Effect of dietary zinc pectin oligosaccharides chelate on growth performance, enzyme activities, Zn accumulation, metallothionein concentration, and gene expression of Zn transporters in broiler chickens¹

Zhong Cheng Wang,^{†,‡,||} Hui Min Yu,^{†,‡,||} Jing Jing Xie,^{†,‡,||} Hu Cui,^{†,‡,||} Hao Nie,^{†,‡,||} Tietao Zhang,[§] and Xiu Hua Gao^{†,‡,||,2}

[†]Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, P. R. China; and [‡]Key Laboratory for Feed Biotechnology of the Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, P. R. China; ^{||}National Engineering Research Center of Biological Feed, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, P. R. China; [§]Institute of Special Wild Economic Animals and Plants, Chinese Academy of Agricultural Sciences, Changchun, Jilin 130112, China

ABSTRACT: This study was to investigate the effect of zinc pectin oligosaccharides chelate (Zn-POS) on growth performance, serum enzyme activities, tissue zinc accumulation, metallothionein (MT) concentrations, and gene expression of *zinc transporters* (ZnT) in broilers. Five hundred forty 1-d-old Arbor Acres broiler chicks were randomly assigned to 5 dietary groups with 6 replicates of 18 birds per replicate. The diets were formulated with the same supplemental Zn level (80 mg/kg diet) but different amount of the Zn-POS: 0, 200, 400, 600, and 800 mg Zn-POS/kg diet. ZnSO₄ was used to adjust to the desired amount of the Zn (80 mg/kg) in the Zn-POS diets. Broilers were fed with the experimental diets for 42 d including the starter (days 1 to 21) and grower (days 22 to 42) phases. Our results showed that dietary supplementation of Zn-POS linearly and quadratically increased ($P < 0.05$) the average daily gain and gain-to-feed ratio during 22 to 42 d and 1 to 42 d as well as body weight on day 42, whereas reduced

($P < 0.05$) the sum of mortality and lag abnormalities in broilers on day 42. Besides, serum alkaline phosphatase and copper-zinc superoxide dismutase activities increased ($P < 0.05$) linearly and quadratically in response to dietary Zn-POS supplemental level on day 42. Dietary Zn-POS supplementation increased Zn accumulation in serum (linear, $P < 0.05$), liver (linear, $P < 0.05$), and pancreas (linear and quadratic, $P < 0.05$). In addition, Zn-POS supplementation linearly and quadratically increased ($P < 0.01$, $P < 0.05$, respectively) MT concentrations in liver and pancreas of broilers. Pancreatic mRNA levels of *MT*, *ZnT-1*, and *ZnT-2* increased ($P < 0.05$) linearly and quadratically, and the mRNA expression of *metal response element-binding transcription factor-1* increased linearly ($P < 0.05$), in response to dietary Zn-POS supplementation. In conclusion, supplementation of Zn-POS in the diet increases Zn enrichment in the metabolic organs such as liver and pancreas and promotes productive performance in broilers.

Key words: broilers, gene expressions, growth performance, metallothionein, zinc pectin oligosaccharides chelate, zinc transporters

© The Author(s) 2019. Published by Oxford University Press on behalf of the American Society of Animal Science. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com.

J. Anim. Sci. 2019.97:2114–2124

doi: 10.1093/jas/skz038

¹This work was financed by the Special Fund for Agro-scientific Research in the Public Interest (Nos. 201403047 and 2011-G7). Grateful acknowledgment was expressed to the staff of Key Laboratory for Feed Biotechnology of the Min-

istry of Agriculture for their valuable help in carrying out these experiments.

²Corresponding author: gaoxiuhua@caas.cn

Received November 29, 2018.

Accepted January 16, 2019.

INTRODUCTION

Zinc (Zn) is the catalytic metal ion in numerous enzymes that are involved in many physiological functions, such as growth performance, immunity, and antioxidant, and it has been well recognized as an essential trace element in broilers (Vallee and Falchuk, 1993; Gaither and Eide, 2001; Park et al., 2004). Zinc source in organic or inorganic forms has been supplemented to broilers diets (Huang, 2008). Pectin oligosaccharides (POS) have been classified as prebiotics (Gullon et al., 2013) and used in animal feed for improving growth performance. For example, supplementation of POS in diets increased the growth performance of weaned rats (Mao et al., 2016) and piglets (Chen et al., 2017). Moreover, pectin oligosaccharides have been demonstrated to promote the absorption of minerals into living organisms via simply mixing it with foods, drinks, or pharmaceutical compositions for oral use (Nakanishi and Kitada, 1999; Yu et al., 2008).

Our previous study have indicated that supplementation of zinc pectin oligosaccharides chelate (Zn-POS), as a new organic source, increased growth performance, nutrients digestibility, and Zn accumulations of broilers (Wang et al., 2016a). Supplementation of Zn-POS in the diet also increased average daily gain (ADG), Zn accumulation in tibia and pancreas of fur-growing female mink (Cui et al., 2017). However, the mechanism of dietary Zn-POS supplementation in increasing growth performance and regulating Zn metabolism is not understood completely. Especially, the role of dietary Zn-POS in induction of metallothionein (MT) and gene expression of *Zinc transporters* (*ZnT*) and its relationships to the status of Zn in tissues have not been assessed. Therefore, this experiment was performed to evaluate the effect of dietary Zn-POS on growth performance, serum Zn metalloenzymatic activities, tissue Zn accumulation, MT concentrations, and gene expressions of *ZnT* in broilers.

MATERIALS AND METHODS

The experimental design and procedures were approved by the Animal Care and Use Committee of the Feed Research Institute of the Chinese Academy of Agricultural Sciences in Beijing, China. The product of Zn-POS used in this experiment was obtained from the Feed Research Institute, Chinese Academy of Agricultural Sciences, same as the product used in the study with fur-growing female mink (Cui et al., 2017).

The product consisted of 30% Zn-POS and 70% corn starch. The Zn content was 7%, and the chelation rate of Zn-POS with 95% of purity was $\geq 96\%$ by analysis (Guo et al., 2013).

