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Nano-sized Zinc in Broiler Chickens: Effects on Growth Performance, Zinc Concentration in Organs, and Intestinal Morphology

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The effects of dietary supplementation of zinc (Zn) sources and concentrations were investigated on growth performance, absorption into tissues, fecal excretion, nutrient retention, and intestinal morphology in broilers fed a corn-soybean meal basal diet. A total of 525 one-day-old chicks (Ross 308) were assigned based on body weight to seven dietary treatments. There were five replicate pens for each treatment and 15 broilers per replicate pen. The dietary treatments included a basal diet (control, without supplementing Zn), and basal diet supplemented with Zn, as inorganic zinc sulfate (ZnS; 110 mg/kg); organic Zn-methionine (ZnM; 110 mg/kg); hot-melt extruded (HME) 25 zinc sulfate (27.5 mg/kg); HME50 zinc sulfate (55 mg/kg); HME75 zinc sulfate (82.5 mg/kg); or HME100 zinc sulfate (110 mg/kg) for 35 days in two phases (d 1−21, phase I and d 22−35, phase II). Bodyweight and feed efficiency of broiler chicks fed diets supplemented with increasing dietary concentrations of HME-Zn improved linearly during the study period ($P<0.05$). Compared to the control treatment, the ZnS, ZnM, and HME diets increased Zn concentrations in the serum and liver. Inorganic ZnS supply resulted in the highest Zn concentration in excreta. Increasing supplemented Zn content in diets as HME linearly increased Zn concentration in the excreta, serum, liver, and tibia. Broiler chicks fed diets supplemented with increasing concentrations of HME increased villus height (VH; linear and quadratic) of the jejunum and VH of the ileum (linear). Increasing concentrations of dietary Zn supplied as HME resulted in linearly enhanced dry matter, gross energy, and nitrogen retention of broilers on day 21. These results suggest that dietary HME-Zn at a lower level (55 ppm) shows the same growth performance as common $ZnSO_4$ at 110 ppm.

Key words: bioavailability, broiler chickens, inorganic, nano-zinc, organic

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Introduction

Zinc (Zn) is an essential mineral for animals and is commonly supplemented to the diet from inorganic (oxides and sulfates) or organic (bioplexes and chelates) sources. In chickens, Zn is required for skin quality, collagen synthesis, skeletal development, and immune responses (Salim *et al*., 2011; Kakhki *et al*., 2016). Zinc also enhances antioxidant activity because it is involved in glutathione peroxidase production (Saleh *et al*., 2018). Thus, Zn deficiency may compromise the growth performance of chickens. Recent guideline by Aviagen (2019) shows a higher Zn requirement (110 mg/kg) for broiler chicks than was previously recommended (40 mg/kg) in NRC (1994). In recent years, the overuse of Zn in the diets to obtain maximum growth has been a subject of increasing concern due to the excessive environmental pollution because of Zn excretion. Previous studies suggest that organic Zn has a higher bioavailability

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compared with inorganic salts because of the ligand, which protects Zn from reacting with phytates (Star *et al*., 2012; Sahraei *et al*., 2013; Swain *et al*., 2016). In addition, in order to find low-cost alternatives, researchers have focused on nano-Zn sources in the animal feed industry (Lee *et al*., 2017). Previous experiments have suggested that Zn in a nano form at lower doses (20 mg/kg) has a greater growth performance compared with the conventional source (60 mg/kg; Zhao *et al*., 2014). Information covering the effects of nano-Zn sources and the optimum level of dietary supplementation to meet Zn requirements in broilers are limited in the poultry science literature. The properties of nano-sized minerals are different from conventional sources because of their smaller size, larger surface area, and higher biological potential (Chokshi and Zia, 2010; Mahmoud *et al*., 2016). However, the kind of nano-process also changes the bioavailability of nanoparticles. The new generation of nanoparticles may have the potential to increase the dispersion of nanoparticles in the final product.

Hot-melt extrusion (HME) is a continuous mixing process to melt or solubilize ingredients and medicines by using a combination of temperature, pressure, moisture, and mechanical shear inside the matrix of polymer to improve solubility and bioavailability (Lee *et al*., 2017). It is an advanced and environmentally friendly production process, which is widely used in the food (Chokshi and Zia, 2010), and pharmaceutical industries (Lee *et al*., 2017). The HME processing in this investigation included Soluplus as the main polymer matrix. Soluplus is a copolymer with binding properties in wet or dry granulation to increase the solubility and dispersion of poorly water-soluble drugs (Lee *et al*., 2017), which may reduce environmental pollution through the excretion of trace minerals by improving their absorption and utilization. The present study was designed to determine the bioavailability of Zn from ZnS, ZnM, and HME sources in broilers fed a Zn-deficient basal diet. In addition, this study measures the effects of supplemented dietary Zn source and concentration on growth performance, absorption into tissues and fecal excretion, nutrient retention, and intestinal morphology in broilers fed a corn-soybean meal-based diet.

