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Abstract

In this research, the growth efficiency, nutritional utilization, fecal microbial levels, and fecal score of weaned pigs were evaluated using therapeutic zinc oxide (ZnO) and zinc aspartic acid chelate (Zn-Asp). In a 42-day feeding trial, 60 weaned pigs [Yorkshire × Landrace) × Duroc] were arbitrarily allotted (age: 21 days; 7.01 ± 0.65 kg preliminary body weight) to 3 different treatment groups with 5 repetitions (2 male and 2 female piglets) in each pen. The trial had 2 different phases, including 1-21 days as phase 1, and 22-42 days as phase 2. The nutritional treatments were: basal diet as control (CON), basal diet incorporated with 3000 ppm ZnO as TRT1, and basal diet incorporated with 750 ppm Zn-Asp as TRT2. In comparison to the CON group, the pigs in the TRT1 and TRT2 groups had greater ($p < 0.05$) body weight on day 42; an average daily gain, and an average daily feed intake on days 22-42. Furthermore, during days 1-42, the average daily gain in the treatment groups trended higher ($p < 0.05$) than in the CON group. Additionally, the fecal score decreased ($p < 0.05$) at week 6, the lactic acid bacteria count tended to increase ($p < 0.05$), and coliform bacteria presented a trend in reduction ($p < 0.05$) in the TRT1 and TRT2 groups compared to the CON group. However, there was no difference in nutrient utilization ($p > 0.05$) among the dietary treatments. Briefly, the therapeutic ZnO and Zn-Asp nutritional approaches could decrease fecal score and coliform bacteria, increase lactic acid bacteria, and improve growth efficiency; moreover, Zn-Asp (750 ppm) can perform a comparable role to therapeutic ZnO (3000 ppm). So we can use Zn-Asp (750 ppm) instead of therapeutic ZnO (3000 ppm) for the better performance of weaning pigs and the reduction of environmental pollution, as therapeutic ZnO is responsible for environmental pollution.

Keywords: feed efficiency, growth performance, weaning pig, zinc acid replacement, zinc aspartic acid chelate

INTRODUCTION

Pharmacological zinc oxide (ZnO; 3000 ppm) has been widely applied in swine industry to ameliorate intestinal disturbances, diarrheas, and growth retardations induced by weaning incidents for the last decades [1, 2]. High amounts of ZnO were believed to have antibacterial properties and could treat diarrhea by lowering the community of microbes in the intestines and enterotoxigenic *E. coli* invasion [3]. Moreover, feeding therapeutic ZnO resulted in 80% of the ZnO being excreted through the feces due to the low absorption rate of ZnO causing a remarkable deterioration in the environment. Therefore, with the emergence of multi-resistant pathogenic microorganisms induced by ZnO supplementation, the utilization of pharmacological ZnO has been limited worldwide [4]. A law implemented by the European Union in 2017 mandates that the utilization of therapeutic ZnO in swine production be taken out by 2022. Nowadays, using a zinc source with higher bioavailability and possessing the ability to reduce drug resistance is a strategy to solve this problem [5, 2].

To this end, amino acid-chelated zinc is developed as a source of zinc for pigs diets. Since amino acid-chelated minerals and inorganic minerals have different absorption pathways, antagonism and interactions among trace minerals are avoided [6]. This makes amino acid-chelated organic minerals more absorbable than inorganic minerals [7, 8]. Organic zinc could increase retention through the small intestinal coagulation process and amino acid or peptide transportation system, as well as reduce soil pollution from heavy metals [9]. An organic type of zinc exhibiting greater accessibility would enable a lower dosage in feed and subsequently lower discharge in the atmosphere, providing advantages to livestock [10]. Low doses of porous and nano ZnO in the diet had a comparable (or even better) impact on weaning piglets' gastrointestinal structure, growth efficiency, reduced diarrhea, and intestinal inflammation than high doses of regular ZnO [5]. According to Jiao et al. [11], feeding growing pigs with a 1000 ppm Zn-Asp-containing diet could raise the lactic acid bacterium, reduce the coliform bacteria in feces, and subsequently improve nutrient digestibility, thus further improving growth performance. As compared to 3,000 mg/kg of conventional ZnO, Wang et al. [12] found that low concentrations (50 and 100 mg/kg) of zinc from zinc glycine chelate could increase growth rate, blood alkaline phosphatase, and copper/zinc antioxidant functions in weaning piglets. According to Mazzoni et al. [8], using 200 ppm of zinc glutamic acid chelate showed equal benefits in enhancing growth efficiency and lowering fecal coliform bacteria (CB) populations in young pigs as 2500 ppm of ZnO. As noted by Ren et al. [13], feeding weaned piglets with a 100 ppm zinc methionine hydroxy analogue chelate-containing diet had similar growth performance to that fed with a 2000 ppm ZnO-containing diet. Additionally, Hollis et al. [14] proved that the growth parameters in weaning pigs administered with 500 ppm zinc methionine chelate-containing diets were similar to those of pigs fed with 2500 ppm ZnO.

