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CELLULAR AND MOLECULAR BIOLOGY

Effect of Supplemental Zinc on Performance, Nutrient Digestibility, Jejunum Architecture, and Immune Response in Broiler chickens Fed Wheat-Soy Diets

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Abstract: The present study was conducted to evaluate the effect of dietary zinc (Zn) levels on growth performance, carcass characteristic, nutrient digestibility, jejunum architecture and immune responses in broiler chickens fed wheat-soy diets. In addition the Zn requirement to optimize responses were estimated through regression models. A total, of 250 day-old male Ross-308 broiler chicks were randomly allocated to a completely randomized design experiment with five dietary treatments of five replicates of 10 birds each. Birds were fed diets containing 30, 70, 110, 150, and 190 mg/kg Zn from 1 to 35 days of age. A Zn level of 70 mg/kg diet was adequate to acquire typical growth performance, the nutrient digestibility, and carcass yield. First antibody titres response to sheep red blood cell inoculation, cutaneous basophil hypersensitivity elicited by phytohemagglutinin-P intradermal injection increased linearly by the increase in dietary Zn level. The Zn requirement estimated by the quadratic and linear broken-line models was varied between 63-70 mg/kg to optimize growth performance criteria. It is concluded the basal Zn concentration in wheat-soy diet is inadequate to fulfill the broiler chickens genetic potential in growth and a minimum of 70 mg/kg dietary Zn concentration is suggested to optimize broiler chickens performance.

Key words: Broiler chickens, performance, wheat, zinc.

INTRODUCTION

Zinc (Zn) is an essential trace element in numerous metabolic pathways. It plays an essential role in a wide variety of processes, including cell proliferation, growth, immune system, reproduction, hormone secretion, antioxidant defense, as well as many other biochemical processes (Abedini et al. 2018, Attia et al. 2019). The fact that more than 300 enzymes activities depend on Zn, makes it absolutely necessary for a large number of cellular vital functions, such as alkaline phosphatase, alcohol dehydrogenase, carboxypeptidase, carbonic anhydrase, and DNA/RNA polymerase (Stephanos & Addison 2014). Because of its effect on the number of peripheral T cells, thymocytes, and activity of natural killer cells, Zn is an essential element for the normal activity of immune system (Sunder et al. 2008, Attia et al. 2013a, b). It has also positive effects on the intestine through increased cell proliferation, enhanced protein synthesis (Tako et al. 2005), intestine crypt cell production, enterocytes integrity, and barrier structures and functions (Lambert et al. 2004).

A wide range of Zn requirement from 10.6 (Emmert & Baker 1995) to 105 mg/kg (Rossi et al. 2007) of diet were reported to optimize

different traits in broiler chickens. The amounts of Zn supply from usual feedstuffs in non-supplemented regular diets ranges between 13-62 mg/kg (Salim et al. 2008). However, Sunder et al. (2008) reported that preparation of 29 mg Zn/kg diet from the organic sources was adequate to optimize growth performance, mineral retention and immune response in broiler chickens. The results of some studies showed marginal supplementation of Zn (20 mg/kg) in practical diets was adequate to meet the Zn requirement of broiler chickens (Ao et al. 2011, Huang et al. 2007). Most of these estimates are less than of supplementary levels of trace elements (100 to 110 mg/kg) recommended by broiler chickens professional manual guide (Aviagen 2016). Excess minerals in diets may cause antagonism affecting other minerals bioavailability (Jahanian & Rasouli 2015).

There are high amounts of soluble non-starch polysaccharides (NSP) in wheat cell wall as well as other cereals such as barley, rye, and triticale (Teimouri et al. 2018). High levels of these compounds in the poultry diets can increase digesta viscosity and decrease physical mixing of intestinal contents, movement of compounds through the gastrointestinal tract, and consequently nutrients bioavailability (Zarghi et al. 2010, Attia et al. 2019). It is hypothesized that Zn supplementation may have a positive effect on performance of broiler chickens when fed wheat-based diet. Therefore, the current experiment was conducted to study the effects of different dietary Zn levels on growth performance, carcass yield, nutrient digestibility, intestinal digesta viscosity, jejunal architecture and immune responses in broiler chickens fed wheat-soy diet.

MATERIALS AND METHODS

Birds and management

The protocol used in this experiment complied with the Animal Care Committee guidelines, Ferdowsi University of Mashhad, Mashhad, Iran (approval no. 271/329/2013). Two hundred fifty day-old Ross 308 male broiler chicks were obtained from a local commercial hatchery. The chicks were weighed and assigned to treatments in a completely randomized design with 5 treatments, 5 replicates and 10 each one. The chicks were kept in floor pen (1 m^2, covered with wood shavings, equipped with a hanging feeder and two nipples water to provide birds with free access to feed and water), and each pen containing 10 birds served as an experimental unit. The house initial temperature was 32±2ºC for 3 days and then gradually decreased (0.5ºC, every day) to reach a constant temperature of 20-22ºC at 24 day of age. The relative humidity was maintained at 50-60% and a 23: 1 hour of light: dark cycle was used throughout the experiment.