Material and Methods

The experimental design for this study was permitted by the Committee of Institutional Animal Care and Use of Kangwon National University (KW-170519-1), Chuncheon 24341, Republic of Korea.

Preparation of HME

$ZnSO_4 \cdot H_2O$ was purchased from TMC Co., Ltd. (Anyang, Korea). Soluplus was prepared from BASF (Ludwigshafen, Germany). Polyethylene glycol, polyvinyl caprolactam, and polyvinyl acetate are the main components of Soluplus, which is highly amphiphilic. Zinc sulfate nanoparticles were designed and produced using hot melting extrusion techniques by maintaining optimum processing conditions. The production assay was carried out as suggested by Lee *et al*. (2017). Briefly, the optimum conditions during HME operations were: temperature of 50−55℃; the speed of screw at 150 rpm; diameter of die at 1.0 mm, and production of 3 kg/h. Soluplus (grafted copolymer) and $ZnSO_4$ were mixed (7:3, w/w) and extruded by a twin-screw hot-melt extruder (STS-25HS, Hankook E.M. Ltd., Pyeongtaek, Korea) equipped with a round-shaped die (2 mm diameter) to produce HME. The speed of the screw was set to 200 rpm and extrudates were cooled and pulverized by the HBL-3500S grinder (Samyang Electronics Co., Gunpo, Korea). The particle size of HME Zn powder was evaluated at pH＝6.8 (small intestine simulation) by dynamic light scattering and laser Doppler methods (ELS-Z1000; Otsuka Electronics, Tokyo, Japan) following the manufacturer's instructions. The mean particle size of $ZnSO_4$ as HME was 76.5±3 nm.

Birds, Diets, and Management

A total of 525 one-day-old broilers (Ross 308; average BW 44.2±0.9 g) were assigned to 7 dietary treatments based on body weight for 35 days in 2 phases (d 1−21, phase I and d 22−35, phase II). As the requirement of Zn for broiler chicks is suggested to be 110 mg/kg (Aviagen, 2019), the dietary treatments consisted of control (without Zn supplementation), ZnS (110 mg Zn as $ZnSO_4$/kg diet), ZnM (110 mg Zn as Zinc methionine/kg diet), HME25 (27.5 mg nano-Zn as $ZnSO_4$/kg diet; 25% of the requirement), HME50 (55 mg nano-Zn as $ZnSO_4$/kg diet; 50% of the requirement), HME75 (82.5 mg nano-Zn as $ZnSO_4$/kg diet; 75% of the requirement), HME100 (110 mg nano-Zn as $ZnSO_4$/kg diet; 100% of the requirement). Analyzed Zn concentrations of experimental diets are presented in Table 1. There were 5 replicate pens in each treatment and 15 broilers per replicate pen. Dietary Zn was added to the diet through mineral supplements based on treatments. The experimental diets were fed in a crumble diet during the first week and pelleted diet for the rest of the study. Supplemental Zn was added to the treatment diets prior to pelleting. In phase I, diets were prepared to contain 3100 kcal/kg metabolisable energy (ME) and 21.50% crude protein (CP). Diets for phase II were formulated to contain 3200 kcal/kg ME and 19.50% CP. The diets were supplemented with vitamins, minerals (excluding Zn), and amino acids (AA) to meet or exceed the nutrient

Table 1. **Zinc (Zn) concentrations in the experimental diets for broilers (phase I and phase II). Values are means (±SD) of *n*＝4**

Treatments[a]	Phase I	Phase II
ZnS	142.1±1.0	132.9±1.3
ZnM	141.2±2.1	131.6±2.2
Control	29.3±0.6	24.6±0.2
HME25	53.9±1.8	51.3±0.6
HME50	84.2±2.0	79.3±0.9
HME75	113±1.3	109.3±1.1
HME100	144.6±1.6	133.2±1.3

[a] ZnS, 110 ppm Zn as zinc sulfate; ZnM, 110 ppm Zn as Zn-methionine; Control, without zinc supplement; HME25, 27.5 ppm Zn as nano-zinc sulfate; HME50, 55 ppm Zn as nano-zinc sulfate; HME 75, 82.5 ppm Zn as nano-zinc sulfate, HME100, 110 ppm Zn as nano-zinc sulfate.