Bird Husbandry, Diets, and Experimental Design

Five hundred forty 1-d-old healthy Arbor Acre broilers were randomly divided into 5 groups with 6 replicates of 18 broilers/replicate (half male and half female) for a 42-d feeding trial including starter (days 1 to 21) and grower (days 22 to 42) phases. The initial body weight (BW) of the broilers did not differ among groups (on average 38.5 ± 0.4 g). The basal diet (Table 1) was formulated with corn, soybean meal, fish meal, and soybean oil to meet the nutrient requirements for starter and grower broilers except for Zn (NRC, 1994; China, 2004). The experimental diets were supplemented with the same amount of Zn (80 mg/kg diet) either from ZnSO_4 (Control) or from 200 (Zn-POS-200), 400 (Zn-POS-400), 600 (Zn-POS-600), and 800 (Zn-POS-800) mg Zn-POS/kg in basal diet, respectively. ZnSO_4 was used to adjust to the desired amount of the Zn (80 mg/kg) in the Zn-POS diets. Thus, the zinc contents (mg) from ZnSO_4 and Zn-POS were 66 and 14 for Zn-POS-200, 52 and 28 for Zn-POS-400, 38 and 42 for Zn-POS-600, and 24 and 56 for Zn-POS-800, respectively (Table 2). Zn requirement from NRC recommendation was 40 mg/kg, which was established more than 40 yr ago with purified diets (NRC, 1994), and it is underestimated for the rapid growth of commercial broiler strains (Huang, 2008; Yogesh et al., 2013; Applegate and Angel, 2014). Recent studies indicated that Zn requirement for broilers varied from 35 to 150 mg/kg in the diet (Huang, 2008). The optimum BW gain was achieved at 80 mg/kg supplemental Zn level in a corn-soybean meal basal diet (Burrell et al., 2004). Therefore, the supplemental level of Zn in the present study was 80 mg/kg in the form of ZnSO_4 or the combination of ZnSO_4 and Zn-POS. The analyzed Zn concentration in the experimental diets was 34.4 (days 1 to 21) and 40.5 mg/kg (days 22 to 42). Zn source from ZnSO_4 or Zn-POS was added into the basal diet to replace the equal amount of the carrier (bentonite) in the premix and mixed with each aliquot of the basal diet.

Broilers were raised in a temperature-controlled room with continuous lighting and managed in accordance with the guidelines of Arbor Acres broilers from Beijing Arbor Acres Poultry Breeding Co., Ltd. (Beijing, China). The temperature of the room was maintained at 35 °C for

Table 1. Ingredient and chemical composition of basal diet (air-dried basis)

Ingredients, %	Days 1 to 21	Days 22 to 42
Corn	52.0	56.1
Soybean oil	2.9	3.3
Soybean meal	36.2	32.7
Fish meal	4	3
Limestone	1.3	1.4
Calcium hydrophosphate	1.9	1.9
DL-Methionine	0.3	0.2
L-Lysine•HCl	0.2	0.11
Salt	0.3	0.3
Premix ¹	1.0	1.0
Analyzed values		
CP	22.1	20.6
Lys	1.2	1.1
Met	0.51	0.39
Met + Cys	0.89	0.75
Ca	0.90	0.85
Zn, mg/kg	34.3	40.5
Calculated values		
ME, MJ/kg	12.2	12.6

¹Contained the following per kilogram of premix: 10,000 IU vitamin A; 2,500 IU vitamin D₃; vitamin E; 15 mg, 3 mg vitamin K₁; 0.01 mg vitamin B₁; 34 mg nicotinic acid; 12 mg calcium pantothenate; 0.5 mg folic acid; 0.2 mg biotin; 1,200 mg choline chloride; 80 mg Fe (from ferrous sulfate); 8 mg Cu (from copper sulfate); 100 mg Mn (from manganese sulfate); 0.7 mg iodine (from calcium iodate iodide); 0.3 mg Se (from sodium selenite).

Table 2. Zn source and level of experimental groups

Groups	Zn source	The amount of Zn-POS, mg/kg	The amount of Zn from each source, mg/kg
Control	ZnSO ₄		80
	Zn-POS	0	0
Zn-POS-200	ZnSO ₄		66
	Zn-POS	200	14
Zn-POS-400	ZnSO ₄		52
	Zn-POS	400	28
Zn-POS-600	ZnSO ₄		38
	Zn-POS	600	42
Zn-POS-800	ZnSO ₄		24
	Zn-POS	800	56

the first 3 d and then reduced by 2 °C per week to a final temperature of 25 to 26 °C. The broilers were allowed ad libitum access to the mash feed and clean water with no detectable Zn and other minerals. Body weight and feed consumption were weighed and recorded weekly to calculate ADG, average daily feed intake (ADFI), and gain-to-feed ratio (G:F) based on the replicates for each treatment. The mortality and leg abnormalities of the broilers were also recorded daily on the replicates for each treatment, and the data were included in the calculation of G:F.

Sample Collection

All broilers were weighed after fasted 12 h at 42 d. Two healthy broilers with the average BW in each replicate were selected and killed by cervical dislocation. Blood samples were collected from the heart into 10 µL of nonheparinized tubes. The blood samples were centrifuged at 1,500 × g for 15 min, and serum were collected and stored at -20 °C until analyzed. The tissue samples from pancreas and liver were also collected and frozen in liquid nitrogen immediately. The middle section of ileum was rinsed with ice-cold saline after excised, and the mucosa was scraped from the underlying submucosa with a sterile glass slide and frozen immediately in liquid nitrogen. All samples were transferred and stored in -80 °C freezer until analyzed.

Analysis of Serum Enzyme Activities

Serum activities of Zn-related enzymes including alkaline phosphatase (ALP), lactate dehydrogenase (LDH), copper-zinc superoxide dismutase (CuZn-SOD), and glutathione peroxidase (GSH-Px) were determined using automatic biochemistry analyzer (Beckman Coulter AU480, Beckman Coulter, Inc., Ibaraki, CA). The test kits were purchased from BioSino Bio-Technology & Science Inc. (Beijing, China).

Determination of Tissue Zn Concentrations

Serum Zn was determined with microplate reader (ST-360, Shanghai Kehua Bio-engineering Co., Ltd, Shanghai, China). The test kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The digestion of samples was conducted using the electric heating method reported by Huang et al. (2009). Zn concentrations in liver and pancreas were determined using an air-acetylene flame on an atomic absorption spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan).

Tissue MT Concentrations

Metallothionein concentrations in liver, pancreas, and ileum mucosa were measured using Chicken MT ELISA Kit (MyBiosource) on ELX808 Absorbance Reader and 405 Touch Microplate washer (BioTek, Vermont, CA) according to the manufacturer's instructions.

Real-Time PCR

Total RNA was isolated from pancreas using RNAiso reagent (Tiangen Biotechnology, Beijing,

China) following the manufacturer's protocol. Purity and concentration of total RNA were determined using NanoPhotometer N60 (Implen, München, GM). The RNA samples were diluted with diethyl pyrocarbonate-treated water to the concentration of 1 ng/μL. Reverse transcription of total RNA was performed using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher, Massachusetts, CA) according to the instructions of manufacturer. Beta-actin was used to normalize the interested genes as recommended (Vandesompele et al., 2002). Primers (Table 3) for *β-actin*, *MT*, *ZnT-1*, *ZnT-2*, *ZnT-5*, and *metal response element-binding transcription factor-1 (MTF-1)* were designed specifically according to the sequences reported in GenBank (Tang et al., 2015). Quantification of mRNA was performed on ABI 7500 Real-Time PCR System (Thermo Fisher, Massachusetts, CA) using Maxima SYBR Green/Fluorescein qPCR Master Mix (2X) (Thermo Fisher, Massachusetts, CA). The samples performed in PCR reaction system (25 μL) contained 12.5 μL Maxima SYBR Green/Fluorescein qPCR Master Mix (2X), 1 μL Forward Primer (0.3 μmol/L), 1 μL of reverse primer (0.3 μmol/L), 1 μL of cDNA template, and 9.5 μL nuclease-free water. Conditions for RT-qPCR were as follows: 50 °C for 2 min, 95 °C for 10 min, 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s. All measurements were completed in triplicates.