Experimental diets

Falat wheat variety was used in this experiment, and obtained from the Khorasan Razavi Agricultural and Natural Resource Research Center, Iran. The chemical compositions of feed ingredients were determined by Evonic "Evonik Nutrition & Care GmbH" office in Tehran, Iran via NIR analysis and the values were used to formulate diets using UFFDA software. Three basal diets were formulated for the starter (d1-10), grower (d11-24), and finisher (d25-35) periods, to meet nutrient requirements according to the Ross-308 recommendation (Aviagen 2016) for a target live body weight of 1.70-2.40 kg/b (Table I). A big batch of basal diet for each period was provided and then divided into five equal portions. The basal (starter, grower and finisher) diets were obtained by using a Zn-free trace

Table I. Feed ingredients and composition of the basal wheat-soy diets[1].

	Periods		
	Starter (d1-10)	**Grower (d11-24)**	**Finisher (d25-35)**
Ingredient (g/kg)			
Wheat grain (13.15% CP)	579.2	613.3	670.8
Soybean meal (45.55% CP)	320.4	280.2	217.9
Soybean oil	52.3	62.8	70.3
Limestone	9.9	9.20	8.5
Dicalcium phosphate	20.0	17.8	16.0
Sodium chloride	1.6	1.6	1.4
Sodium bicarbonate	1.5	1.5	1.5
Vitamin premix[2]	2.5	2.5	2.5
Mineral premix[3]	2.5	2.5	2.5
DL-methionine	3.9	3.4	3.1
L-Threonine	1.9	1.6	1.5
L-Lysine HCL	4.3	3.6	4.0
Nutrient composition[4], as-fed basis			
Metabolizable energy, MJ/kg	12.6	13.0	13.4
Crude protein, g/kg	230.0	215.0	19.5
Calcium, g/kg	9.6	8.7	7.9
Available phosphorus, g/kg	4.8	4.4	4.0
Sodium, g/kg	1.6	1.6	1.6
Lysine, g/kg	14.4	12.9	11.6
Methionine, g/kg	7.0	6.3	5.7
Methionine + Cystine, g/kg	10.8	9.9	9.1
Threonine, g/kg	9.7	8.8	7.8
Zinc, mg/kg	30.1	29.2	28.9

[1]A batch of basal diet for each period was made and then divided into 5 equal portions, the Zn supplement as reagent-grade "ZnSO4.7H2O, 22.60% Zn" was added at the rate of 0, 177, 354, 531 and 708 mg/kg on top of each portion and mixed to provide five diets with 30 (basal diet), 70, 110, 150, and 190 mg/kg Zn concentration.
[2]Vitamin premix supplied the followings per kilogram of diet: vitamin A (all-trans-retinol), 12000 IU; vitamin D3 (cholecalciferol), 5000 IU; vitamin E (α-tocopherol), 18 IU; vitamin K3 (menadione), 2.65 mg; vitamin B1 (thiamin), 2.97 mg; vitamin B2 (riboflavin), 8.0 mg; vitamin B3 (niacin), 57.42 mg; vitamin B5 (pantothenic acid), 17.86 mg; vitamin B6 (pyridoxine), 4.45 mg; vitamin B9 (folic acid), 1.9 mg; vitamin B12 (cyanocobalamin), 0.02 mg; vitamin H2 (biotin), 0.18 mg; choline chloride, 487.5 mg, and antioxidant 1.0 mg.
[3]Mineral premix supplied the followings per kilogram of diet: Zn (zinc sulfate), 0.00 mg; Mn (manganese sulfate), 120.6; Fe (iron sulfate), 40.5; Cu (copper sulfate), 16.1; I (calcium iodate), 1.26; Se (Sodium Selenite), 0.31; choline chloride, 474.0.
[4]The determined ingredient analysis was used to calculate nutrients composition.

mineral mixture and contained 28.9-30.1 mg/kg of Zn from raw materials, as measured by Atomic Absorption Spectrometry analysis. A Zn of reagent-grade ($ZnSO_4.7H_2O$, 22.60% Zn-Sigma-Aldrich Chemical Co., St. Louis, MO) was added at the rate of 0, 177, 354, 531 and 708 mg/kg to the top of each portion and mixed to provide five diets with 30 (basal diet), 70, 110, 150, and 190 mg Zn/kg of diet, as fed basis. The supplemental and analysed zinc in experimental diets is shown in Table II.

Performance traits

All birds (pen groups) were weighed at 1, 10, 24 and 35d of age 4h after feed withdrawal. Daily dead birds in each pen were weighed and recorded to correct the growth performance traits. Average daily weight gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated after adjustment for mortality in each pen and period.

Nutrient digestibility

Chromic oxide was used at the rate of 3 g/kg diet as an inert marker during 24-30d of ages. Excreta samples were collected three times daily for 72h from a plastic sheet that was placed on the litter of each pen after 3 days adaptation period (24-27d). Collected excreta samples for each pen was pooled, thoroughly mixed and about 100g of the uniform mix was immediately stored at –20°C. A 100g excreta samples from each pen was later dried in a forced air oven at 60°C for 72h. The feed and dried excreta samples ground to pass through a 40-mesh screen and mixed thoroughly before analysis. Apparent digestibility coefficients of DM, organic dry matter (ODM) and crude protein (CP) were calculated according to formula 1, using the marker ratio in the diet and excreta (Tiemouri et al. 2019).