Table 2. **Ingredient and chemical composition of basal diet (as-fed basis)**

Item	Phase I (d 1−21)	Phase II (d 22−35)
Ingredients (%)		
Corn	50.67	57.71
Soybean meal (44.5%)	40.05	28.37
Corn gluten meal (60%)	—	5
Animal fat	5.23	5.02
Choline chloride (50%)	0.05	0.05
Limestone	1.45	1.36
Salt	0.3	0.3
Mono-Di-Calcium Phosphate	1.45	1.33
Vitamin premix[1]	0.1	0.1
Mineral premix[2]	0.15	0.15
Threonine (98%)	0.13	0.1
Lysine (55%)	0.06	0.21
Methionine (80%)	0.31	0.25
Phytase	0.05	0.05
Total	100	100
Chemical composition, calculated		
Metabolizable energy (kcal/kg)	3100	3200
Crude protein (%)	21.5	19.5
Calcium (%)	0.89	0.79
Available phosphorus (%)	0.44	0.4
Lysine (%)	1.15	1.03
Methionine (%)	0.56	0.51
Zinc (mg/kg)	32.95	25.2

[1] Provided per kg of diet: 9,000 IU vitamin A (palmitate), 1,800 IU vitamin D_3 (cholecalciferol), 30 mg vitamin E (dl-α-tocopheryl acetate), 1 mg vitamin K_3 (menadione), 1 mg vitamin B_1 (thiamin), 10 mg vitamin B_2 (riboflavin), 4 mg vitamin B_6 (pyridoxine), 0.02 mg vitamin B_{12} (cyanocobalamin), 30 mg niacin, 12 mg pantothenic acid, 0.50 mg folic acid, 0.20 mg biotin

[2] Provided per kilogram of diet: 80 mg Fe, 20 mg Cu, 120 mg Mn, 1.40 mg I, and 0.30 mg Se.

requirements (Table 2) for Ross 308 chickens (Aviagen, 2019).

The broilers were housed and reared in floor pens covered with fresh and dried rice hulls (8 cm depth). All broiler pens (1.43×1.84 m) were arranged with a round self-feeder (6.11 cm of feeder space per chick) and available nipple drinkers for easy access to feed and water. Plastic feeders and drinkers were used to minimize Zn contamination from the environment. During the first week, the humidity and temperature of the broiler house was controlled at 53% and 34℃, respectively. After the first week, the temperature was gradually decreased by 3℃/week and the humidity was decreased to 41%. After the temperature reached 23℃ it was maintained until the end of the study. The broilers were provided with 23-hour/day lighting. The light intensity was adjusted at 35 lux for the first week, and at 5 lux thereafter.

Experimental Procedure and Sampling

The one-day-old chicks were weighed individually at the start of the trial and on day 35. Feed that was not consumed was weighed at the end of the experiment and feed intake was calculated for phase I, phase II and, overall. Broiler chicken body weight gain, feed intake, and feed conversion ratio (FCR) were corrected for the weight of dead broilers. Nutrient retention trials were conducted during the phase I and phase II of the feeding trial to determine the retention of dry matter (DM), gross energy (GE), and CP. From day 18 and day 33 onwards, two birds from each replicate around the average body weight of the pen were allocated to individual cages (2 birds/cage; 68.5×46 cm) to facilitate the collection of excreta samples. The diets containing 2.5 g/kg chromium as an indigestible marker was given. Two broilers from each replicate were separated and reared in the cage for excreta collection and excreta samples were collected using individual trays from each cage during d 18−21 and d 33−35. The excreta samples were dried in a forced air drying oven at 65℃ for 48 hours, feathers were removed from the samples, and the samples were ground using a 1-mm screen in a Wiley mill (Thomas Wiley® Mill, Model 4, Thomas scientific, USA). The nutrient retention was calculated as: nutrient retention (%)=100−[100×(% Cr in feed/% Cr in excreta)×(% nutrient in excreta/% nutrient in feed)].

On day 35, the anticoagulant sodium heparin (Becton Dickinson, NJ) containing disposable vacutainer tubes was used to collect blood samples (10 mL) by jugular vein puncture for measuring Zn concentration from 2 randomly selected healthy broilers in each pen. Serum samples were separated by centrifugation (at 4℃, 3,000×*g* for 15 min) and stored at −20℃ for measuring Zn concentrations later. On the last day (day 35) of the experiment, 35 birds (1 bird/replicate) were randomly picked and sacrificed by cervical dislocation. Liver and right tibia were collected from one bird of average weight from each cage (5 birds per treatment) for Zn concentration analysis. To study morphological changes in the small intestine of broilers samples from each region of the intestinal segments (duodenum, jejunum, and ileum) were collected after flushing and fixating the tissues. The contents of the small intestine were flushed with physiological saline, and the tissues were then soaked in a fixative standard solution (pH 7.3, 0.1 M collidine buffer) containing 20 g/L paraformaldehyde, 15 g/L acrolein and 30 g/L glutaraldehyde, and brought to the laboratory for further analysis.

Chemical Analyses

Dry matter (method 930.15) and nitrogen (N; method 990.03) were measured in feed and excreta triplicate samples following AOAC (2007). An oxygen bomb calorimeter was used to determine the GE of diets and excreta (Model 1261, Parr Instrument Co., IL). The concentration of chromium in diet and excreta was measured with an automated spectrophotometer (Jasco V-650, Jasco Corp., Japan) following the Fenton and Fenton (1979) procedure.