Statistical Analysis

Data were analyzed by 1-way ANOVA using SPSS statistical software (version22 for windows; SPSS Inc., Chicago, IL). Data of mortality and leg abnormalities were transformed to arcsines before

analysis. Differences among groups was evaluated by Tukey's test. Results were presented as means with pooled SEM. Orthogonal polynomials were used to assess the linear and quadratic effects of dietary Zn-POS supplemental level. *P* values less than 0.05 were considered significant difference and $0.05 < P < 0.10$ was considered a trend.

RESULTS

Growth Performance

The effect of dietary Zn-POS on growth performance is listed in Table 4. During the starter phase, broilers fed Zn-POS diets quadratically increased ($P < 0.05$) BW of broilers on day 42. Compared with the Control, broilers fed Zn-POS-400 diet increased ($P < 0.05$) BW on day 21. No linear and quadratic effects of dietary treatment with Zn-POS on ADG, ADFI, G:F, and mortality were observed compared with the Control during days 1 to 21 ($P > 0.05$).

During the grower phase, Zn-POS supplementation in diets linearly and quadratically increased ($P < 0.05$) ADG, ADFI, and G:F, whereas tended to linearly decrease ($P = 0.08$) the mortality of broilers. The highest ADG was observed in the Zn-POS-800 ($P < 0.05$). Average daily feed intake in the Zn-POS-800 was higher ($P < 0.05$) than that in the Control and Zn-POS-200. There was no significant response to the increasing dietary Zn-POS level in mortality ($P > 0.05$).

Over the whole experimental period, supplementation of Zn-POS linearly and quadratically increased ($P < 0.05$) ADG, ADFI, G:F, and BW of broilers. In addition, supplementation of Zn-POS in broiler diets linearly and quadratically decreased ($P < 0.05$) leg abnormalities as well as the sum

Table 3. Primers information for quantitative real-time PCR

Gene ¹	GenBank ID ²	Primer sequence, sense/antisense	Product size, bp
<i>β-actin</i>	NM_205518	AACACCCACACCCCTGTGAT TGAGTCAAGCGCCAAAAGAA	100
<i>MT</i>	NM_205275.1	CTCCTGCTCCTGTGCTGGGTCGTGC CGGTTCCCTTGACACACAGCCCTT	175
<i>ZnT-1</i>	AJ619980.1	TGCGAGTGCCTTCTTCTCCT AAGGAGCTGTCAGGTCTGTAAT	131
<i>ZnT-2</i>	XM_423325.1	TCTTCTCCGTGCTGGTGCT CGGCGTTGAAATCCATCC	99
<i>ZnT-5</i>	XM_424760.1	ATGCTGTTGTGGGATGTA TGTCTTGGCTGGTCTCTC	159
<i>MTF-1</i>	MN_001031495.1	TGTCTTACTGATGAGGTGTC GCTCTTCAAAGTCCCAAATG	158

¹ZnT-1 = Zn transporter 1; ZnT-2 = Zn transporter 2; ZnT-5 = Zn transporter 5; MT = metallothionein; MTF-1 = metal response element-binding transcription factor-1.

²GenBank ID for those genes are according to Tang et al. (2015).

Table 4. Effects of dietary zinc pectin oligosaccharides chelate on growth performance of broilers during 1 to 42 d

Item ¹	Groups ²					SEM ³	P-value ⁴		
	Control	Zn-POS-200	Zn-POS-400	Zn-POS-600	Zn-POS-800		ANOVA	Linear	Quadratic
Days 1 to 21									
ADG, g	39.0	39.7	40.9	40.0	39.8	0.33	0.49	0.41	0.26
ADFI, g	53.7	54.9	54.6	54.4	54.4	0.29	0.80	0.71	0.61
G:F	0.73	0.72	0.75	0.73	0.73	0.01	0.60	0.71	0.61
BW, g	863 ^b	873 ^{ab}	889 ^a	879 ^{ab}	875 ^{ab}	2.6	0.03	0.10	0.01
Mortality, %	1.96	0.98	2.94	0.98	1.96	0.64	0.88	1.00	1.00
Days 22 to 42									
ADG, g	71.4 ^b	72.4 ^a	76.4 ^a	78.1 ^a	80.4 ^a	1.1	0.04	<0.01	0.01
ADFI, g	121 ^b	121 ^b	128 ^{ab}	125 ^{ab}	130 ^a	1.2	0.05	0.01	0.03
G:F	0.59	0.60	0.60	0.63	0.62	0.01	0.24	0.01	0.03
Mortality, %	7.2	3.9	4.0	3.1	2.0	0.90	0.24	0.08	0.20
Days 1 to 42									
ADG, g	55.2 ^b	56.0 ^a	58.7 ^a	59.1 ^a	60.0 ^a	0.60	0.02	<0.01	0.01
ADFI, g	87.2	88.2	91.2	89.6	92.4	0.66	0.07	0.01	0.04
G:F	0.65	0.65	0.66	0.68	0.67	0.01	0.29	0.01	0.04
BW, g	2,382 ^b	2,518 ^a	2,529 ^a	2,531 ^a	2,598 ^a	21.9	0.02	<0.01	0.01
Mortality, %	8.8	4.9	6.9	3.9	3.9	1.0	0.31	0.14	0.33
Leg abnormalities, %	8.8	4.0	3.4	4.0	2.2	0.85	0.13	0.03	0.05
Mortality + Leg abnormalities, %	16.0 ^a	8.8 ^b	8.8 ^b	7.8 ^b	6.0 ^b	1.2	0.03	0.01	0.03

^{a,b}Means within rows with different letter superscripts differ significantly ($P < 0.05$).

Data are means of 6 replicates with 18 broilers each replicate.

¹Mortality = number of dead birds/number of birds placed $\times 100\%$. Statistical inferences for percentage data were based on analysis after arcsine transformation. Leg abnormalities = number of leg disorders/number of birds placed $\times 100$, the average percentage of chicks within a cage with visual swelling of the tibiotarsal joint. Statistical inferences for percentage data were based on analysis after arcsine transformation, same with the data of mortality + Leg abnormalities.

²Control = basal diet + 80 mg Zn from ZnSO₄/kg diet; Zn-POS-200 = basal diet + 200 mg/kg zinc pectin oligosaccharides (66 mg Zn from ZnSO₄ + 14 mg Zn from zinc pectin oligosaccharides/kg diet); Zn-POS-400 = control diet + 400 mg/kg zinc pectin oligosaccharides (52 mg Zn from ZnSO₄ + 28 mg Zn from zinc pectin oligosaccharides/kg diet); Zn-POS-600 = control diet + 600 mg/kg zinc pectin oligosaccharides (38 mg Zn from ZnSO₄ + 42 mg Zn from zinc pectin oligosaccharides/kg diet); Zn-POS-800 = control diet + 800 mg/kg zinc pectin oligosaccharides (24 mg Zn from ZnSO₄ + 56 mg Zn from zinc pectin oligosaccharides/kg diet).

³Values listed for means and pooled SEM of all percentage data are actual data.