Formula 1: Apparent nutrient digestibility % = $\{(N/M)_d - (N/M)_e\}/ (N/M)_d \times 100$,

Where $(N/M)_d$ = Ratio of nutrient to marker in diet, and $(N/M)_e$ = Ratio of nutrient to marker in excreta.

Table II. Supplemental and analysed Zn concentrations in starter, grower and finisher diets[1].

Zn Supplement, mg/kg (ZnSO4.7H2O, 22.60% Zn)	Analysed diets Zn (mg/kg)		
	Starter	Grower	Finisher
0	30.10	29.20	28.90
177	70.20	69.50	70.10
354	110.50	111.60	109.60
531	149.50	150.50	151.30
708	189.50	190.50	191.0

[1] **The basal experimental diets were analysed for Zn content by atomic absorption. Each value based on triplicate determinations.**

Chemical analysis

The Cr2O5, DM, and nitrogen contents in feed and excreta samples were determined using the standard procedure of AOAC (Latimer Junior 2012). The Zn in feed sample was determined in an Air-Acetylene flame on an atomic absorption spectrophotometer (Perkin Elmer A Analyst 100, Massachusetts, Wellesley, USA) after digestion with tri-acid mixture; HNO3: H2SO4: HClO4 in the ratio of 15:2:4 (Jahanian & Rasouli 2015).

Humoral immune response

Sheep red blood cells (SRBC) inoculation, as a non-pathogenic antigen, was used for evaluating the humoral immune response in broiler chickens. Two birds/replicate close to the average pen weight were marked with dye and injected with 0.1 ml of 0.5% SRBC suspension into the brachial vein on the 21th and 27th days, and blood samples were taken seven day after each inoculation (Allahdo et al. 2018). Subsequently, the micro hemagglutination activity of serum

was estimated and the antibody titre (log2) measured following the standard procedure reported by Bartlett & Smith (2003).

Cellular immune response

The cell-mediated immune (CMI) response was assessed by measuring the response of cutaneous basophil hypersensitivity (CBH) to the mitogen phytohemagglutinin-P (PHA-P) by intradermal injection. Two birds/replicate close to the average pen weight were marked at 35d of age, the thickness of the web between the third and fourth inter-digital space on the left and right feet were measured with a micrometre and the web of right foot was injected with 100µg of PHA-P suspended in 0.1 ml of phosphate buffered saline (PBS), while in each bird the left web (Control) was injected with 0.1 ml of PBS. The web swelling of both feet was measured at 8, 16 and 24 h after injection (Allahdo et al. 2018). The CBH response to PHA-P was determined by subtracting the web thickness of the first measurement (pre-injection) from the second (post-injection) and the values of left foot (control) from the right foot (Corrier & Deloach 1990).

Carcass and visceral organs relative weight

Two birds/replicate close to the average pen weight were weighed and slaughtered after 4 hours of feed withdrawal, then plucked, and gastrointestinal tract, giblets, and other inner organs excised. Carcass yield and its cuts were weighed after chilled for 24 h at 4°C (Zarghi et al. 2020). Carcass, breast, legs, abdominal fat, and visceral organ were weighed by a weighing scale (0.001-g, model GF 400, A&D Weighing, San Jose, CA, USA), to calculate their relative weights (g/100 LBW).

Intestinal contents viscosity

The intestinal tract of the slaughtered birds was immediately removed to obtain small intestine contents by gentle finger stripping of the intestinal segments. For the viscosity measurement, the contents were taken from the jejunum and/ or ileum and each divided into two sub-samples, homogenized thoroughly and approximately 1.5 g wet weight centrifuged at 12700 g for 5 min to obtain the supernatants (Zarghi et al. 2010). The supernatant was withdrawn and its viscosity in centipoises (1/100 dyne-second per square centimetre) was measured in a Brookfield digital Viscometer (Model LVDVII + CP, Brookfield Engineering Labs, Inc., Stoughton, MA 02072) at 37°C. The average value obtained from two subsamples was used for the statistical analysis.

Jejunum architecture

One tissue sample (0.5-1.0 cm) was taken from jejunum midpoint of each slaughtered bird and then immersed in a 10% buffered formalin solution for 72h. Then, samples treated in the tissue processor apparatus and embedded in paraffin wax. Transverse sections were cut (6 µm) using a rotary microtome (Leica RM 2145), placed on a glass slide and stained with Hematoxylin and Eosin (H&E), then they were analysed under a light microscope to determine morphological indices (Gangali et al. 2015). All chemicals were purchased from Sigma chemical company. Micrographs were taken with an Olympus BX41 microscope (Olympus Corporation, Tokyo, Japan) fitted with a digital video camera. The images were analysed using image software (Image-Pro Plus v 4.5) on 9 villi chosen from each slide and only vertically oriented villus were selected for measuring. The morphometric traits were: villus height (VH), villus width (VW), crypt depth (CD), muscular thickness (MT) and villus surface area

(VSA) which was calculated according to Formula 2 (Teimouri et al. 2018).