Zinc Determination

Zinc content in feed, excreta, serum, liver, and tibia was determined in dissolved ashes prepared following AOAC (2007) using inductively coupled plasma emission spectroscopy (ICP). Feed and excreta samples were measured in triplicates for Zn determination and 1 g of ground feed and fecal samples were dry ashed for 1 h in a muffle furnace at

600℃. Then, the ashed samples were cooled, dissolved by adding 10 mL 50% HCl (v/v), and kept covered overnight. The samples were filtered using Whatman filter paper in a 100 m*l* volumetric flask by washing crucibles 2−3 times and diluted with deionized distilled water and Zn concentrations were measured by ICP.

For serum analysis, 1 mL of sample was dispensed into a porcelain crucible, oven-dried for 4 hours at 105℃, and then ashed for 1 h at 600℃ in a muffle furnace. Liver samples were dried for 24 h at 105℃, ground in a stainless-steel blade grinder, and 1 g of liver samples were dry ashed at 600℃ for 1 h in a muffle furnace. After removing the soft tissues from tibia bones, the bones were dried for 24 h at 105℃ and ashed at 600℃ for 1 h in a muffle furnace. Then dry ashed serum, liver and 0.5 g of tibia ash samples were dissolved by adding 10 mL 50% HCl (v/v) and kept covered overnight. The samples were filtered using Whatman filter in a 100 mL volumetric flask by washing crucibles 2−3 times and diluted with deionized distilled water and Zn concentrations were measured by ICP.

Small Intestinal Morphology

Three cross-sections for each intestinal sample were prepared after staining with azure A and eosin using standard paraffin embedding procedures (Hosseindoust *et al*., 2017a). Well-oriented crypt-villus groups (total 10 intact) were chosen in triplicates for analyzing each intestinal cross-section. Crypt depth was characterized as the depth of the invagination between two villi, the width of the villi was determined at the mid of the villus, the height of the villi was determined from the villus-crypt junction to the edge of the villi. By using an image processing and analyzing system all of the morphological characteristics were measured (crypt depth or villus height) in 10-μm increments (Media Cyber genetics, Optimus software version 6.5, North Reading, MA).

Statistical Analyses

Statistical analysis of the current experimental data was completed by using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) in a completely randomized design. Significant differences among the treatment means were partitioned by using Tukey's Honestly Significant Difference test. The effects of increasing dietary HME concentrations in diets (0, 25, 50, 75, and 100%) were compared using orthogonal polynomial effects to assess linear and quadratic contrasts. Pens were considered the experimental unit for growth performance, and broiler chickens were experimental units for measuring the retention of nutrients and intestinal morphology. Data on the bioavailability of Zn (RBV) in excreta, serum, liver, and tibia from organic and HME Zn sources relative to zinc sulfate (analyzed; mg/kg) were analyzed by the slope-ratio assay method (Finney 1978). Probability values of <0.05 were considered significant.

Results

Growth Performance

The effects of dietary Zn source and concentration on chick growth performance are shown in Table 3. In phase I, there was a linear improvement in the weight gain and FCR as HME inclusion level increased ($P<0.01$). Dietary supplementation of ZnM and HME100 showed an increase in weight gain compared with the Zn-deficient diet (control) ($P<0.05$). Broiler chicks fed the diet supplemented with HME25 and control diets showed a higher FCR ($P<0.05$). There was no difference in feed intake among the treatments. In phase II, there was a linear and quadratic increase ($P<0.01$) in weight gain and a linear decrease ($P<0.05$) in FCR by the addition of HME to the diet. Broiler chicks in the HME25 and control treatments had the lowest ($P<0.05$) weight gain compared with the other treatments. Broiler chicks fed HME75 showed a lower FCR than chicks fed the

Table 3. **Effect of dietary Zn concentration and source on growth performance in broilers**