⁴Values for the probability of all percentage data values based on arcsine-transformed values.

of mortality and leg abnormalities of broilers. Compared with the Control, dietary Zn-POS supplementation at dosage of 200 to 800 mg/kg increased ($P < 0.05$) ADG and ADFI, and reduced ($P < 0.05$) the sum of mortality and leg abnormalities in broilers. No difference of mortality in broilers was observed individually among all groups ($P > 0.05$).

Serum Activities of Zn Metabolism-Related Enzymes

The activities of enzymes related to Zn metabolism in serum were significantly affected by dietary Zn-POS supplemental levels (Table 5). Dietary supplementation of Zn-POS linearly and quadratically increased ($P < 0.01$, $P < 0.05$, respectively) the enzyme activity of ALP in serum of broilers. Compared with the Control,

dietary supplementation of 400, 600, or 800 mg Zn-POS/kg increased ($P < 0.05$) serum ALP activity. Dietary supplementation of Zn-POS-400 had higher ($P < 0.05$) serum LDH activity than that in the Zn-POS-200 and Zn-POS-800. In addition, the activity of CuZn-SOD in serum was increased ($P < 0.01$) linearly and quadratically with the increase in Zn-POS levels in the diet. The enzyme activity of CuZn-SOD was much higher ($P < 0.05$) in broilers fed diet with Zn-POS-800 than that of the Control, Zn-POS-200 and Zn-POS-400. However, dietary supplementation of Zn-POS did not affect serum GSH-Px activity ($P > 0.05$).

Tissue Zn Accumulation

Zinc accumulation in tissues from the broilers is summarized in Table 6. Zn concentrations in serum

Table 5. Effects of dietary zinc pectin oligosaccharides chelate on serum zinc metalloenzymatic activities of broilers on day 42

Item ¹	Groups ²					SEM	P-value		
	Control	Zn-POS-200	Zn-POS-400	Zn-POS-600	Zn-POS-800		ANOVA	Linear	Quadratic
ALP, U/L	2,373 ^b	2,833 ^{ab}	3,311 ^a	3,656 ^a	3,433 ^a	151	0.04	<0.01	0.01
LDH, U/L	1,117 ^{ab}	937 ^b	1,175 ^a	1,055 ^{ab}	946 ^b	30.0	0.03	0.30	0.41
CuZn-SOD, U/ml	165 ^b	167 ^b	174 ^b	178 ^{ab}	196 ^a	3.5	0.02	<0.01	<0.01
GSH-Px, U/ml	362	351	361	367	364.50	2.2	0.16	0.15	0.29

^{a,b}Means within rows with different letter superscripts differ significantly ($P < 0.05$).

Data are expressed as mean with pooled standard error for 6 replicates of 2 broilers each replicate.

¹ALP = alkaline phosphatase; LDH = lactate dehydrogenase; CuZn-SOD = copper-zinc superoxide dismutase; GSH-Px = glutathione peroxidase.

²Control = basal diet + 80 mg Zn from ZnSO₄/kg diet; Zn-POS-200 = basal diet + 200 mg/kg zinc pectin oligosaccharides (66 mg Zn from ZnSO₄ + 14 mg Zn from zinc pectin oligosaccharides/kg diet); Zn-POS-400 = control diet + 400 mg/kg zinc pectin oligosaccharides (52 mg Zn from ZnSO₄ + 28 mg Zn from zinc pectin oligosaccharides/kg diet); Zn-POS-600 = control diet + 600 mg/kg zinc pectin oligosaccharides (38 mg Zn from ZnSO₄ + 42 mg Zn from zinc pectin oligosaccharides/kg diet); Zn-POS-800 = control diet + 800 mg/kg zinc pectin oligosaccharides (24 mg Zn from ZnSO₄ + 56 mg Zn from zinc pectin oligosaccharides/kg diet).

Table 6. Effects of dietary zinc pectin oligosaccharides chelate on Zn accumulation in broilers on day 42

Item	Groups ¹					SEM	P-value		
	Control	Zn-POS-200	Zn-POS-400	Zn-POS-600	Zn-POS-800		ANOVA	Linear	Quadratic
Serum, μ mol/L	42.0 ^b	48.8 ^{ab}	46.8 ^{ab}	52.5 ^a	45.4 ^{ab}	1.2	0.05	0.20	0.04
Liver, mg/kg	32.3 ^b	35.4 ^{ab}	41.2 ^a	40.4 ^{ab}	36.3 ^{ab}	1.2	0.09	0.13	0.03
Pancreas, mg/kg	41.4 ^b	50.5 ^b	55.4 ^{ab}	51.9 ^{ab}	65.5 ^a	2.6	0.05	0.01	0.02

^{a,b}Means within rows with different letter superscripts differ significantly ($P < 0.05$).

Data are expressed as mean with pooled SEM for 6 replicates of 2 broilers each replicate.

¹Control = basal diet + 80 mg Zn from ZnSO₄/kg diet; Zn-POS-200 = basal diet + 200 mg/kg zinc pectin oligosaccharides (66 mg Zn from ZnSO₄ + 14 mg Zn from zinc pectin oligosaccharides/kg diet); Zn-POS-400 = control diet + 400 mg/kg zinc pectin oligosaccharides (52 mg Zn from ZnSO₄ + 28 mg Zn from zinc pectin oligosaccharides/kg diet); Zn-POS-600 = control diet + 600 mg/kg zinc pectin oligosaccharides (38 mg Zn from ZnSO₄ + 42 mg Zn from zinc pectin oligosaccharides/kg diet); Zn-POS-800 = control diet + 800 mg/kg zinc pectin oligosaccharides (24 mg Zn from ZnSO₄ + 56 mg Zn from zinc pectin oligosaccharides/kg diet).

and liver increased ($P < 0.05$) linearly in response to dietary Zn-POS supplemental levels. Compared with the Control, Zn concentrations were higher ($P < 0.05$) in serum from Zn-POS-600, liver from Zn-POS-400, and pancreases from Zn-POS-800. Moreover, Zn content in pancreas of broilers was increased ($P < 0.05$) linearly and quadratically with the increase of Zn-POS level in the diet. The concentration of pancreatic Zn in the Zn-POS-800 was higher than that of the Zn-POS-200 ($P < 0.05$).

Tissue MT Concentrations

As shown in Table 7, supplementation of Zn-POS linearly and quadratically increased ($P < 0.01$, $P < 0.05$, respectively) hepatic and pancreatic MT concentrations. Broilers fed with Zn-POS-600 and Zn-POS-800 increased ($P < 0.05$) the MT concentrations in liver and pancreas compared with those from the Control. However, the ileum MT concentrations were not linearly or quadratically affected by dietary Zn-POS supplementation ($P > 0.05$).

Messenger RNA Expression

Table 8 presents the relative mRNA enrichment of Zn transporters in pancreas of broilers. Increasing dietary Zn-POS induced a linear and quadratic increase ($P < 0.05$) in gene expression of *MT* as well as *ZnT-1* and *ZnT-2* in broilers. Zn-POS-600 and 800 supplementation increased ($P < 0.05$) the mRNA levels of *MT*, *ZnT-1*, and *ZnT-2* in pancreas when compared with those of the Control. The mRNA levels of *MT* and *ZnT-1* ($P < 0.05$) were higher ($P < 0.05$) in Zn-POS-600 than the Control. Moreover, the *MT* mRNA levels in pancreas were higher ($P < 0.05$) in the broilers fed in Zn-POS-800 than Zn-POS-400 diet, and the *ZnT-1* mRNA levels were higher ($P < 0.05$) in the broiler fed with Zn-POS-800 than Zn-POS-200 and Zn-POS-400. The *ZnT-2* mRNA levels from Zn-POS-800 were higher ($P < 0.05$) than those from Zn-POS-400. Zn-POS supplementation linearly increased the mRNA levels of *ZnT-5* ($P < 0.05$) and tended to linearly increase ($P = 0.09$) *MTF-1* mRNA levels of broilers.