Formula 2: $$Villus\ surface\ area = 2\pi \times \frac{VW}{2} \times VH$$

Where π = 3.14, VW = Villus width, and VH = Villus height

Statistical analysis

All data were analysed for normality using SAS 9.1 software through the Univariate plot normal procedure (SAS 2003). Then, data were analysed by the General Linear Model of SAS 9.1 software with diets as independent variables. Linear and quadratic polynomial models were analysed to describe the relationships between dietary Zn levels and variables. Zinc requirement for optimal growth performance response parameters were determined by using NLIN procedure of SAS 9.1 software, through linear and quadratic broken-lines regression fit models (Robbins et al. 2006). Two mathematical models were used to estimate the optimal dietary Zn level to maximize broiler responses base on a gradient dietary Zn levels. Linear broken-line (LBL) and quadratic-broken line (QBL) models were expressed as formula 3-4, respectively (Zarghi et al. 2020).

Formula 3: $$Y = \beta_0 + \beta_1 (\beta_2 - X),\ (\beta_2\text{-}X) = 0 \text{ for } X > \beta_2$$

Where: Y is the dependent variable, X is the dietary Zn concentration, β_0 is the value at the plateau, β_1 is the slope and β_2 is the Zn concentration at the breakpoint.

Formula 4: $$Y = \beta_0 + \beta_1 (\beta_2 - X)^2,\ (\beta_2\text{-}X) = 0 \text{ for } X > \beta_2$$

Where: Y is the dependent variable, X is the dietary Zn concentration, β_0 is the value at the plateau, $\beta 1$ is the slope and $\beta 2$ is the Zn concentration at the breakpoint.

To assist in choosing an appropriate model, R^2 values and mean absolute error were calculated using the formula 5 and 6, respectively.

Formula 5: $$R^2 = \frac{(CTSS - SSE)}{CTSS}$$

Where: R^2 is the fraction of variation in the dependent variable explained by the model, residual values were used to compute the necessary sums of squares for error and corrected total sums of squares (CTSS) were used as the baseline.

Formula 6: $$MAE = \frac{1}{n} \sum_{1}^{i} \left| Y_i + \acute{Y}_i \right|$$

Where: MAE is the mean absolute error, n is the number of observation, Y_i is the observation and $\acute{Y}_i$ is the predicted value.

RESULTS

Performance traits

The initial average weight of chicks was 44.42±2.26g and were not significant for the assigned treatment groups (P<0.05). The Overall mortality mean was 3.95% and was not significantly influenced by dietary Zn concentration. Increasing dietary Zn levels quadratically affect BW, ADG and FCR, whereas linearly ADFI was increased in the starter period. Birds fed diet contained 70 mg/kg Zn performed higher than the other groups. Live body weight was 8.2% higher in birds fed diet with 70 mg/kg Zn compared to those fed non-supplemented diet at the end of grower period (P<0.002). In spite of large numerical differences the ADG, ADFI and FCR were not significantly affected by dietary Zn levels in the grower period. Supplemental dietary Zn produce a quadratic (P<0.001) dose response for final BW and linear (P<0.001) responses for ADFI and ADG in the finisher period, but not effect on FCR. Increased dietary Zn levels quadratically enhanced final BW and ADG; and linearly increased ADFI in the whole rearing period (d1-35). Live body weight

was 7.0, 5.4, 5.4 and 4.6% higher in birds fed diets contained 70, 110, 150 and 190 mg/kg Zn as compared to those fed the basal diet (dietary Zn level, 30 mg/kg) at the end of the trial. Similarly, a significant quadratic effect of increasing dietary Zn level was observed for ADG. As dietary Zn level increased from 30 to 190 mg/kg, the ADFI linearly increased. The FCR was not affected by dietary Zn levels (Table III).

Nutrient digestibility

There was a significant linear response in nutrients digestibility to increasing dietary Zn concentration. The increase in Zn concentration from 30 mg/kg (basal diet) up to 150 mg/

Table III. Effect of dietary zinc concentration on the performance of broiler chickens fed wheat-soy diets 1-35d of age[1].