Item[1]	ZnS	ZnM	Control	HME[2]				SEM[3]		*P*-value[4]		
				25	50	75	100	TRT	LQ	TRT	L	Q
Phase I (d 1−21)												
Weight gain (g/bird)	731[ab]	758[a]	693[b]	694[b]	736[ab]	744[ab]	759[a]	6.2	7.5	<0.01	<0.01	0.917
Feed intake (g/bird)	1,019	1,045	1,045	1,022	1,021	1,036	1,032	6.4	8.0	0.871	0.878	0.438
FCR	1.40[b]	1.38[b]	1.51[a]	1.47[a]	1.39[b]	1.39[b]	1.36[b]	0.01	0.01	<0.01	<0.01	0.145
Phase II (d 22−35)												
Weight gain (g/bird)	1,227[a]	1,233[a]	1,189[b]	1,196[b]	1,225[a]	1,236[a]	1,231[a]	3.3	4.1	<0.01	<0.01	0.026
Feed intake (g/bird)	1,974	2,003	1,980	1,946	1,959	1,983	1,976	7.1	12.3	0.501	0.958	0.939
FCR	1.61[ab]	1.62[ab]	1.67[a]	1.63[ab]	1.60[ab]	1.60[b]	1.60[ab]	0.01	0.01	0.038	0.025	0.437
Overall (d 1−35)												
Weight gain (g/bird)	1,958[a]	1,991[a]	1,882[b]	1,891[b]	1,960[a]	1,980[a]	1,990[a]	7.7	9.5	<0.01	<0.01	0.235
Feed intake (g/bird)	2,993	3,048	3,025	2,968	2,979	3,019	3,008	8.9	16.5	0.215	0.958	0.645
FCR	1.53[b]	1.53[b]	1.61[a]	1.57[a]	1.52[b]	1.53[b]	1.51[b]	0.01	0.01	<0.01	<0.01	0.224

[1] Zn content (mg/kg diet): control, 0; ZnS (110 mg Zinc sulfate); ZnM (110 mg Zinc methionine); HME, 25% (27.5 mg), 50% (55 mg); 75% (82.5 mg); 100% (110 mg).
[2] Hot melt extrusion zinc sulfate.
[3−4] Standard error of means and *P*-value for all treatments (TRT), and linear (L) and quadratic (Q) effects of HME concentrations.
[ab] Means within a column with unlike superscripts differ significantly ($P<0.05$).

Table 4. **Effect of dietary Zn concentration and source on Zn content and bioavailability of excreta, serum, liver, and tibia in broilers (d 35)**

Item[1]	ZnS	ZnM	Control	HME[2]				SEM[3]		*P*-value[4]			RBV[5]	
				25	50	75	100	TRT	LQ	TRT	L	Q	ZnM	HME100
Excreta Zn (mg/kg)	524.5^{a}	430.1^{b}	169.3^{e}	183.6^{e}	272.6^{d}	328.9^{c}	422.9^{b}	19.4	17.6	<0.01	<0.01	<0.01	—	—
Serum Zn (mg/L)	2.00^{ab}	2.11^{a}	1.25^{d}	1.66^{c}	1.71^{bc}	2.14^{a}	2.13^{a}	0.06	0.07	<0.01	<0.01	0.281	—	—
Liver Zn (mg/kg)	113.7^{b}	139.6^{a}	102.8^{c}	113.2^{b}	117.2^{b}	134.4^{a}	137.3^{a}	2.21	2.59	<0.01	<0.01	<0.01	125 (119, 133)*	122 (115, 129)*
Tibia Zn (mg/kg)	183.5^{a}	184.5^{a}	144.2^{d}	148.4^{d}	161.9^{c}	173.7^{b}	185.3^{a}	2.68	2.22	<0.01	<0.01	<0.01	101 (96, 106)	101 (96, 106)

[1] Zn content (mg/kg diet): control, 0; ZnS (110 mg Zinc sulfate); ZnM (110 mg Zinc methionine); HME, 25% (27.5 mg), 50% (55 mg); 75% (82.5 mg); 100% (110 mg).
[2] Hot melt extrusion zinc sulfate.
[3–4] Standard error of means and *P*-value for all treatments (TRT), and linear (L) and quadratic (Q) effects of HME concentrations.
[5] RVB, relative bioavailability values relative to ZnS; Liver and tibia Zn content from zinc sulfate is 100%. * Different from the reference substance (100%), $P<0.05$.
[ae] Means within a column with unlike superscripts differ significantly ($P<0.05$).

control diet ($P<0.05$). The overall results showed that increasing dietary HME levels from 0 to 110 mg/kg improved weight gain by 5.7% (linear effect, $P<0.01$) and FCR by 6.2% (linear effect, $P<0.01$). The lowest weight gain and the highest FCR appeared in control and HME25 treatment groups ($P<0.01$). There was no difference between the treatment groups in the feed intake of broiler chicks.

Zn Concentrations and Bioavailability in Excreta, Serum, Liver, and Tibia

The effects of dietary Zn source and concentration on excreta, serum, liver, and tibia ash in broilers are shown in Table 4. There was a linear increase in the concentration of Zn in excreta, serum, liver, and tibia as the HME inclusion level increased ($P<0.01$). Similar quadratic trends were observed in the concentration of Zn in the excreta, liver, and tibia ($P<0.1$). Inorganic ZnS had greater Zn concentration in excreta than the other treatments ($P<0.01$). Broiler chicks fed the control diet had the lowest concentration of Zn in the serum and liver ($P<0.01$). Broiler chicks in the ZnM treatment group showed a higher ($P<0.01$) concentration of Zn in the liver compared with the ZnS, HME25, and HME50. The tibial Zn concentration of the broilers fed ZnM was not different from the ZnS and HME100 diets; however, these treatments had a higher ($P<0.05$) tibia Zn content compared with the control, HME25, HME50, and HME75 groups. ZnS was used as the reference at 100% to estimate the RBV of Zn in the ZnM and HME. The relative zinc RBV in the liver was significantly increased by the HME100 and ZnM compared with the chicks fed ZnS ($P<0.05$). Supplementation with the HME100 and ZnM did not influence Zn RBV in the tibia.

