz-Esser S. Xylanase modulates the microbiota of ileal mucosa and digesta of pigs fed corn-based arabinoxylans likely through both a stimbiotic and prebiotic mechanism. PLOS ONE. 2021;16:e0246144. https://doi.org/10.1371/journal.pone.0246144
64. Petry AL, Patience JF. Xylanase supplementation in corn-based swine diets: a review with emphasis on potential mechanisms of action. J Anim Sci. 2020;98:skaa318. https://doi.org/10.1093/jas/skaa318
65. Mkabayi L, Malgas S, Wilhelmi BS, Pletschke BI. Evaluating feruloyl esterase—xylanase synergism for hydroxycinnamic acid and xylo-oligosaccharide production from untreated, hydrothermally pre-treated and dilute-acid pre-treated corn cobs. Agronomy. 2020;10:688. https://doi.org/10.3390/agronomy10050688
66. Mafa MS, Malgas S, Pletschke BI. Feruloyl esterase (FAE-1) sourced from a termite hindgut and GH10 xylanases synergy improves degradation of arabinoxylan. AMB Express. 2021;11:21. https://doi.org/10.1186/s13568-021-01180-1
67. Arzola-Alvarez C, Hume ME, Anderson RC, Latham EA, Ruiz-Barrera O, Castillo-Castillo Y, et al. Influence of sodium chlorate, ferulic acid, and essential oils on Escherichia coli and porcine fecal microbiota. J Anim Sci. 2020;98:skaa059. https://doi.org/10.1093/jas/skaa059
68. Kim SW, Chen H, Parnsen W. Regulatory role of amino acids in pigs fed on protein-restricted diets. Curr Protein Pept Sci. 2019;20:132-8. https://doi.org/10.2174/1389203719666180517100746