Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Jan - Mar 2018 / v.20 / n.1 / 127-132

http://dx.doi.org/10.1590/1806-9061-2017-0604

Effects of Dietary Zinc Levels on the Growth Performance, Organ Zinc Content, and Zinc Retention in Broiler Chickens

Author(s)

Zhang TY[I]
Liu JL[I]
Zhang JL[II,III]
Zhang N[II,III]
Yang X[II,III]
Qu HX[II]
Xi L[II]
Han JC[II]

[I] Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, 100193, China
[II] Department of Animal Science, College of Life Science, Shangqiu Normal University, Shangqiu, Henan, 476000, China
[III] College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou, Henan, 450002, China

Mail Address

Corresponding author e-mail address
Zhang TY
Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, 100193, China
Tel: (010)62816012
Email: zhty999@163.com

Keywords

Broiler chickens, growth, requirement, retention, zinc.

Submitted: 11/August/2017
Approved: 19/October/2017

ABSTRACT

The objective of this study was to investigate the optimal zinc (Zn) requirement of broiler chickens based on Zn retention. On the day of hatch, 350 male Ross 308 broilers were randomly assigned to seven treatments with five replicates of ten birds each. Zinc was supplemented as $ZnSO_4 \cdot 7H_2O$ at 0, 20, 40, 60, 80, 100, or 120 mg/kg in the starter diet (fed from 1 to 21 d of age) and at 0, 16, 32, 48, 64, 80, or 96 mg/kg in the grower diet (fed from 22 to 42 d of age). The analyzed Zn levels were 34.98 and 27.57 mg/kg in the basal starter and grower diets, respectively. Supplemental Zn levels did not influence body weight gain, feed intake, feed conversion ratio, or liver Zn content of broilers at 21 and 42 d of age ($p>0.05$). Tibia ash Zn content of 21-d-old broilers increased when Zn supplementation level increased from 0 to 40 mg/kg Zn in ($p<0.05$). The highest breast muscle Zn content in 42-d-old broilers was observed when 100 and 80 mg Zn/kg was supplemented in the starter and grower diets, respectively. Fecal Zn content, Zn intake, Zn excretion, and Zn retention of 31- to 33-d-old broilers linearly increased with supplemental Zn levels ($p<0.05$). Zinc retention values, calculated as the difference between Zn intake and Zn excretion, were negative, about zero, and positive when starter/grower diets were supplemented with 0/0 and 20/16, 40/32, and 60/48 and 120/96 mg/kg, respectively. These results indicate that supplementing 40 and 32 mg Zn/kg in starter and grower diets, respectively, promote the growth performance of broiler chickens, while reduce Zn excretion in the environment.

INTRODUCTION

Zinc (Zn) is an essential trace element for poultry, and contributes for the maintenance of growth performance (Liu *et al*., 2011), skeletal development (Ao *et al*., 2007; Tomaszewska *et al*., 2017), and immune function (Kidd *et al*., 1996) of broiler chickens. The Feeding Standards of Chickens in China (Ministry of Agriculture of P. R. China, 2004) indicate that Zn levels of 100 and 80 mg/kg are suitable for broiler chickens from 1 to 21 and 22 to 42 d of age, respectively. However, the NRC (1994) recommends 40 mg/kg Zn for broilers from 1 to 42 d of age. Recent research has shown that the optimal levels of supplemental Zn for the performance of broiler chickens range between 12 and 60 mg/kg for (Ao *et al*., 2007; Huang *et al*., 2007; Liu *et al*., 2011). These data suggest that the Zn supplementation recommended by the Feeding Standards of Chickens in China (Ministry of Agriculture of P. R. China, 2004) may be higher than the Zn requirement for broiler chickens and should be revised.

Excessive Zn supplementation in chicken diets may cause environmental pollution due to Zn excretion in the feces, as fecal Zn content linearly increases with Zn dietary levels in broiler chickens and laying hens (Burrell *et al*, 2004; Kim & Patterson, 2004, 2005). Moreover, when Zn load in the soil increases, the Zn content in plant tissues also increases, hindering plant growth (Schmidt, 1997).

Therefore, this study was conducted to investigate the optimal Zn requirement of broiler chickens from 1 to 42 d of age based on Zn retention.

MATERIALS AND METHODS

All procedures applied in this study were approved by the Institute of Animal Science of the Chinese Academy of Agricultural Sciences and Shangqiu Normal University.