	Dietary Zn level, mg/kg as-fed					SEM[2]	Significance, P-value		
	30	70	110	150	190		ANOVA	Linear	Quadratic
Starter (d1-10)									
LBW d1, g	45.2	43.5	44.7	44.9	43.8	1.058	0.758	0.685	0.922
LBW d10, g	198^{cd}	228^{a}	204^{bc}	207^{b}	195^{d}	2.287	<0.001	0.145	0.004
ADG, g/d	15.26^{c}	18.45^{a}	15.94^{bc}	16.18^{b}	15.13^{c}	0.252	<0.001	0.185	0.008
ADFI, g/d	19.82^{b}	21.36^{a}	21.79^{a}	22.55^{a}	22.80^{a}	0.473	0.002	<0.001	0.202
FCR	1.300^{b}	1.160^{c}	1.367^{b}	1.394^{b}	1.512^{a}	0.039	<0.001	0.131	<0.001
Grower (d11-24)									
LBW d24, g	842^{b}	911^{a}	848^{b}	860^{b}	839^{b}	11.936	0.002	0.279	0.114
ADG, g/d	46.00	48.82	46.02	46.69	45.98	0.790	0.086	0.444	0.421
ADFI, g/d	65.32	72.53	68.31	71.66	69.31	1.758	0.064	0.266	0.158
FCR	1.423	1.486	1.485	1.541	1.510	0.052	0.607	0.159	0.299
Finisher (d25-35)									
LBW d35, g	1805^{c}	1932^{a}	1902^{ab}	1902^{ab}	1889^{b}	12.598	<0.001	0.053	<0.001
ADG, g/d	96.32^{b}	102.09^{a}	105.33^{a}	104.13^{a}	105.04^{a}	1.574	0.003	0.002	0.123
ADFI, g/d	185.27^{c}	194.80^{b}	198.86^{ab}	203.45^{a}	203.25^{a}	2.101	<0.001	<0.001	0.112
FCR	1.924	1.908	1.888	1.955	1.940	0.032	0.624	0.439	0.878
Total (d1-35)									
ADG, g/d	50.28^{c}	53.97^{a}	53.06^{ab}	53.05^{ab}	52.73^{b}	0.354	<0.001	0.051	<0.001
ADFI, g/d	84.72^{b}	90.77^{a}	90.37^{a}	93.23^{a}	92.31^{a}	1.094	<0.001	<0.001	0.115
FCR	1.685	1.682	1.703	1.758	1.751	0.026	0.142	0.145	0.326

[1]The value are means of 5 replicates of 10 chickens each.
[2]SEM, Standard error of the means.
LBW, Live body weight; ADG, Average daily gain; ADFI, Average daily feed intake; FCR, Feed conversion ratio.
[a, ...c]The means within each row with uncommon superscript letters are significantly different (P<0.05).

kg, apparent digestibility of dry matter (DM) and crude protein (CP) increased linearly. In birds fed diet with 150 mg/kg Zn, the apparent digestibility of DM and CP were 2.2% and 4.3% higher than those fed non supplemented basal diet, but remained almost constant at higher Zn level, 190 mg/kg of diet (Table IV).

Relative carcass and visceral organs weights and intestine content viscosity

Effects of dietary Zn levels on carcass and cuts yield, abdominal fat, relative small intestine weights (g/100g of LBW) and small intestine content viscosity are shown in Table V. An increase in dietary Zn concentration linearly enhanced relative carcass and breast weights, and decreased relative small intestine weight (P<0.01). Supplementation of diet with Zn caused a significant linear reduction in ileum content viscosity (P<0.05), but did not affect jejunum content viscosity.

Table IV. Effect of dietary Zn concentration on apparent dry matter, organic dry matter and crude protein digestibility in broiler chickens fed wheat-soy diets[1].

	Apparent nutrient digestibility (%)		
	Dry matter	Organic dry matter	Crude protein
Dietary Zn level, mg/kg			
30	65.82	68.27	60.54
70	66.08	69.04	61.49
110	67.00	69.29	63.16
150	67.23	69.37	63.18
190	67.09	69.33	62.72
SEM[2]	0.666	0.914	1.121
Significance, P-value			
ANOVA	0.081	0.785	0.095
Linear	0.027	0.193	0.040
Quadratic	0.178	0.586	0.134

[1]The value are means of 5 replicates of 10 chickens each.
[2]SEM= Standard error of the means.

Jejunum architecture

Effects of dietary Zn levels on jejunum morphometric traits are shown in Table VI. A significant linear response to increasing dietary Zn concentration was observed on VH, CD and VH/CD ratio (P<0.001). Increasing dietary Zn levels from 30 to 190 mg/kg the VH increased from 1092 to 1369 µm (approximately 25% longer) and CD decreased from 220 to 174 µm (approximately 25% diminish) with a linear manner. Therefore, birds fed diet with 190 mg/kg Zn had the highest VH and lowest CD. In line with those, the VH/CD ratio linearly increased from 5.1 to 7.9 as dietary Zn level increased from 30 to 190 mg/kg. Villus width, muscular thickness and villus surface area were not significantly affected by diet Zn level.

Immune response

The effect of dietary Zn supplementation on the relative weight of lymphoid organs, humoral and cellular immune responses are shown in Table VII. The humoral and cellular immune responses linearly were improved as dietary Zn concentration increased (P<0.05). First antibody titres to sheep red blood cell (SRBC) inoculation and cutaneous basophil hypersensitivity (CBH) elicited by phytohemagglutinin-P (PHA-P) intradermal injection were increased linearly by the increase in dietary Zn levels (P<0.05). Relative lymphoid organs (liver, Bursa of Fabricius and Spleen) weights were not affected by dietary Zn levels (P>0.05).

Estimated Zn requirement

The Zn requirement estimated by a linear broken line (LBL) and quadratic broken line (QBL) regression fit models to optimize growth performance responses are shown in Table VIII. The predicted Zn requirement for optimal LBW, ADG, ADFI and FCR by the LBL model were 68.09, 68.04, 64.79, and 62.75 mg/kg of diet, and by the

Table V. Effect of dietary Zn concentration on relative weight (g/100g live body weight) of the carcass, abdominal fat and visceral organs and gut contents viscosity (centipoise) in broiler chickens fed wheat-soy diets[1].