However, the effects of the Zn-Asp inclusion on the performance of growth, nutritional utilization, fecal bacterium levels, and fecal score in weaned piglets are still limited. We hypothesized that the administration of Zn-Asp could increase fecal beneficial bacteria counts and decrease fecal harmful microorganisms, enhance nutrient utilization, and lower the fecal score, thus ameliorating growth efficiency, as well as generate comparable effects to those of pharmacological ZnO. Therefore, the level of Zn-Asp at 750 ppm was used to compare the outcomes of pharmacological ZnO and Zn-Asp on the performance of growth, nutritional utilization, fecal bacterium levels, and fecal score in weaned piglets in the current study.

MATERIAL AND METHODS

In a 42-day feeding trial, 60 21-day-old weaned piglets [(Yorkshire × Landrace) × Duroc] with 7.01 ± 0.65 kg of preliminary body weight were erratically distributed to 5 replicate pens, with 4 piglets (2 males and 2 females) per pen. The trial period was divided into 2 phases: phase 1, days 1–21; phase 2, days 22–42. Dietary treatments were comprised of CON, TRT1 (a basal diet incorporating 3000 ppm ZnO), and TRT2 (a basal diet incorporating 750 ppm Zn-Asp). The ZnO utilized in our trial was feed-grade. The Zn-Asp was acquired from a commercial corporation (BTN Co., Asansi, Korea). In this experiment, aspartic acid made up 95% of the volume. Aspartic acid was directly linked to zinc2+ at a molecular concentration of 1:2 in the Zn-Asp compound, which contained 35% zinc [11]. The diet (1) was designed to meet or surpass the NRC's nutritional requirements (NRC, 2012) [15]. Additives and feed were thoroughly mixed using a feed mixer (Daedong Tech, DDK801F, Anyang-si, South Korea). Living conditions of pigs were environmentally maintained with slatted plastic flooring (0.6 m × 2.0 m × 0.5 m) and a one-sided stainless steel self-feeder and a nipple drinker installed to provide feed and water ad libitum in pigs. Beginning room temperatures were kept at 30℃ ± 1 and 60% relative humidity, and the light was regularly adjusted to provide twelve hours of artificially created light each day. The Dankook University Animal Care and Use Committee in Cheonan, Republic of Korea, authorized the research protocol (DK-1-2039) for this study.

Sample collection and measurements

Growth performance

All pigs were weighted individually on days 1, 21, and 42 to estimate the average daily gain (ADG). Values were presented on an average data in pen basis. On a pen-by-pen basis, the average daily feed intake (ADFI) was assessed each day. Values of ADG and ADFI were utilized to calculate the feed efficiency (gain to feed ratio, G/F).