Table 7. Effects of dietary zinc pectin oligosaccharides chelate on tissue MT concentration in broilers on day 42

Tissue	Groups ¹					SEM	P-value		
	Control	Zn-POS-200	Zn-POS-400	Zn-POS-600	Zn-POS-800		ANOVA	Linear	Quadratic
Liver, ng/ml	21.5 ^b	27.4 ^{ab}	26.7 ^{ab}	30.0 ^a	32.5 ^a	1.2	0.03	<0.01	0.01
Pancreas, ng/ml	32.3 ^b	40.5 ^{ab}	39.4 ^{ab}	45.8 ^a	51.2 ^a	2.0	0.03	<0.01	0.01
Ileum, ng/ml	11.6	9.5	10.4	10.5	10.5	0.38	0.60	0.68	0.54

^{a,b}Means within rows with different letter superscripts differ significantly ($P < 0.05$).

Data are expressed as mean with pooled SEM for 6 replicates of 2 broilers each replicate.

¹Control = basal diet + 80 mg Zn from ZnSO₄/kg diet; Zn-POS-200 = basal diet + 200 mg/kg zinc pectin oligosaccharides (66 mg Zn from ZnSO₄ + 14 mg Zn from zinc pectin oligosaccharides/kg diet); Zn-POS-400 = control diet + 400 mg/kg zinc pectin oligosaccharides (52 mg Zn from ZnSO₄ + 28 mg Zn from zinc pectin oligosaccharides/kg diet); Zn-POS-600 = control diet + 600 mg/kg zinc pectin oligosaccharides (38 mg Zn from ZnSO₄ + 42 mg Zn from zinc pectin oligosaccharides/kg diet); Zn-POS-800 = control diet + 800 mg/kg zinc pectin oligosaccharides (24 mg Zn from ZnSO₄ + 56 mg Zn from zinc pectin oligosaccharides/kg diet).

Table 8. Effects of dietary zinc pectin oligosaccharides chelate on gene expression of Zn transporters in pancreas of broilers on day 42

Gene ¹	Groups ²					SEM	P-value		
	Control	Zn-POS-200	Zn-POS-400	Zn-POS-600	Zn-POS-800		ANOVA	Linear	Quadratic
MT	1.5 ^c	1.7 ^{abc}	1.5 ^{bc}	1.7 ^{ab}	1.8 ^a	0.04	0.04	0.01	0.03
ZnT-1	0.86 ^c	0.89 ^{bc}	0.89 ^{bc}	0.94 ^{ab}	0.97 ^a	0.01	0.03	<0.01	0.01
ZnT-2	1.4 ^b	1.5 ^{ab}	1.4 ^b	1.6 ^{ab}	1.7 ^a	0.03	0.03	0.01	0.02
ZnT-5	1.0	1.1	1.1	1.1	1.1	0.02	0.54	0.09	0.24
MTF-1	0.89	0.93	0.92	0.96	1.0	0.02	0.29	0.03	0.10

^{a,b} Means within rows with different letter superscripts differ significantly ($P < 0.05$).

Data are expressed as mean with pooled SEM for 6 replicates of 2 broilers each replicate.

¹MT = Metallothionein; ZnT-1 = Zinc transporter 1; ZnT-2 = Zinc transporter 2; ZnT-5 = Zinc transporter 5; MTF-1 = Metal response element-binding transcription factor-1.

²Control = basal diet + 80 mg Zn from ZnSO₄/kg diet; Zn-POS-200 = basal diet + 200 mg/kg zinc pectin oligosaccharides (66 mg Zn from ZnSO₄ + 14 mg Zn from zinc pectin oligosaccharides/kg diet); Zn-POS-400 = control diet + 400 mg/kg zinc pectin oligosaccharides (52 mg Zn from ZnSO₄ + 28 mg Zn from zinc pectin oligosaccharides/kg diet); Zn-POS-600 = control diet + 600 mg/kg zinc pectin oligosaccharides (38 mg Zn from ZnSO₄ + 42 mg Zn from zinc pectin oligosaccharides/kg diet); Zn-POS-800 = control diet + 800 mg/kg zinc pectin oligosaccharides (24 mg Zn from ZnSO₄ + 56 mg Zn from zinc pectin oligosaccharides/kg diet).

DISCUSSION

Results from previous studies have shown that Zn is an important trace element for the growth of broilers (Brooks et al., 2013; Sahraei et al., 2013; Mwangi et al., 2017). Zn deficiency in chicks results in a decreased appetite, stunted growth, immunological dysfunctions, and various bone abnormalities (Naz et al., 2016). Zn from the organic source would be better than inorganic sources such as ZnSO₄ in promoting growth performance and reducing environmental pollution due to its bioavailability. Therefore, the organic Zn has been recommended widely as feed additives in broilers (Zhao et al., 2014). The current data showed that Zn-POS at 400 mg/kg, as an organic Zn source, replacing partial of ZnSO₄ with the equal amount of Zn in the diet increased BW of broilers on day 21, supplementation of 200 to 800 mg/kg increased ADG during days 22 to 42 and 1 to 42 d as well as BW on day 42, which was in agreement

with previous studies suggesting that supplement Zn-POS at 600 mg/kg had higher ADG than those of the broilers fed the control diet (Wang et al., 2016a). In addition, dietary Zn-POS supplementation linearly and quadratically increased G:F of broilers during days 22 to 42 and 1 to 42. The superior effect of Zn-POS on growth performance may be attributed to higher availability of Zn-POS as an organic source of Zn. In support of this, the results from the current study clearly showed that MT (the protein associated with Zn absorption) and the gene expression of Zn transporters such as *ZnT-1* were increased in pancreas. Moreover, the mortality of broilers is influenced by the leg abnormalities and sudden death syndrome. Manganese (Mn) deficiency is closely related to leg abnormalities of broilers, whereas Zn has a synergistic effect on the absorption of Mn (Gajula et al., 2011). Therefore, increasing Zn absorption in the diet could promote the Mn absorption. POS just has been reported to

promote absorption of minerals including Zn into living organisms (Nakanishi and Kitada, 1999). In our study, we found that the sum of mortality and leg abnormality was decreased with Zn-POS supplementation especially at the high level of Zn-POS, implying that the Mn absorption might be increased also with the Zn-POS supplementation in the diet although we did not measure the Mn status. The component of POS in Zn-POS may contribute to the decrease of the mortality plus leg abnormality by increasing absorption of Zn and Mn in broilers as well. Therefore, the findings from our study demonstrated that Zn-POS would be a beneficial substitute of ZnSO_4 as Zn source for the growth performance of broilers.