Intestinal Morphology

The effects of dietary Zn source and concentration on small intestinal morphology in broilers are shown in Table 5. At day 35, there was no difference in villus height or crypt depth of duodenum. Broiler chicks fed diets supplemented with increasing concentrations of HME increased villus height (linear and quadratic, $P<0.05$), crypt depth (linear, $P<0.05$) and villus height to crypt depth ratio (VH:CD; linear, $P<0.05$) in the jejunum, as well as villus height and VH:CD in the ileum (linear, $P<0.05$). Broiler diets supplemented with the ZnS, ZnM, and HME75 sources increased villus height of jejunum compared with broiler chicks fed the control, HME75, and HME50 diets ($P<0.05$). Moreover, the jejunal crypt depth of broiler chicks was lower in the HME25 and HME50 groups than the ZnS group. A greater VH:CD in the jejunum was observed in the HME75 group than in the ZnS, control, and HME25 groups. The study also revealed that both VH and VH:CD of the ileum was not statistically different between the ZnS, ZnM, and HME treatments. However, broiler chicks fed the control diet showed a lower ileal VH and VH:CD compared with the HME100.

Nutrient Retention

The effects of dietary Zn source and concentration on nutrient retention of broilers are shown in Table 6. In phase I, an increase in concentration of HME in diets resulted in linearly improved DM, GE, and N retention of broilers on day 21 (linear, $P<0.05$). Dietary supplementation of Zn as HME50 and HME75 significantly improved DM retention compared with the control group. Broiler chicks fed a ZnS diet showed a greater N retention ($P<0.05$) compared with broiler chicks fed the control diet. In phase II, N retention tended to increase (linear, $P=0.07$) with a dietary increasing concentration of HME. However, there was no difference in retention of DM, GE, and N among the treatments.

Discussion

In the present study, the Zn deficient (control) and HME25 diets negatively affected the overall weight gain. These results may suggest that the Zn levels in the control or HME25 diet groups were not sufficient for optimal growth perform-

Table 5. **Effect of dietary Zn concentration and source on intestinal morphology in broilers (d 35)**

Item[1]	ZnS	ZnM	Control	HME[2]				SEM[3]		*P*-value[4]		
				25	50	75	100	TRT	LQ	TRT	L	Q
Duodenum												
Villus height (μm)	1,637	1,595	1,579	1,610	1,630	1,554	1,648	16.8	22.4	0.779	0.587	0.963
Crypt depth (μm)	250	220	246	253	247	229	254	4.6	5.7	0.357	0.903	0.953
VH:CD[5]	6.58	7.29	6.46	6.39	6.69	6.79	6.6	0.10	0.11	0.295	0.468	0.952
Jejunum												
Villus height (μm)	1,319^{a}	1,324^{a}	1,187^{bc}	1,132^{c}	1,182^{bc}	1,281^{a}	1,275^{ab}	14.4	14.3	<0.01	<0.01	<0.01
Crypt depth (μm)	194^{a}	178^{ab}	174^{ab}	159^{ab}	140^{b}	144^{b}	170^{ab}	4.4	4.4	<0.01	0.147	0.019
VH:CD	6.88^{c}	7.51^{abc}	6.87^{c}	7.16^{bc}	8.55^{ab}	8.91^{a}	7.65^{abc}	0.18	0.23	<0.01	<0.01	0.246
Ileum												
Villus height (μm)	516^{ab}	614^{a}	462^{b}	579^{ab}	518^{ab}	620^{a}	616^{a}	15.0	19.9	<0.01	<0.01	0.914
Crypt depth (μm)	128	140	125	138	129	138	130	3.1	2.2	0.087	0.244	0.175
VH:CD	4.02^{ab}	4.39^{ab}	3.72^{b}	4.18^{ab}	4.01^{ab}	4.47^{ab}	4.72^{a}	0.05	0.12	0.035	<0.01	0.452

[1] Zn content (mg/kg diet): control, 0; ZnS (110 mg Zinc sulfate); ZnM (110 mg Zinc methionine); HME, 25% (27.5 mg), 50% (55 mg); 75% (82.5 mg); 100% (110 mg).
[2] Hot melt extrusion zinc sulfate.
[3–4] Standard error of means and *P*-value for all treatments (TRT), and linear (L) and quadratic (Q) effects of HME concentrations.
[5] villus height to crypt depth
[ac] Means within a column with unlike superscripts differ significantly ($P<0.05$).