Nutrient utilization

During days 35 to 41, a 0.20% chromium oxide (indigestible marker)-containing experimental diet was used to feed animals for measuring the nutrient utilization of dry matter (DM), nitrogen (N), and energy (E). After being combined, feed specimens were taken from every treatment group. On day 42, two randomly selected pigs from each pen were used to gather fecal samples using the rectal massage technique. Then, feed and fecal specimens were dried in an electric oven (70 ℃) for 72 h, and later they were crushed to pass through a 1-mm sieve and collected. The DM, N, and E in feed and feces were assessed using the AOAC [16] method. The concentration of chromium was determined using ultraviolet spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan). The energy was measured as the heat of combustion in the specimens, utilizing a bomb calorimeter (Parr 6100; Parr Instrument Co., Moline, IL). The indirect ratio methods were used to calculate the apparent total tract digestibility (ATTD) using Park et al. [17]'s procedure. $\text{ATTD} (\%) = [1 - (Nf \times Cd)/ (Nd \times Cf)] \times 100$, where Nf denotes the nutrient concentration in feces (% DM), Nd denotes the nutrient concentration in diet (% DM), Cd denotes the chromium concentration in diet (% DM), and Cf denotes the chromium concentration in feces (% DM).

Fecal bacteria counts

During day 42, 2 pigs were chosen arbitrarily from every pen to collect feces using the rectal massage technique to count the coliform bacteria (CB) and lactic acid bacteria (LAB) present in the feces. Then the samples were gathered on a pen basis, put in an ice box, and then moved to the experimental laboratory. The combined fecal specimens from every pen were mixed after being diluted with 9 mL of 1% peptone broth. The microbial counts were determined by 10-fold dilution and cultured on MacConkey agar for CB (Difco Laboratories, Detroit, MI) and *Lactobacilli* medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) for LAB. The *Lactobacilli* medium III agar plates were incubated under an anaerobic atmosphere for 48 hours at 39 ℃ while the MacConkey agar plates were incubated under an anaerobic atmosphere for 24 hours at 37 ℃. Colony amounts were then totaled, and the results were presented as log10 transformed data.

Fecal score

At 8:00 and 20:00 h, the fecal score was calculated on days 1, 21, and 42. Using a 5-grade scoring method, the average value of four pigs from each pen served as the basis for calculating the fecal score. The fecal scoring method is standardized as follows: 1: hard, dry pellets in a small, hard mass; 2: firm, formed, remaining solid and soft; 3: soft, formed, and moist, maintaining its shape; 4: loose, unformed, taking the shape of the container; 5: watery, liquid, pourable feces.

Statistical analysis

Using the one-way ANOVA, the variables were statistically examined in a randomly selected complete block design with the feeding strategies as the classifying variable. Duncan's multiple comparison tests were done to find out if the means were very different. The standard error of the means (SEM) was a way of expressing the data's variability. Significant differences were examined at $p < 0.05$ and trends were examined at $p < 0.10$.

RESULTS

Growth performance

Pigs in ZnO and Zn-Asp had higher body weight on day 42 ($p < 0.05$), ADG on days 22–42 ($p < 0.05$), and ADFI at days 22–42 ($p < 0.05$) compared to the CON group (Table 2). Furthermore, at days 1-42, ADG showed a trend ($p < 0.05$) of an increase in the ZnO and Zn-Asp groups compared to the CON group. However, there was no substantial change ($p > 0.05$) in the G/F ratio among treatments.

Nutrient utilization

Nutrient utilization is shown in Table 3. Feeding methods had no impact ($p > 0.05$) on the nutrient utilization of DM, N, and E.

Fecal bacteria counts

As shown in Table 4, feeding pigs ZnO and Zn-Asp included diet showed a trend in reduction ($p < 0.05$) on CB counts, along with, LAB tended to increase ($p < 0.05$) in ZnO and Zn-Asp group compared to CON.

Fecal score

The outcome of ZnO and Zn-Asp inclusion into the weaning pig diet is presented in Table 5. Dietary administration of ZnO and Zn-Asp reduced the fecal score ($p < 0.05$) at week 6 than CON.