	Carcass portion[2]				Abdominal fat[2]	Small intestine[2]	Digesta viscosity(Cps[3])	
	Carcass	Breast	Legs	Other[4]			Jejunum	Ileum
Dietary Zn level, mg/ kg								
30	71.75[b]	24.06[b]	20.66	27.03	1.93	3.09[a]	0.59	4.02[a]
70	71.76[ab]	24.01[b]	20.45	27.31	1.89	2.98[a]	0.62	3.47[ab]
110	72.22[ab]	24.81[ab]	20.24	27.18	1.60	2.85[ab]	0.64	2.80[b]
150	72.69[ab]	25.19[ab]	19.66	27.84	1.96	2.85[ab]	0.63	2.67[b]
190	73.95[a]	26.44[a]	20.00	27.50	1.92	2.70[b]	0.62	2.92[b]
SEM[5]	0.565	0.618	0.511	0.703	0.224	0.094	0.048	0.418
Significance, P-value								
ANOVA	0.068	0.067	0.678	0.936	0.793	0.047	0.956	0.045
Linear	0.005	0.004	0.181	0.488	0.943	0.022	0.637	0.035
Quadratic	0.011	0.012	0.384	0.779	0.732	0.221	0.699	0.038

[1]The value are means of 5 replicates of 2 samples each.
[2]Calculated as a percentage of live body weight.
[3]Cps=centipoise, 1/100 dyne-second per square centimetre.
[4]Back and neck.
[5]SEM, Standard error of the means.
[a, b]The means within each column with uncommon superscript letters are significantly different (P<0.05).

QBL model were 67.85, 70.00, 70.00 and 68.01 mg/kg of diet, respectively. The Zn requirement estimated to optimize LBW and ADG responses by the LBL and QBL models were approximately equal, but the estimated Zn requirement by the QBL model to optimize FI and FCR were 1.08% higher than those estimated by the LBL fit model.

DISCUSSION

Dietary Zn and performance

The results of this experiment indicated that ADFI and ADG increased by the increase in dietary Zinc level. It seems that feeding broiler chickens with a basal diet (non Zn supplemented) lead to reduce appetite. The reduction in appetite may be signaled by decrease in brain neurotransmitter "gamma-amino-butyric acid" concentrations and key enzyme carbohydrates metabolism "pyruvate kinase" (Salim et al. 2008). Improvement in weight gain of the birds fed diet supplemented with Zn has been observed in earlier study (Sunder et al. 2008). The increase in body weight in birds fed Zn supplemented diet may be linked with higher production of digestive enzymes (Korének et al. 2007), improvement of nucleic acid biosynthesis and protein synthesis (Salim et al. 2008). In our study, the maximum of ADG was observed in chickens fed diet contained 70 mg of Zn/kg, and then gradually decreased in a quadratic manner as supplemental Zn increased further (P<0.001). This downward may be resulted by Zn antagonism with other nutrients. It has been reported that increasing dietary Zn levels more than 80 mg/ kg impair phosphorus bioavailability (Sunder et al. 2008). In contrast, some studies reported dietary Zn supplementation did not affect the ADFI and ADG of broiler chickens (Burrell et

Table VI. Effect of dietary Zn concentration on jejunum histomorphological measurements in broiler chickens fed wheat-soy diets[1].

	VH[2]	VW[2]	CD[2]	MT[2]	VSA[2]	VH/CD[2]
Dietary Zn level, mg/kg	**..... µm .....**				**10000 µm²**	
30	1092[c]	207	220[a]	164	70.61	5.10[c]
70	1113[c]	180	235[a]	153	63.13	4.75[c]
110	1212[bc]	185	184[b]	164	69.89	6.60[b]
150	1241[b]	163	173[b]	174	63.22	7.25[ab]
190	1369[a]	158	174[b]	170	63.34	7.90[a]
SEM[3]	39.834	16.092	10.342	9.948	2.592	0.355
Significance, P-value						
ANOVA	0.001	0.181	0.001	0.528	0.255	<0.001
Linear	<0.001	0.211	<0.001	0.875	0.162	<0.001
Quadratic	<0.001	0.321	0.002	0.579	0.121	<0.001

[1] The value are means of 5 replicates.
[2]VH=Villus Height; VW=Villus Width; CD=Crypt Depth; MT=Muscular thickness; VSA= Villus surface area; and VH/CD=Villus height/ Crypt depth.
[3]SEM, Standard error of the means.
[a, ...c]The means within each column with uncommon superscript letters are significantly different (P<0.05).

al. 2004, Sunder et al. 2008). Increased dietary Zn level through organic (Rossi et al. 2007), inorganic and their mixtures (Burrell et al. 2004, Attia et al. 2019) did not significantly affect the feed efficiency in broiler chickens. In contrast, the beneficial effect of supplemental Zn on feed efficiency has been demonstrated in other study (Hess et al. 2001), they showed that the increase in dietary Zn up to 95 mg/kg improved feed efficiency in broiler chickens.