Table 6. **Effect of dietary Zn concentration and source on nutrient digestibility (%) in broilers**

Item[1]	ZnS	ZnM	Control	HME[2]				SEM[3]		*P*-value[4]		
				25	50	75	100	TRT	LQ	TRT	L	Q
d 21												
Dry matter	71.4^{ab}	71.6^{ab}	69.4^{b}	70.5^{ab}	72.1^{a}	72.2^{a}	71.2^{ab}	0.25	0.41	0.032	<0.01	0.266
Gross energy	72.7	72.9	71.3	72.1	72.8	73.1	72.5	0.19	0.40	0.187	0.042	0.504
Crude protein	62.8^{a}	62.6^{ab}	59.2^{b}	60.1^{ab}	60.7^{ab}	62.2^{ab}	62.2^{ab}	0.35	0.52	0.019	<0.01	0.518
d 35												
Dry matter	66.4	65.6	65.1	64.7	66.4	65.9	65.4	0.26	0.32	0.605	0.317	0.688
Gross energy	68.5	69.8	68.5	67.4	68.5	67.1	68.3	0.26	0.29	0.148	0.380	0.272
Crude protein	57.3	57.6	56.5	56.2	56.2	58.5	57.3	0.28	0.57	0.256	0.068	0.196

[1] Zn content (mg/kg diet): control, 0; ZnS (110 mg Zinc sulfate); ZnM (110 mg Zinc methionine); HME, 25% (27.5 mg), 50% (55 mg); 75% (82.5 mg); 100% (110 mg).
[2] Hot melt extrusion zinc sulfate.
[3–4] Standard error of means and *P*-value for all treatments (TRT), and linear (L) and quadratic (Q) effects of HME concentrations.
[ab] Means within a column with unlike superscripts differ significantly ($P<0.05$).

ance. Zinc is a fundamental component of over 300 enzymes, playing a crucial role in cellular growth, reproduction, and carbohydrate and protein metabolism (Rossi *et al*., 2007). Consistent with previous research, diets low in Zn reduce weight gain (Sahraei *et al*., 2013). In addition, there was a linear increase in weight gain as dietary HME increased. However, growth performance alone cannot be considered as a sole factor to evaluate Zn requirement for broiler chicks, particularly because the control diet was based on corn and soybeans with a very low content of Zn. Evidence in the literature has shown that nano-Zn improves growth performance, feed efficiency and reduces production cost in pigs and poultry (Swain *et al*., 2016; Kim *et al*., 2017). However, an earlier study reported decreased growth performance and increased Zn concentration in the tibia of broilers fed a diet supplemented with nano-Zn compared to ZnM and conventional $ZnSO_4$ (Mohammadi *et al*., 2015). The lower performance in nano-Zn supplemented diets may be attributed to the high absorption of Zn owing to lower particle size and higher biological availability. Therefore, they compete with other minerals such as Fe and Cu, which share the same receptors for absorption (Skrivan *et al*., 2005). Indeed, the performance traits in chickens fed 50 mg/kg HME, was comparable to those of chickens fed 75 or 100 mg/kg HME, ZnS or ZnM. None of the performance traits were affected by the sources of Zn administration at standard level, showing that HME100 was likely to maintain more Zn than required for optimal growth. Therefore, the growth performance shows that a lower level of nano-Zn (HME50) can be a better option than conventional ZnS due to lower Zn excretion.

Zinc concentration in the excreta indicated a main effect of

dietary Zn content and source. Broiler chicks fed the ZnS diet showed the highest Zn concentration in excreta compared with the other treatments. Recent studies have shown that high levels of supplemental dietary Zn in broiler chickens consistently increased the amount of excreted Zn (Yuan *et al*., 2011). Mwangi *et al*. (2017) reported that the concentration of Zn in the excreta of birds fed the control diet supplemented with 40 mg/kg of Zn was higher than that from birds fed the control diet supplemented with 8 mg/kg of Zn. In fact, the higher Zn content in excreta of chicks fed ZnS in comparison to chicks fed HME100 or ZnM diets is attributed to the lower absorption efficiency. It seems that the bioavailability of nanoparticles can be evaluated with higher accuracy by measuring the concentration of minerals in the organs such as liver and tibia rather than in excreta of chicks because excreta is a mix of feces and urine. Several studies on broiler chicks have shown that nano-Zn has a higher bioavailability compared with the conventional Zn source due to the higher concentration of Zn in tibia or liver (Yuan *et al*., 2011; Zhao *et al*., 2014; Mohammadi *et al*., 2015). Nanoparticles possess a large active surface area and a high catalytic efficiency; they are easily absorbed and distributed into the heart, liver, kidney, spleen, lung, and blood (Swain *et al*., 2016). Therefore, nanoparticles can be transported directly to the target organs. The Zn level linearly increased in plasma as the level of dietary Zn increased. In contrast, Bartlett and Smith (2003) reported that the concentration of Zn in plasma was similar when different levels of Zn were added into the diet; however, tibia Zn concentration increased with increasing Zn levels. The analysis of Zn concentrations in liver showed that the liver Zn content increased in broiler chicks fed the HME75 and HME100 diets compared with broiler chicks fed the ZnS diet. These findings are consistent with those of Zhao *et al*. (2014) who found a greater concentration of Zn in the hepatic tissue of broiler chicks fed nano-Zn compared with conventional Zn. This result may be correlated with the lower Zn concentration in excreta of chicks fed ZnS, which confirms a lower Zn absorption.