DISCUSSION

Organic sources of minerals for dietary administration, such as amino acid chelate, have gained popularity in feed commerce over the last two decades owing to their greater accessibility [18]. According to Jiao et al. [11], growing piglets' BW, ADG, and G:F were substantially enhanced by a dietary Zn-ASP-supplemented diet. Our study's therapeutic ZnO and Zn-Asp methods of feeding showed an increase in ADG and ADFI, which helped to enhance growth performance by allowing the animals to consume more nutrient components. In line with our study, the administration of ZnO and Zn-Gly chelate into the weaning pig diet enhanced ADG and ADFI while not differing in feed/gain ratio (F:G) [12]. In comparison to the CON diet, organic sources of Zn did not increase gain, feed intake, or feed efficiency in weaning pigs, in contrast to our study [14]. Similarly, Liu et al. [7] stated that the administration of organic trace minerals had no impact on the growth performance of pigs. Piglets in the nano ZnO groups demonstrated significantly greater ADG than the negative CON group from weaning to 28 days after weaning [5]. The dietary supplementation of Zn amino acid in the weaning pig diet increased ADG and decreased F:G [19]. Therefore, dietary incorporation of pharmacological ZnO or Zn-Asp was helpful to enhance the growth efficiency of weaning pigs, and this was associated with the improvement of feed intake.

Since the 1990s, weaning piglets' diets have included zinc oxide to reduce weaning stress, strengthen impaired immune systems, and treat problems with digestion. As mentioned by Oh et al. [9], nutrient digestibility was considerably greater in groups receiving zinc supplements that were chelated with glycine than in groups receiving other treatments. Jiao et al. [11] showed that feeding a Zn-Asp diet to growing pigs improved apparent DM digestibility, but the ATTD of N and E did not differ significantly. The administration of ZnO in the weaning pig diet increased nutrient utilization of DM, but gross energy did not affect the animals significantly [20]. Hu et al. [21] showed that adding zinc to diets could enhance the function of enzymes responsible for digestion in the gut and intestinal tissue, leading to better digestibility. Pigs given a low dose of the diet containing coated zinc oxide had a higher coefficient of DM digestibility than other treatment groups [22]. In our study, the ZnO or Zn-Asp feeding strategies had no significant effects on the nutrient digestibility of weaning pigs. To some extent, differences in results may be explained by animal breed, dosage, and the source of the ZnO and Zn-Asp.

To ensure beneficial nutritional absorption and/or efficient utilization of feed in newborn pigs, a decreased number of harmful microorganisms and/or a greater proportion of helpful microbes in the gut are necessary [23]. In addition, the fecal bacteria regulation effects of Zn-Asp supplementation have also been observed by Jiao et al. [11], who stated that the addition of 1000 or 2000 ppm Zn-Asp could result in higher lactic acid bacterium levels and lower CB levels of feces in growing pigs, which agrees with our study. Lee et al. [20] stated that dietary inclusion of 3400 ppm ZnO could increase intestinal total anaerobic bacteria counts and decrease CB levels. Upadhaya et al. [24]

demonstrated that providing weaning pigs with a 2500 ppm ZnO-included diet improved fecal LAB amounts and decreased fecal CB amounts. The excellent effects of pharmacological ZnO supplementation on the reduction of fecal coliform bacteria and the increase of fecal lactic acid bacteria in weaning pigs have been reported widely [20, 25]. In the current experiment, we also found that pigs fed the diet with pharmacological ZnO and Zn-Asp supplemented diets had reduced fecal CB counts and increased fecal LAB counts. In weaning pigs, *Lactobacillus* counts were enhanced by zinc-chelated inclusion compared to the treatment group [9]. Zinc oxide inclusion lowers membrane absorption by increasing the production and synthesis of adhesion molecules and prevents gut adhesion molecules breakdown by preventing dangerous germs from adhering to vascular endothelium in the intestine thus increasing beneficial bacteria and decreasing harmful bacteria [26].