Zinc is an essential component in ALP and CuZn-SOD, and the activities of these enzymes in serum were valuable parameters of hepatic function and nutrient digestion (Sun et al., 2014). Serum ALP activity was considered as a good indicator of evaluating Zn uptake status and comparing the biological activities of the inorganic and organic trace elements in poultry (Idowu et al., 2011). The current study found that replacing partial of ZnSO_4 with equal Zn-leveled Zn-POS at 400, 600, or 800 mg/kg in the diet increased serum activity of ALP in broilers, which was consistent with Singh et al. (Singh et al., 2015), who reported that supplying adequate amounts of Zn in laying hens' diets resulted in lower serum ALP activity and typical leg abnormality. Therefore, we inferred that the improvement in performance of the broilers in this study might be associated with the increasing ALP activity in serum which would contribute to the uptake and biological availability of Zn. Furthermore, we found that dietary levels of replacing partial of ZnSO_4 with Zn-POS positively correlated with the enzyme activity of CuZn-SOD, which were able to reduce the reactive oxygen species induced by cellular injury resulting in higher antioxidant capacity of broilers (Song et al., 2018). The highest activity of CuZn-SOD in serum was observed in the broilers fed diet with Zn-POS-800 and the result agreed with the result reported in our previous study with broilers (Wang et al., 2016b). Our results demonstrated again that serum antioxidant capacity may be greater from the organic form, Zn-POS than ZnSO_4 in broilers. Interestingly, the effect of Zn-POS-800 on CuZn-SOD activity in broilers was similar to that observed in broilers fed with Zn-POS-600, suggesting that excess Zn-POS in diets may not have a benefit to the broilers.

Zinc from different Zn sources was digested by the enzymes secreted from pancreas, absorbed

in intestinal-mucosal cells, and subsequently transported to the portal blood of liver. Thus, Zn content in these tissues can accurately reflect the differences in Zn absorption and utilization from different Zn sources and levels. Plasma and pancreatic Zn concentrations increased linearly as dietary Zn concentration increased in Zn sulfate source at 6 or 14 d of age in broilers (Li et al., 2015). Tang et al. (2015) found that Zn-bearing zeolite clinoptilolite supplementation to diets increased Zn accumulation in liver and tibia of birds compared with diet with Zn in broilers. Cao et al. (2003) demonstrated that Zn concentration in liver and pancreas increased with the increase in Zn from Zn proteinate. In the present trial, supplementation of Zn-POS quadratically increased hepatic Zn concentrations, linearly and quadratically increased Zn concentrations in pancreas, supplementation at 400 mg/kg in the diet increased Zn concentrations in liver, and supplementation of Zn-POS at 800 mg/kg increased Zn concentrations in pancreas compared with ZnSO_4 supplementation. This result was in consistent with our previous study that supplemental Zn-POS at 600 mg/kg increased hepatic Zn concentrations, and 900 mg/kg Zn-POS increased pancreatic Zn concentrations in broilers (Wang et al., 2016a). Moreover, our data further indicated that dietary Zn-POS supplementation quadratically increased Zn concentrations in serum; highest Zn concentrations was observed in the Zn-POS-600. Although the response of different tissues to dietary Zn-POS level was different, our data indicated that the increase of Zn accumulation in serum, liver, and pancreas appeared to be associated with diets where ZnSO_4 is replaced partial with Zn-POS in the equal Zn level. Therefore, our results demonstrated again that the Zn utilization (based on the deposition in the tissues) is greater from the organic form, Zn-POS than ZnSO_4 in broilers. The increased Zn accumulation from Zn-POS in tissue may be attributed to the direct minerals absorption promoted by POS (Nakanishi and Kitada, 1999). Indirectly, this can be partly explained that POS could be fermented extensively by colonic microflora such as *Lactobacillus* and *Bifidobacterium*, resulting in the generation of short-chain fatty acids. The generated short-chain fatty acid could decrease the pH, resulting in an increase in the solubility of minerals in the intestinal tract. The increased solubility of minerals could increase the absorption of minerals in intestine (Gullon et al., 2013).

Metallothionein is a low molecular protein with Zn-binding capacity and plays a very important role in Zn absorption, transportation, and storage

in animal body (Kambe, 2013). The ileum is the main site of Zn absorption in chickens (Yu et al., 2008). In the present study, ileal MT concentration was not affected by Zn-POS levels, which was in agreement with the result observed in rat fed with high Zn diet (Reeves, 1995). In contrast with ileum, liver and pancreas are the primary organs for Zn metabolism. MT concentrations in pancreas were positively associated with the Zn concentration in chickens (Huang et al., 2009). In consistent with Huang et al., the present results further indicated that supplemented Zn in the form of Zn-POS (600 to 800 mg/kg diet) increased pancreatic and hepatic MT concentrations, which suggested that dietary supplementation of Zn-POS at the dosage of 600 to 800 mg/kg might have an effect on enhancing Zn metabolism in broilers. Moreover, our data suggested that hepatic and pancreatic MT concentrations could be a sensitive and optimal index for evaluating Zn-POS bioavailability in broilers.

Intestinal Zn absorption is a carrier-mediated process (Condomina et al., 2002). The family of Zn transporters including ZnT-1, ZnT-2, ZnT-5, and MT are known to be the key regulators of Zn absorption and transportation (Inoue et al., 2002; Kambe, 2013). Meanwhile, *MTF-1* is also involved in Zn transportation by regulating the expressions of *MT* and *ZnT-1* genes (Langmade et al., 2000; Laity and Andrews, 2007). Because Zn accumulation is associated with the intestinal Zn absorption and transportation in animals (Kambe, 2013), and the gene enrichment of Zn transporters and their regulators are important for evaluating the Zn utilization in the body. We examined the gene expressions of *ZnT* and *MTF-1* in pancreases of broiler after fed with Zn-POS in this study. The present data showed that replacing partial of ZnSO₄ with Zn-POS (800 mg/kg) in the equal Zn level in the diet linearly and quadratically enriched gene expression of *MT* in pancreas of broilers. Because increasing Zn concentration induced the gene expression of *MT* in pancreas that has been observed in chicks fed with diets supplemented with ZnSO₄ (Huang et al., 2007, 2009; Shen et al., 2013), the increased enrichment of *MT* expression in pancreases could be due to the increased availability of Zn in pancreas of broilers fed diets with Zn-POS. This result indicated that replacement of ZnSO₄ with Zn-POS would stimulate Zn absorption. Similar results were reported in pancreas of chicks fed with diets supplemented with ZnSO₄ (Huang et al., 2007, 2009; Shen et al., 2013), demonstrating that Zn-POS stimulates Zn absorption. Like the transporter *MT*, *ZnT-1*, and *ZnT-2* mRNA levels in pancreas were also

linearly and quadratically increased in the broilers fed diets with 600 or 800 mg Zn-POS supplementation. We found that the increase of the transporters measured in pancreas indeed was agreed with the increase of Zn accumulation in pancreatic tissue, suggesting that the expression of Zn transporter genes, *MT*, *ZnT-1*, and *ZnT-2* could be activated when the Zn concentration was increased in tissues.