Our data confirmed that the increase in dietary Zn, increased relative carcass and breast weights (Table V). Similarly, other researchers revealed that an increase in carcass yield of broiler chickens when they fed diet supplemented with Zn (Ao et al. 2011). The increase in carcass yield of quails by dietary Zn supplementation has also been reported (Sahin et al. 2009). It is suggested that higher concentrations of Zn improved meat yield by helping to promote protein synthesis, collagen formation and optimal activity of the enzymes (Saenmahayak et al. 2010). Although, some investigators did not find any effect of dietary Zn supplementation on relative carcass weight of broiler chickens (Rossi et al. 2007, Zakaria et al. 2017, Sunder et al. 2008, Attia et al. 2013a, b).

Dietary Zn affect GIT responses

The result of our study revealed, a significant linear increase in apparent digestibility of dry matter and crude protein by the increase in dietary Zn concentration (Table IV). Zinc serves as a cofactor for many digestive enzymes, such as metalloproteinase and metallocarboxypeptidases (Sahin et al. 2009, Attia et al. 2019). It is reported that Zn can be found in high concentrations in various glandular organs, especially digestive tract secretions organs (Hedemann et al. 2006) and the pancreatic enzyme activity was increased by dietary Zn supplementation (Szabo et al. 2004).

Our study showed that as dietary Zn concentration increased, the relative small

Table VII. Effect of dietary Zn concentration on antibody titres responses to sheep red blood cell (SRBC) inoculation, cutaneous basophil hypersensitivity (CBH) responses elicited by phytohemagglutinin-P (PHA-P) intradermal injection, and relative weight of lymphoid organs in broiler chickens fed wheat-soy diet[1].

	Antibody titres responses to SRBC first and second inoculation at 28 and 35 d of age, respectively (log2)						CBH-responses[2] (µm)				lymphoid organs relative weight (%LBW[3])		
	7d after the 1st injection			7d after the 2nd injection			Hours Post-injection						
	Total Antibody[4]	IgG[4]	IgM[4]	Total Antibody[4]	IgG[4]	IgM[4]	8	16	24	Mean	liver	Bursa	Spleen
Dietary Zn level, mg/kg													
30	7.67[b]	5.50	2.17[b]	8.33	6.83	1.50	443[b]	324[b]	305[b]	357[b]	2.54	0.14	0.16
70	8.17[ab]	5.33	2.83[ab]	8.67	6.83	1.83	522[ab]	521[a]	503[a]	515[a]	2.52	0.14	0.16
110	8.33[ab]	5.67	2.67[ab]	8.83	7.00	1.83	583[a]	515[a]	423[ab]	507[a]	2.25	0.15	0.14
150	8.33[ab]	5.33	3.00[ab]	9.00	7.33	1.67	593[a]	514[a]	467[a]	525[a]	2.29	0.17	0.17
190	8.50[a]	5.00	3.50[a]	8.83	6.83	2.00	614[a]	509[a]	465[a]	529[a]	2.42	0.17	0.14
SEM[5]	0.235	0.252	0.351	0.262	0.382	0.311	35.39	38.34	46.58	37.10	0.092	0.023	0.012
Significance, P-value													
ANOVA	0.150	0.444	0.139	0.464	0.859	0.826	0.024	0.009	0.069	0.024	0.098	0.111	0.209
Linear	0.017	0.213	0.013	0.108	0.671	0.387	0.001	0.023	0.100	0.013	0.125	0.256	0.398
Quadratic	0.038	0.264	0.049	0.162	0.766	0.686	0.196	0.210	0.161	0.061	0.145	0.693	0.425

[1] The value are means of 5 replicates of 2 samples each.
[2]CBH = (skin thickness, PHA-injected foot) - (skin thickness, PBS-injected foot).
[3]LBW=live body weight.
[4]Data represent means of $\log_2$ of the reciprocal of the last dilution exhibiting agglutination.
[5]SEM= Standard error of the means.
[a, b]The means within each column with uncommon superscript letters are significantly different (P<0.05).

intestine weight and ileum viscosity linearly decreased (Table V). The decrease in the relative small intestine weight may be related to enhanced gut endogenous enzyme excretion and enzyme activity under adequate supply of Zn (Hedemann et al. 2006) which led to decrease the adverse effect of wheat anti-nutrients. Since Zn functions as a cofactor for many

metalloenzymes, decreasing intestinal digesta viscosity in Zn supplemented groups may be linked with higher production of digestive enzymes (Korének et al. 2007). The use of viscos cereal grains such as wheat, barley, rye and triticale in broiler feed formulation is the cause of high digesta viscosity (Zarghi et al. 2010, Attia et al. 2013a, b). So by the increase in dietary Zn level, the adverse effects of high NSP levels on digesta viscosity may be reduced (Sahin et al. 2009, Szabo et al. 2004).

The VH and VH/CD improved numerically with increase in dietary Zn level (Table VI), this beneficial effect may be attributed to the reduced NSP stress caused by wheat based diet. It is reported that the addition of inorganic Zn in diet led to increase in the VH and VH/CD ratio (Shah et al. 2019), and or Zn supplemented at the rate of 80 and 120 mg/kg resulted in a higher VH:CD in broilers challenged with Salmonella typhimurium (Zhang et al. 2012). Zinc is a trace mineral having antioxidant properties by metallothionein production (Shah et al. 2019). It changes intestinal morphology by enhancing intestinal absorptive capacity and promotes intestinal health (Liu et al. 2015). Additionally, the increased VH in Zn supplemented groups may be linked to increase the proliferation of crypt cells due to Zn bioavailability (Tako et al. 2005).