Birds, Diets, and Management

On the day of hatch, 350 male Ross 308 broilers were weighed and randomly allotted to seven treatments with five replicates of ten birds each. Broilers were reared in stainless steel cages (190 cm × 50 cm × 35 cm) in a room with controlled temperature of 33 °C from d 0 to 3, 30 °C from d 4 to 7, 27 °C from d 8 to 14, and 24 °C from d 15 to 42. A lighting program of 20 h of light and 4 h of dark was adopted.

Birds were fed according to a two-phase feeding period: starter (1 to 21 d of age) and grower (22 to 42 d of age). The basal diets formulated for each phase were based on corn-soybean meal and supplemented with a trace-mineral premix with no Zn (Table 1). The experimental diets (treatments) consisted of the supplementation of 0, 20, 40, 60, 80, 100, or 120 mg of Zn/kg diet in the starter phase, and of 0, 16, 32, 48, 64, 80, and 96 mg Zn/kg diet in the grower phase. The source of zinc supplemented to the basal diet was $ZnSO_4 \cdot 7H_2O$, and the feeds were fed as mash. The birds were provided *ad libitum* access to feed and water during the entire experimental period.

Growth performance parameters

Feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) were calculated for the periods 1 to 21 and 1 to 42 d of age. All dead birds were weighed and their weight was used to correct the FI.

Sample Collection

Fecal samples were collected from trays placed under each cage using the total fecal collection method from 31 to 33 d of age, and stored at –20 °C (Han *et al*., 2009). Before analysis, fecal samples were dried at 65 °C for 48 h and then ground to pass through a 1-mm mesh screen.

Table 1 – Ingredients and nutrient composition of experimental diets.

Item	Day 1–21	Day 22–42
Ingredients (%)		
Corn	58.02	63.18
Soybean meal (43% CP)	32.07	27.52
Soybean oil	2.20	3.00
Soybean protein isolate (65% CP)	3.50	2.74
Limestone	1.36	1.45
Dicalcium phosphate	1.94	1.36
L-Lysine·HCl (98%)	0.14	0.14
DL-Methionine (98%)	0.14	0.08
Trace-mineral premix[1]	0.10	0.10
Vitamin premix[2]	0.03	0.03
Choline chloride (50%)	0.20	0.10
Sodium chloride	0.30	0.30
Total	100.00	100.00
Nutrient composition (%)		
Metabolizable energy (kcal/kg)	2950.00	3054.00
Crude protein	21.07	19.08
Calcium	1.00	0.90
Total phosphorus	0.69	0.58
Non-phytate phosphorus	0.45	0.35
Lysine	1.10	0.90
Methionine	0.50	0.41

[1] The trace-mineral premix (without zinc) provided the following (per kg of diet): 80 mg iron; 8 mg copper; 60 mg manganese; 0.35 mg iodine; and 0.15 mg selenium.

[2] The vitamin premix provided the following (per kg of diet): 8,000 IU vitamin A; 1,000 IU vitamin D_3; 20 IU vitamin E; 0.5 mg menadione; 2.0 mg thiamine; 8.0 mg riboflavin; 35 mg niacin; 3.5 mg pyridoxine; 0.01 mg vitamin B_{12}; 10.0 mg pantothenic acid; 0.55 mg folic acid; and 0.18 mg biotin.

All broilers were weighed per cage on d 21 and 42. Ten birds per treatment were randomly selected for the collection of the tibia, liver, and breast muscle. Liver and breast muscle samples were frozen at –20 °C until further analysis. Tibial bones were boiled for 5 min, and the soft tissues were removed. The bones were then dried for 24 h at 105 °C and ashed in a muffle furnace at 600 °C for 24 h.

Zn Content Analysis

Zn content in the feed, feces, tibia ash, liver, and breast muscle were determined by inductively coupled plasma atomic emission spectroscopy (IRIS Intrepid II, Thermal Jarrell Ash, Waltham, MA, USA). Follwing the method reported by Huang *et al*. (2009), approximately 0.5 g of the samples was weighed in triplicate, digested with 10 mL HNO_3 and 0.4 mL $HClO_4$ at 200 °C in a 50-mL calibrated flask until the solution became clear, evaporated to almost dryness, and diluted to 1:20 (vol/vol) with 2% HNO_3 before analysis.

Zn retention was calculated as follows:

Zn retention (mg/bird) = [feed intake (g/bird) × feed Zn content (mg/g)] – [fecal output (g/bird) × fecal Zn content (mg/g)]. The feed and fecal Zn contents were based on their analyzed values. The Zn content in the basal diet was also included in the Zn intake.