Zinc transporter 5 mediated cellular Zn absorption by transporting Zn from the intestinal lumen into the enterocytes, which is upregulated at low concentration of Zn in the enteric cavity to maintain Zn homeostasis (Cragg et al., 2005). Zn supplementation increased the ZnT-5 mRNA levels in the jejunum of broilers. Similarly, the current study found that dietary Zn-POS supplementation tended to linearly increase the ZnT-5 mRNA levels in pancreas of broilers. Moreover, *MTF-1* is activated by Zn via binding with metal response elements in the gene promoter and regulated the *MT* and *ZnT-1* gene expressions at transcription level (Langmade et al., 2000; Laity and Andrews, 2007). In this study, Zn-POS supplementation linearly increased the gene expression of *MTF-1*, which was in agreement with the result of *MT* and *ZnT-1* and indicated that Zn-POS supplementation increased the Zn absorption and transcription by *MTF-1*-mediated Zn transport pathways in broilers.

In conclusion, the replacement of ZnSO₄ with Zn-POS in the diet has a positive effect on growth performance in broilers. In addition to the positive effect on growth performance, Zn-POS increased the Zn metalloenzymatic activities in serum, the Zn deposition and MT concentrations in liver and pancreas, and the gene expression of *MT*, *ZnT-1*, and *ZnT-2* in pancreas in broilers. However, the substitution of Zn-POS for ZnSO₄ on growth performance and the Zn metabolic and/or physiological status in serum, liver, and pancreas are associated with the supplementation level of Zn-POS in the diet. A beneficial level would be over 600-mg Zn-POS/kg diet based on the indices measured in this study.

LITERATURE CITED

- Applegate, T. J., and R. Angel. 2014. Nutrient requirements of poultry publication: history and need for an update. *J. Appl. Poult. Res.* 23:567–575. doi:10.3382/japr.2014-00980
- Brooks, M. A., J. L. Grimes, K. E. Lloyd, S. Verissimo, and J. W. Spears. 2013. Bioavailability in chicks of zinc from zinc propionate. *J. Appl. Poult. Res.* 22:153–159. doi:10.3382/japr.2012-00525
- Burrell, A. L., W. A. Dozier, III, A. J. Davis, M. M. Compton, M. E. Freeman, P. F. Vendrell, and T. L. Ward. 2004. Responses of broilers to dietary zinc concentrations and sources in relation to environmental implications. *Br.*

- Poult. Sci. 45:255–263. doi:10.1080/00071660410001715867
- Cao, J. Y., X. G. Luo, S. R. Davis, P. R. Henry, R. J. Cousins, R. D. Miles, and C. B. Ammerman. 2003. Tissue zinc and metallothionein concentrations and metallothionein gene expression as criteria for relative bioavailability assays of zinc sources in chicks. *Chin. J. Anim. Vet. Sci.* 34:227–231.
- Chen, H., H. Hu, D. Chen, J. Tang, B. Yu, J. Luo, J. He, Y. Luo, J. Yu, and X. Mao. 2017. Dietary pectic oligosaccharide administration improves the growth performance and immunity in weaned pigs infected by rotavirus. *J. Agric. Food Chem.* 65:1–31. doi:10.1021/acs.jafc.7b00039
- China, M. O. A. O. 2004. Chicken feeding standard. NY/T33-2004 ed. Standards Press of China, Beijing.
- Condomina, J., T. Zornoza-Sabina, L. Granero, and A. Polache. 2002. Kinetics of zinc transport in vitro in rat small intestine and colon: interaction with copper. *Eur. J. Pharm. Sci.* 16:289–295. doi:10.1016/S0928-0987(02)00125-2
- Cragg, R. A., S. R. Phillips, J. M. Piper, J. S. Varma, F. C. Campbell, J. C. Mathers, and D. Ford. 2005. Homeostatic regulation of zinc transporters in the human small intestine by dietary zinc supplementation. *Gut* 54:469–478. doi:10.1136/gut.2004.041962
- Cui, H., T. T. Zhang, H. Nie, Z. C. Wang, X. L. Zhang, B. Shi, F. H. Yang, and X. H. Gao. 2017. Effects of sources and concentrations of zinc on growth performance, nutrient digestibility, and fur quality of growing-furring female mink (*Mustela vison*). *J. Anim. Sci.* 95:5420–5429. doi:10.2527/jas2017.1810
- Gaither, L. A., and D. J. Eide. 2001. Eukaryotic zinc transporters and their regulation. *Biomaterials* 14:251–270. doi:10.1023/A:1012988914300
- Gajula, S. S., V. K. Chelasani, A. K. Panda, V. L. Mantena, and R. R. Savaram. 2011. Effect of supplemental inorganic Zn and Mn and their interactions on the performance of broiler chicken, mineral bioavailability, and immune response. *Biol. Trace Elem. Res.* 139:177–187. doi:10.1007/s12011-010-8647-8
- Gullon, B., B. Gomez, M. Martinez-Sabajanes, R. Yanez, J. C. Parajo, and J. L. Alonso. 2013. Pectic oligosaccharides: manufacture and functional properties. *Trends Food Sci. Technol.* 30:153–161. doi:10.1016/j.tifs.2013.01.006
- Guo, F. N., C. C. Li, L. M. Jin, Y. T. Fu, W. Z. Hu, and M. X. Tian. 2013. Synthesis and antioxidant activity of chitoooligosaccharide-Fe complex. *Sci. Technol. Food Ind.* 34:119–121. doi:10.13386/j.issn1002-0306.2013.20.045
- Huang, Y. 2008. Research advances in the nutrition requirement of zinc for broilers. *China Feed.* 11:28–30. doi:10.15906/j.cnki.cn11-2975/s.2008.11.002
- Huang, Y. L., L. Lu, S. F. Li, X. G. Luo, and B. Liu. 2009. Relative bioavailabilities of organic zinc sources with different chelation strengths for broilers fed a conventional corn-soybean meal diet. *J. Anim. Sci.* 87:2038–2046. doi:10.2527/jas.2008-1212
- Huang, Y. L., L. Lu, X. G. Luo, and B. Liu. 2007. An optimal dietary zinc level of broiler chicks fed a corn-soybean meal diet. *Poult. Sci.* 86:2582–2589. doi:10.3382/ps.2007-00088
- Idowu, O. M. O., R. O. Ajuwon, A. O. Oso, and O. A. Akinloye. 2011. Effects of zinc supplementation on laying performance, serum chemistry and Zn residue in tibia bone, liver, excreta and egg shell of laying hens. *Int. J. Poult. Sci.* 10:225–230.
- Inoue, K., K. Matsuda, M. Itoh, H. Kawaguchi, H. Tomoike, T. Aoyagi, R. Nagai, M. Hori, Y. Nakamura, and T. Tanaka. 2002. Osteopenia and male-specific sudden cardiac death in mice lacking a zinc transporter gene, *znt5*. *Hum. Mol. Genet.* 11:1775–1784. doi:10.1093/hmg/11.15.1775
- Kambe, T. 2013. Overview of and update on the physiological functions of mammalian zinc transporters. *Nihon Eiseigaku Zasshi.* 68:92–102. doi:10.1265/jjh.68.92
- Laity, J. H., and G. K. Andrews. 2007. Understanding the mechanisms of zinc-sensing by metal-response element binding transcription factor-1 (MTF-1). *Arch. Biochem. Biophys.* 463:201–210. doi:10.1016/j.abb.2007.03.019
- Langmade, S. J., R. Ravindra, P. J. Daniels, and G. K. Andrews. 2000. The transcription factor MTF-1 mediates metal regulation of the mouse *znt1* gene. *J. Biol. Chem.* 275:34803–34809. doi:10.1074/jbc.M007339200
- Li, W. X., X. Y. Ma, L. Lu, L. Y. Zhang, and X. G. Luo. 2015. Relative bioavailability of tribasic zinc sulfate for broilers fed a conventional corn-soybean meal diet. *J. Integr. Agr.* 14:2042–2049. doi:10.1016/S2095-3119(15)61033-4
- Mao, X. B., X. H. Feng, D. W. Chen, B. Yu, B. Shi, H. Chen, J. He, J. Yu, and J. Q. Luo. 2016. Effect of dietary apple pectic oligosaccharide supplementation on growth performance, antioxidant capacity and intestinal health of weaned rats. *Chin. J. Anim. Nutr.* 28:2872–2877. doi:10.3969/j.issn.1006-267x.2016.09.026
- Mwangi, S., J. Timmons, T. Ao, M. Paul, L. Macalintal, A. Pescatore, A. Cantor, M. Ford, and K. A. Dawson. 2017. Effect of zinc imprinting and replacing inorganic zinc with organic zinc on early performance of broiler chicks. *Poult. Sci.* 96:861–868. doi:10.3382/ps/pew312
- Nakanishi, N., and Y. Kitada. 1999. Mineral absorption promoting agent. Pola Chemical Industries Inc., Shizuoka, JP. US Pat. No. 5,952,308.
- Naz, S., M. Idris, M. A. Khalique, Zia-Ur-Rahman, I. A. Alhidary, M. M. Abdelrahman, R. U. Khan, N. Chand, U. Farooq, and S. Ahmad. 2016. The activity and use of zinc in poultry diets. *Worlds Poult. Sci. J.* 72:159–167. doi:10.1017/S0043933915002755
- NRC. 1994. Nutrient requirements of poultry. 9th ed. Natl. Acad. Press, Washington, DC.
- Park, S. Y., S. G. Birkhold, L. F. Kubena, D. J. Nisbet, and S. C. Ricke. 2004. Review on the role of dietary zinc in poultry nutrition, immunity, and reproduction. *Biol. Trace Elem. Res.* 101:147–163. doi:10.1385/BTER:101:2:147
- Reeves, P. G. 1995. Adaptation responses in rats to long-term feeding of high-zinc diets: emphasis on intestinal metallothionein. *J. Nutr. Biochem.* 6:48–54. doi:10.1016/0955-2863(94)00008-A
- Sahraei, M., H. Janmmohamadi, A. Taghizadeh, G. A. Moghadam, and S. A. Rafat. 2013. Estimation of the relative bioavailability of several zinc sources for broilers fed a conventional corn-soybean meal diet. *J. Poult. Sci.* 50:53–59. doi:10.2141/jpsa.0120022
- Shen, S. F., R. L. Wang, L. Lu, S. F. Li, S. B. Liu, J. X. Xie, L. Y. Zhang, M. L. Wang, and X. G. Luo. 2013. Effect of intravenously injected zinc on tissue zinc and metallothionein gene expression of broilers. *Br. Poult. Sci.* 54:381–390. doi:10.1080/00071668.2013.787158