Reducing diarrhea is additionally an approach for enhancing the growth efficiency of weaning pigs because weaning is the most dangerous stage for affecting diarrhea. Coliform bacteria are the predominant pathogenic strain causing post-weaning diarrhea and could produce one or more enterotoxins when colonizing the cell membranes of the gut, thus inducing increased gut permeability, which was manifested in diarrhea [27]. So, it is essential to maintain the number of CB and enhance the number of LAB (beneficial bacteria) in the intestine to reduce the chance of causing diarrhea. In agreement with the current study, Castillo et al. [10] stated that feeding weaning pigs' organic zinc, which is linked to amino acid residues and a variety of polypeptides, tends to decrease the enterobacteria counts in the jejunum, and significantly decrease the fecal score. Similarly, zinc oxide nanoparticle supplementation has excellent effects on the amelioration of post-weaning diarrhea, as indicated by a reduced fecal score [28]. Conversely, the dietary incorporation of a higher dose (3000 ppm) or lower dose (300 ppm) of ZnO had no significant impact on the fecal score [29]. Weaning pigs were fed low dosages of porous and nano ZnO, which had a lower incidence of diarrhea than high amounts of regular ZnO [5]. Weanling pigs' diarrhea rates and diarrhea indices did not vary when Zn-amino acid was included in the diet [19]. Therefore, the reduction of fecal CB counts and increased LAB counts induced by pharmacological ZnO or Zn-Asp feeding strategies were considered the reason for reducing diarrhea, as reflected in the fecal score.

CONCLUSION

Taken together, our results suggest that supplementation of ZnO (3000 ppm) and Zn-Asp (750 ppm) could enhance growth performance, regulate fecal bacteria amounts, and decrease fecal scores. Zn-Asp was implemented as a substitute for medicinal ZnO since nutritional incorporation with Zn-Asp had comparable outcomes to that of medicinal ZnO on growth performance, fecal microbial counts, and fecal score in weaning pigs. Weaning pigs on diets containing Zn-Asp would be advantageous in economic and environmental aspects. Therefore, Zn-Asp could be utilized as a stimulant of growth and a potential environmental pollution reducer in weaned piglets instead of the medicinal ZnO.

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Table 1. Formula and composition of experimental diet (as fed-basis)

	Phase 1 (days 1-21)			Phase 2 (days 22-42)		
	CON	**TRT1**	**TRT2**	**CON**	**TRT1**	**TRT2**
Ingredients, %						
Corn	52.05	51.67	52.02	58.86	58.48	58.83
Soybean meal (48% crude protein)	16.74	16.74	16.70	22.60	22.60	22.56
Fermented soybean meal (45% crude protein)	4.00	4.00	4.00	3.00	3.00	3.00
Spray-dried porcine plasma	3.00	3.00	3.00	-	-	-
Tallow	2.82	2.82	2.74	2.77	2.77	2.69
Lactose	7.78	7.78	7.78	3.18	3.18	3.18
Sugar	3.00	3.00	3.00	3.00	3.00	3.00
Whey protein	7.00	7.00	7.00	3.00	3.00	3.00
Monocalcium phosphate	1.08	1.08	1.08	1.15	1.15	1.15
Limestone	1.20	1.20	1.20	1.22	1.22	1.22
Salt	0.10	0.10	0.10	0.10	0.10	0.10
Methionine	0.15	0.15	0.15	0.08	0.08	0.08
Lysine	0.65	0.65	0.65	0.61	0.61	0.61
Mineral mixture[1]	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin mixture[2]	0.20	0.20	0.20	0.20	0.20	0.20
Choline (50%)	0.03	0.03	0.03	0.03	0.03	0.03
Zinc oxide	-	0.38	-	-	0.38	-
Zinc aspartic acid chelate	-	-	0.15	-	-	0.15
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated value, %						
Metabolizable energy, MJ/kg	14.24	14.24	14.24	14.03	14.03	14.03
Crude protein	18.00	18.00	18.00	18.00	18.00	18.00

Calcium	0.80	0.80	0.80	0.80	0.80	0.80
Phosphorus	0.60	0.60	0.60	0.60	0.60	0.60
Lysine	1.50	1.50	1.50	1.40	1.40	1.40
Methionine	0.40	0.40	0.40	0.35	0.35	0.35
Crude fat	4.91	4.91	4.83	5.14	5.07	5.07

[1]Provided per kg diet: Fe, 100 mg as ferrous sulfate; Cu, 17 mg as copper sulfate; Mn, 17 mg as manganese oxide; I, 0.5 mg as potassium iodide; and Se, 0.3 mg as sodium selenite.