Statistical Analysis

Replicates were the experimental units used in the statistical analysis. Data were analyzed using the GLM procedure of SAS software (SAS Institute, 2002). Polynomial comparisons were performed to determine the linear and quadratic effects of supplemental Zn levels on the growth performance, organ Zn contents, and Zn retention. Means were compared by Tukey test when probability values were significant ($p<0.05$).

RESULTS AND DISCUSSION

Zn contents in the basal diets

The analyzed Zn contents in the basal starter (fed between 1 to 21 days of age) and grower (fed between 22 and 42 days) diets were 34.98 and 27.57 mg/kg, respectively (Table 2). Bartlett and Smith (2003), Ao *et al*. (2007), Huang *et al*. (2007), Sunder *et al*. (2008), Star *et al*. (2012), and Pacheco *et al*. (2017) determined that the Zn content of corn-soybean basal diets for broilers ranges from 25 to 35 mg/kg, indicating an average Zn content of 30 mg/kg. The Zn content of the basal diet cannot be overlooked when formulating broiler chicken feeds.

Table 2 – Calculated and analyzed Zn levels in experimental diets.

Day 1–21		Day 22–42	
Calculated Zn (mg/kg)	Analyzed Zn (mg/kg)	Calculated Zn (mg/kg)	Analyzed Zn (mg/kg)
0	34.98	0	27.57
20	51.22	16	39.16
40	84.15	32	56.71
60	104.22	48	67.80
80	118.13	64	86.99
100*	140.52	80*	121.79
120	144.88	96	130.89

*Zn levels recommended by the Feeding Standards of Chickens in China (Ministry of Agriculture of P. R. China, 2004).

Growth Performance

The BWG, FI, and FCR of 21- and 42-d-old broilers were not influenced by supplemental Zn levels ($p>0.05$, Table 3). No linear or quadratic relationships were observed between supplemental Zn levels and growth performance parameters ($p>0.05$).

It is well known that Zn is an essential element for animals. Zn deficiency results in growth inhibition in animals (MacDonald, 2000). However, broiler performance was not affected by supplemental Zn levels in the present study. Previous studies had also observed that broiler growth was not affected by dietary Zn concentrations (Bartlett and Smith, 2003; Rossi *et al*., 2007; Sunder *et al*., 2008; Owens *et al*., 2009; Yang *et al*., 2011; Star *et al*., 2012; Liao *et al*., 2013; Pacheco *et al*., 2017; Zakaria *et al*., 2017). This is attributed to the Zn content of basal diets (about 30 mg/kg), which is adequate to maintain the growth of broiler chickens from 1 to 42 d of age. Therefore, in the present study, growth performance could not be used as a criterion to evaluate Zn requirement of broiler chickens. However, other studies showed that broilers fed diets with no supplemental Zn presented lower BWG and FI than those Zn-supplemented diets (Huang *et al*., 2007; Liu *et al*., 2011; Sarvari *et al*., 2015). Moreover, it was observed that high levels of supplemental Zn (500 to 1500 mg/kg) impaired the growth performance of broilers (Sandoval *et al*., 1998; Kim & Patterson, 2004).

Table 3 – Effects of supplemental Zn levels on the growth performance of broiler chickens.

Supplemental Zn (mg/kg)		Body weight gain (g)		Feed intake (g)		Feed conversion ratio	
1–21 d	22–42 d	1–21 d	1–42 d	1–21 d	1–42 d	1–21 d	1–42 d
0	0	631	2411	1073	4651	1.70	1.93
20	16	679	2415	1163	4571	1.71	1.89
40	32	705	2472	1199	4703	1.70	1.90
60	48	667	2388	1126	4588	1.69	1.92
80	64	668	2417	1127	4681	1.69	1.94
100	80	619	2422	1075	4632	1.74	1.91
120	96	683	2338	1176	4532	1.72	1.94
SEM		8	24	13	40	0.01	0.01
p value							
Treatment		0.051	0.910	0.067	0.935	0.579	0.972
Zn	Linear	0.995	0.483	0.709	0.674	0.400	0.636
	Quadratic	0.224	0.463	0.402	0.575	0.302	0.732