- Singh, A. K., T. K. Ghosh, and S. Haldar. 2015. Effects of methionine chelate- or yeast proteinate-based supplement of copper, iron, manganese and zinc on broiler growth performance, their distribution in the tibia and excretion into the environment. *Biol. Trace Elem. Res.* 164:253–260. doi:10.1007/s12011-014-0222-2
- Song, Z. H., K. Cheng, X. C. Zheng, H. Ahmad, L. L. Zhang, and T. Wang. 2018. Effects of dietary supplementation with enzymatically treated artemisia annua on growth performance, intestinal morphology, digestive enzyme activities, immunity, and antioxidant capacity of heat-stressed broilers. *Poult. Sci.* 97:430–437. doi:10.3382/ps/pex312
- Sun, L. H., N. Y. Zhang, Q. H. Zhai, X. Gao, C. Li, Q. Zheng, C. S. Krumm, and D. Qi. 2014. Effects of dietary tin on growth performance, hematology, serum biochemistry, antioxidant status, and tin retention in broilers. *Biol. Trace Elem. Res.* 162:302–308. doi:10.1007/s12011-014-0129-y
- Tang, Z. G., G. Y. Chen, L. F. Li, C. Wen, T. Wang, and Y. M. Zhou. 2015. Effect of zinc-bearing zeolite clinoptilolite on growth performance, zinc accumulation, and gene expression of zinc transporters in broilers. *J. Anim. Sci.* 93:620–626. doi:10.2527/jas.2014-8165
- Vallee, B. L., and K. H. Falchuk. 1993. The biochemical basis of zinc physiology. *Physiol. Rev.* 73:79–118. doi:10.1152/physrev.1993.73.1.79
- Vandesompele, J., K. De Preter, F. Pattyn, B. Poppe, N. Van Roy, A. De Paepe, and F. Speleman. 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol.* 3:RESEARCH0034.
- Wang, Z. C., X. Z. Wu, H. Cui, C. M. Wan, T. T. Zhang, Q. Peng, H. M. Yu, and X. H. Gao. 2016b. Effects of different dietary zinc-pectic oligosaccharide chelate supplemental levels on growth performance, immune function and serum antioxidant capacity of broilers. *Chin. J. Anim. Nutr.* 28:1757–1764. doi:10.3969/j.issn.1006-267x.2016.06.017
- Wang, Z., H. Yu, X. Wu, T. Zhang, H. Cui, C. Wan, and X. Gao. 2016a. Effects of dietary zinc pectin oligosaccharides chelate supplementation on growth performance, nutrient digestibility and tissue zinc concentrations of broilers. *Biol. Trace Elem. Res.* 173:475–482. doi:10.1007/s12011-016-0654-y
- Yogesh, K., C. Deo, H. P. Shrivastava, A. B. Mandal, A. Wadhwa, and I. Singh. 2013. Growth performance, carcass yield, and immune competence of broiler chickens as influenced by dietary supplemental zinc sources and levels. *Agric. Res.* 2:270–274. doi:10.1007/s40003-013-0067-5
- Yu, Y., L. Lu, X. G. Luo, and B. Liu. 2008. Kinetics of zinc absorption by in situ ligated intestinal loops of broilers involved in zinc transporters. *Poult. Sci.* 87:1146–1155. doi:10.3382/ps.2007-00430
- Zhao, C. Y., S. X. Tan, X. Y. Xiao, X. S. Qiu, J. Q. Pan, and Z. X. Tang. 2014. Effects of dietary zinc oxide nanoparticles on growth performance and antioxidative status in broilers. *Biol. Trace Elem. Res.* 160:361–367. doi:10.1007/s12011-014-0052-2