In the present study, Zn source at the same level (ZnS, ZnM, and HME100) did not affect the tibia ash percentage. Similarly, Mwangi *et al*. (2017) reported that chicks supplied with feed containing ZnO and Bioplex (organic source) did not show any significant treatment effects on Zn content in tibia ash. However, Mohammadi *et al*. (2015) stated that Zn concentration in tibia significantly increased when chicks were fed diets supplemented with nano-Zn compared with conventional $ZnSO_4$. In addition, this study showed that lower doses of dietary Zn decreased tibia ash content. In contrast, Sahraei *et al*. (2012) reported that the Zn content in tibia ash from chicks did not vary with the supplementation of 100, 150, or 200 mg/kg of Zn supplied as organic Zn (Bioplex) or inorganic Zn (ZnO) in a corn-soybean diet. The inconsistency of Zn content in tibia ash in the literature may be associated with some other factors such as chicks' age or health status, environmental stress, and the quality of dietary Zn supplementation. Bioavailability of Zn may influence early bone development due to its importance in collagen synthesis, which largely influences the control of hydroxyapatite crystallization and the development of gene transcription with a role in ossification and cellular aggression of the cartilage matrix via the osteoblasts (Dibner *et al*., 2007). Bone mineralization can be affected by the dietary level of trace minerals (Mohammadi *et al*., 2015). The amount of bone Zn mineralization may be the best predictor of Zn bioavailability in the broiler (Star *et al*., 2012; Swain *et al*., 2016). The mineral metabolism is quite dynamic, and the bone Zn constitutes a critical reserve to be mobilized when Zn deficiency occurs (Mwangi *et al*., 2017). Star *et al*. (2012) reported that the Zn concentration in tibia ash increased when chicks were fed higher supplemental Zn. Similarly, Vieira *et al*. (2013) fed broilers with feed containing different levels of Zn and observed that chicks fed a diet with 0 ppm supplemental Zn had lower Zn concentration in tibia ash. The result of the present study shows that the dietary Zn level affects tibia Zn content more than the Zn source itself.

The present study demonstrated that chicks fed with 110 mg of Zn/kg as ZnS or ZnM had a greater villus height in the jejunum than those fed with the control diet. Furthermore, villus height in jejunum and ileum linearly increased by increasing dietary HME-Zn levels. In agreement, Ma *et al*. (2011) reported that the supplementation of Zn-Glycine Chelate at 90 ppm into the diet of broiler chicks increased villus height and decreased crypt depth of the jejunum in a 42-day feeding trial. Dietary Zn is known to have fundamental effects on repairing the epithelial cell by increasing the apoptotic resistance and proliferation index, which leads to improved villus height in the ileum of broiler chicks (Shao *et al*., 2014). The gut health status and poor nutrient absorption in a close relationship with the intestinal mucosa structure. Hosseindoust *et al*. (2017b) concluded that improved villus height in the duodenum of weaning piglets fed high dietary Zn enhanced the surface area for absorption, in turn leading to increased nutrient digestion capacity. In addition, the results of the present study showed that supplementation of dietary HME-Zn linearly increases the retention of DM, GE, and N in phase I. Dietary Zn supplementation improves the digestibility of nutrients by strengthening the development of intestinal morphology (Hosseindoust *et al*., 2017b). Studies have shown that nano-Zn plays an important role in the modification of tight junctions and improving the barrier roles of intestinal epithelial monolayers in rats (Lee *et al*., 2017). In agreement, the digestibility of DM was significantly increased in pigs supplemented with high concentrations of dietary Zn (Lee *et al*., 2016). They also reported a greater jejunal villus height in pigs fed a high Zn level. The increase in nutrient retention in response to Zn levels might have contributed to improved gut morphology.

In conclusion, the addition of Zn as ZnS, ZnM, and HME-Zn into the diet can improve the growth performance, Zn concentration in blood and liver, retention of nutrients, and gut morphology in broiler chickens. Dietary supplementa-

tion of HME-Zn at the level of 55 mg/kg (HME-50) has the potential to be used as an alternative to conventional $ZnSO_4$ at 110 mg/kg (ZnS) without any adverse effect on growth performance of broiler chickens.

Conflicts of Interest

The authors declare no conflict of interest.

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