Zn contents in bone, breast muscle, and liver

Tibial ash Zn content of 21-d-old broilers linearly increased between 0 and 40 mg Zn per kg addition ($p<0.05$, Table 4), but no differences in tibial ash Zn were observed when supplemental Zn levels ranged from 40 to 120 mg/kg. There was no effect of Zn levels on the tibial ash content of 42-d-old broilers ($p>0.05$). Research has shown that supplemental Zn increases blood Zn concentration in laying hens and broiler chickens (Tsai *et al.*, 2016; Zakaria *et al.*, 2017). A linear relationship was observed between 0 and 20 mg/kg supplemental Zn and bone Zn content in broiler chickens from 1 to 21 d of age (Ao *et al.*, 2007; Star *et al.*, 2012). Consistent results were observed between dietary levels of 0 and 40 mg Zn/kg in starter broilers in the present study. No significant differences were detected between 60–140 mg/kg supplemental Zn and bone Zn content (Huang *et al.*, 2007; Liao *et al.*, 2013). These data suggest that supplemental Zn increases Zn absorption in the blood and Zn deposition in bone, but that bones are not capable of further retaining Zn when supplemental Zn level is higher than 40 mg/kg.

Supplemental Zn levels did not influence breast muscle Zn content of 21-d-old broilers ($p>0.05$). On the other hand, in 42-d-old broilers, the supplemental Zn levels of 100 and 80 mg/kg diet promoted the highest breast muscle Zn contents ($p<0.01$), while the birds fed the highest supplemental Zn level (120 mg/kg) presented similar ($p>0.05$) breast muscle Zn contents as those fed the diets containing 0, 20, 40, and 60 mg supplemental Zn/kg. Sandoval *et al*. (1998) found that supplemental Zn levels increase muscle Zn content in broilers from 1 to 21 d of age. By contrast, Kim & Patterson (2004) found that supplemental Zn levels had no effect on the Zn level in breast muscles of broilers from 6 to 18 d of age. The effect of supplemental Zn level on muscle Zn content should be further clarified.

Supplemental Zn levels did not affect ($p>0.05$) broiler liver Zn content in the present study. In contrast, Sandoval *et al*. (1998) and Sunder *et al*. (2008) found that supplemental Zn levels linearly increased Zn content in the liver and kidneys of broilers. These differences may be due to supplemental Zn levels evaluated among studies. The present experiment evaluated supplemental Zn levels of 0 to 120 mg/kg, whereas Sandoval *et al*. (1998) and Sunder *et al*. (2008) used supplemental Zn levels of 0-320 and 0-1500 mg/kg, respectively.

Table 4 – Effects of supplemental Zn levels on organ Zn contents of broiler chickens

Supplemental Zn (mg/kg)		Zn in tibial ash (µg/g)		Zn in fresh breast muscle (µg/g)		Zn in fresh liver (µg/g)	
Day 1–21	Day 22–42	Day 21	Day 42	Day 21	Day 42	Day 21	Day 42
0	0	296.81 ^{c}	349.58	6.79	7.82^{bc}	39.35	28.13
20	16	371.71 ^{b}	355.23	6.79	7.69^{bc}	42.96	33.85
40	32	454.16 ^{a}	380.77	7.16	7.64^{bc}	41.02	32.93
60	48	465.96 ^{a}	355.83	7.37	7.05^{c}	41.93	31.15
80	64	445.76 ^{a}	361.63	7.06	8.71^{ab}	46.42	31.67
100	80	450.57 ^{a}	399.23	8.06	9.10^{a}	43.95	29.41
120	96	437.75 ^{a}	365.57	7.42	7.55^{c}	44.26	29.99
SEM		10.85	4.96	0.14	0.14	1.30	0.90
p value							
Treatment		<0.001	0.083	0.194	<0.001	0.866	0.676
Zn	Linear	<0.001	0.076	0.027	0.024	0.259	0.726
	Quadratic	<0.001	0.502	0.735	0.874	0.722	0.226

$^{a-c}$Means in the same column without a common superscript differ ($p<0.05$).