Dietary Zn affects immune competence

A significant linear response to increase in dietary Zn concentration was observed for total antibody and IgM (Table VII). The primary response of birds received 190 mg Zn/kg diet was a significantly higher titers of total and IgM antibodies compared to those fed diet containing 30 mg Zn/kg. However, this effect was not observed when measured at the 14d post immunization. These results are in agreement with the previous study that showed diets supplemented with zinc tend to improve the immune status in broiler chickens (Bartlett & Smith 2003, Kidd et al. 1996). Other researchers found an improvement in antibody titer against SRBC in broiler chickens fed a diet with Zn supplementation (Sunder et al. 2008). Abnormal T-lymphocyte development is thought to be the primary consequence of Zn deficiency (Sahin et al. 2009). Diet supplemented with Zn over 40 mg/kg enhanced antibody production (Kidd et

Table VIII. Summary of Zn requirements (mg/kg of diet) for optimization of performance responses estimated by the liner and quadratic broken-line regression fit models in broiler chickens fed wheat-soy diet.

	Linear broken-line regression analysis					Quadratic broken-line regression analysis				
	Estimated Zn requirement	Approximate confidence limits		P-Value	R^2	Estimated Zn requirement	Approximate confidence limits		P-Value	R^2
		Lower 95%	Upper 95%				Lower 95%	Upper 95%		
LBW	68.09	58.49	77.69	0.001	0.94	67.85	60.49	75.22	0.001	0.92
ADG	68.04	58.76	77.32	0.001	0.94	70.00	62.54	77.45	0.001	0.94
ADFI	64.79	37.77	91.81	0.005	0.70	70.00	50.53	89.47	0.004	0.71
FCR	62.75	13.14	94.36	0.010	0.98	68.01	31.71	105.30	0.030	0.90

LBW, Live body weight; ADG, Average daily gain; ADFI, Average daily feed intake; FCR, Feed conversion ratio.

al. 1996). As dietary Zn concentration increased the CBH response elicited by the mitogen PHA-P intradermal injection linearly increased. Cutaneous basophil hypersensitivity elicited in chickens by intradermal injection of PHA-P is a thymus-dependent response mediated by thymic cells and is a useful method for assessing CMI in vivo (Corrier & Deloach 1990). Specific CMI involves many effector mechanisms including the actions of cytotoxic lymphocytes "cytotoxic T lymphocytes and natural killer cells" and macrophages whose functions are greatly enhanced by or dependent on type-1 cytokines (Erf 2004). Sunder et al. (2008) reported CMI responses in the broiler chickens fed diet supplemented with 80 mg/kg or greater which were significantly higher than those fed diet with lower Zn concentration.

Our result showed the relative lymphoid organs (liver, Bursa of Fabricius & Spleen) weights were not affected by dietary Zn levels (P>0.05). Our finding is consistent with previous reported, lymphoid organs such as thymus, bursa of Fabricius, and spleen did not significantly affected by the dietary zinc level in broiler chickens (Bartlett & Smith 2003, Attia et al. 2013a, b). In contrast with the above finding, Sunder et al. (2008) reported the weights of the bursa of Fabricius and spleen were significantly higher in broilers fed a diet with 40 mg/kg supplemental Zn than those fed lower level. On the other hand, low levels of supplemental Zn showed a relative reduction in the size of lymphoid organs with a possible decrease in T-cell function (Kidd et al. 1996).

Zinc requirement

The Zn requirements estimated by the linear and quadratic broken-line models varied between 63-70 mg/kg for performance optimization (Table VIII). Zinc requirements obtained in this study to optimize performance traits in broiler chickens fed wheat soy diet was almost 75% higher than those (40 mg/kg of diet) recommended by the National Research Council (NRC 1994), but was 36% lower than those (110 mg/kg of diet) recommended by Ross 308 manual (Aviagen 2016). In various studies, the different optimum level of dietary Zn was reported to improve the performance of broiler chickens including 29 mg/kg (Sunder et al. 2008), 45 mg/kg (Salim et al. 2008), 84 mg/kg (Huang et al. 2007) and 95 mg/kg of diet (Hess et al. 2001). The differences in Zn requirement in various reports may be due to genetic, diets and rearing conditions used in different studies (Zakaria et al. 2017, Attia et al. 2019).

In conclusion, supplementation of Zn in the wheat-soy diet increased growth performance, immune response, nutrient digestibility, and carcass yield in broiler chickens raised to 35 d of age. Maximum performance in broiler chickens was achieved with 63-70 mg/kg dietary Zn, which was higher than that recommended by NRC (40 mg/kg). Overall, the natural presence of Zn in the wheat-soy diets from the raw materials is not enough alone to adequately sustain the optimum performance and health of broiler chickens. Therefore it is necessary to supply dietary Zn concentration up to 70 mg/kg, as fed basis.

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