[2]Provided per kilograms of diet: vitamin A, 10,800 IU; vitamin D_3, 4,000 IU; vitamin E, 40 IU; vitamin K_3, 4 mg; vitamin B_1, 6 mg; vitamin B_2, 12 mg; vitamin B_6, 6 mg; vitamin B_{12}, 0.05 mg; biotin, 0.2 mg; folic acid, 2 mg; niacin, 50 mg; D-calcium pantothenate, 25 mg.

Table 2. Comparison of the effects of zinc oxide and zinc aspartic acid chelate on growth performance in weaning pigs[1]

Items	CON	TRT1	TRT2	SEM	p-value
Body weight, g					
Day 1	7.02	7.01	7.00	0.16	0.999
Day 21	14.08	13.69	13.72	0.17	0.624
Day 42	23.54^{b}	24.5^{a}	24.77^{a}	0.22	0.042
ADG, g					
Days 1-21	336	318	320	5.32	0.321
Days 22-42	450^{b}	517^{a}	526^{a}	12.44	0.006
Days 1-42	393	417	423	5.97	0.084
ADFI, g					
Days 1-21	395	374	374	6.72	0.357
Days 22-42	614^{b}	685^{a}	703^{a}	15.29	0.021
Days 1-42	516	529	538	5.68	0.308
Feed efficiency					
Days 1-21	0.85	0.85	0.85	0.004	0.935
Days 22-42	0.73	0.75	0.74	0.005	0.267
Days 1-42	0.76	0.78	0.78	0.005	0.125

[1] Abbreviation: CON, basal diet; TRT1, basal diet + 3000 ppm ZnO; TRT2, basal diet + 750 ppm Zn-Asp. ADG, average daily gain; ADFI, average daily feed intake. SEM, Standard error of the mean.

^{a,b} Means in the equivalent row show the superscripts differ ($p < 0.05$).

Table 3. Comparison of the effects of zinc oxide and zinc aspartic acid chelate on apparent total tract digestibility in weaning pigs[1]

Items, (%)	CON	TRT1	TRT2	SEM	p-value
Dry matter	80.13	80.82	81.40	0.58	0.710
Nitrogen	78.69	78.95	79.00	0.54	0.974
Energy	79.36	79.92	79.95	0.28	0.677

[1] Abbreviation: CON, basal diet; TRT1, basal diet + 3000 ppm ZnO; TRT2, basal diet + 750 ppm Zn-Asp. SEM, Standard error of the mean.

[a,b] Means in the equivalent row show the superscripts differ ($p < 0.05$).

Table 4. Comparison of the effects of zinc oxide and zinc aspartic acid chelate on fecal bacteria counts in weaning pigs[1]

Items, log10cfu/g	CON	TRT1	TRT2	SEM	p-value
CB	6.36	6.30	6.19	0.03	0.087
LAB	9.40	9.50	9.62	0.03	0.070

[1] Abbreviation: CON, basal diet; TRT1, basal diet + 3000 ppm ZnO; TRT2, basal diet + 750 ppm Zn-Asp. CB, coliform bacteria; LAB, lactic acid bacteria; SEM, Standard error of the mean.

[a,b] Means in the equivalent row show the superscripts differ ($p < 0.05$).

Table 5. Comparison of the effects of zinc oxide and zinc aspartic acid chelate on fecal score in weaning pigs

Item	CON	TRT1	TRT2	SEM	p-value
Fecal score					
Initial	4.0	4.0	3.8	0.05	0.276
Week 3	3.8	3.6	3.4	0.08	0.158
Week 6	3.7[a]	3.0[b]	2.9[b]	0.14	0.028

[1] Abbreviation: CON, basal diet; TRT1, basal diet + 3000 ppm ZnO; TRT2, basal diet + 750 ppm Zn-Asp. SEM, Standard error of the mean.

[a,b] Means in the equivalent row show the superscripts differ ($p < 0.05$).