Zn retention

Increasing supplemental Zn levels linearly increased fecal Zn content, Zn intake, Zn excretion, and Zn retention of 31- to 33-d-old broilers ($p<0.05$, Table 5). When supplemental Zn levels increased from 0 to 120 and 96 mg/kg in the starter and grower diets, respectively, fecal Zn content, Zn intake, and Zn excretion increased from 137.42 to 388.84 µg/g, from 6.09 to 32.99 mg, and from 7.49 to 26.69 mg, respectively. Zn intake was lower than the Zn excretion at 0 to 20 and 16 mg/kg supplemental Zn in the starter and grower diets, respectively. Zn intake was approximately equal to Zn excretion when in the starter and grower diets were supplemented with 40 and 32 mg Zn/kg, and higher than Zn excretion when supplemental Zn levels were increased from 60 to 120 and 48 to 100 mg/kg in the starter and grower diets, respectively. Zinc retention was calculated as the difference between Zn intake and Zn excretion. Broilers presented negative Zn retention values when fed 0 and 20 mg Zn/kg in the

Table 5 – Effects of supplemental Zn levels on fecal Zn and Zn retention of 31- to 33-d-old broilers based on the Zn levels analyzed in the feeds and feces.

Supplemental Zn (mg/kg)		Fecal Zn (mg/kg)	Zn intake[1] (mg/bird)	Zn excretion (mg/bird)	Zn retention (mg/bird)
Day 1–21	Day 22–42				
0	0	137.42 ^{d}	6.09 ^{f}	7.49 ^{c}	-1.40 ^{b}
20	16	203.43 ^{cd}	11.16 ^{ef}	13.24 ^{bc}	-2.08 ^{b}
40	32	206.56 ^{cd}	15.20 ^{de}	15.49 ^{bc}	-0.29 ^{ab}
60	48	250.12 ^{bc}	17.90 ^{cd}	15.37 ^{bc}	2.53 ^{ab}
80	64	294.93 ^{abc}	24.01 ^{bc}	21.00 ^{ab}	3.01 ^{ab}
100	80	324.47 ^{ab}	28.99 ^{ab}	20.84 ^{ab}	8.15 ^{a}
120	96	388.84 ^{a}	32.99 ^{a}	26.69 ^{a}	6.30 ^{ab}
SEM		15.30	1.61	1.26	0.90
p value					
Treatment		<0.001	<0.001	<0.001	0.004
Zn	Linear	<0.001	<0.001	<0.001	<0.001
	Quadratic	0.521	0.623	0.998	0.722

[a–f]Means in the same column without a common superscript differ ($p<0.05$).

[1]The Zn content in the basal diet was included in the Zn intake calculation.

starter diet and 0 and 16 mg Zn/ kg in the grower diet, approximately zero when fed 40 and 32 mg Zn in the starter and grower diets, and positive values when fed 60 and 120 mg Zn/kg in starter diet and 48 and 96 mg Zn/kg in the grower diet respectively.

Burrell *et al*. (2004) and Kim & Patterson (2004, 2005) verified that fecal Zn content and Zn excretion linearly increased with supplemental Zn levels. Similar results were also detected in the present study. These data suggest that excess Zn is excreted with the feces in the environment, posing pollution risks. Burrell *et al*. (2004) found that the Zn intake was lower than the Zn excretion when supplemental Zn ranged from 0 to 40 mg/kg in broilers from 29 to 31 d of age. Body Zn loss resulted in lower bone Zn content when the Zn intake was lower than the Zn excretion in the present study. The Zn intake was approximately equal to the Zn excretion when supplemental Zn was 80 mg/kg (Burrell *et al*., 2004). Similar results were observed in this study and the Zn intake was approximately equal to the Zn excretion when supplemental Zn levels were 40/32 mg/kg. These data suggest that there is a threshold value of Zn deposition in broiler chickens. When the Zn retention reaches this threshold, Zn intake and Zn excretion are balanced.

The present study also showed that supplemental Zn did not significantly increase Zn content in the muscle, bone, or organs of 42-d-old broiler chickens. Therefore, most of the supplemental Zn was not retained in the chickens' bodies. Excessive Zn is excreted in the environment by the feces if Zn intake is higher than Zn requirements. Zn excretion and its effects on the environment should be considered when supplementing Zn in broiler chicken diets.

CONCLUSIONS

The corn-soybean basal diet contained approximately 30 mg/kg Zn. Supplemental Zn levels of 40 and 32 mg/kg in starter (1 to 21 days of age) and grower (22 to 42 days of age) diets, respectively, are sufficient to promote adequate growth performance of broiler chickens, also taking into consideration that Zn excretion in the feces may pose environmental pollution risks.

ACKNOWLEDGMENTS

This study was supported by the Special Fund for Agro-Scientific Research in the Public Interest of China (201303091), the Innovation Scientists and Technicians Troop Construction Projects of Henan Province (C20130058), and the Foundation of the Education Department of Henan Province (16A230003).

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