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Zinc Requirements of Japanese Quails *(Coturnix coturnix japonica)* by Assessing Dose- Evaluating Response of Zinc Oxide Nano-Particle Supplementation

Abbasi M[1], Dastar B[1], Afzali N[2], Shams Shargh M[1] & Hashemi SR[3]

[1]Department of Animal and Poultry Nutrition, Faculty of Animal Science, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
[2]Department of Animal Science, Faculty of Agricultural Sciences and Natural Resources, University of Birjand, Iran
[3]Department of Animal and Poultry Physiology, Faculty of Animal Science, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

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Corresponding author
Behrouz Dastar
dastar@gau.ac.ir

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Abstract

This study was conducted to determine the effects of various doses and particle sizes (micro or nano) of dietary zinc and zinc oxide on growth performance, serum enzyme activities, carcass characteristics, and zinc requirements in Japanese quails. A total of 576 day-old Japanese quails (both sexes) were housed in 36 deep litter floor pens. Birds received a basal corn–soybean meal diet that was deficient in zinc (27 mg zinc/kg) for 10 days post-hatching in order to deplete them from zinc reserves. Then, quails were randomly allocated to nine dietary treatments: a control treatment (27 mg of Zn/kg of diet), or one of four levels of Zn (25, 50, 75, and 100 mg/kg of diet) that were one of two ZnO particle sizes (micro or nano ZnO). Birds were fed the experimental diets from 10 to 40 days of age. Body weight and feed intake per pen were measured every 10 days and feed conversion ratio was calculated. On day 40, two males per replicate were slaughtered and carcass characteristics were measured. A quadratic increase in body weight gain ($P < 0.01$) and feed conversion ratio ($P < 0.05$) were found in zinc-supplemented quails between 20 to 30 d. Increasing dietary Zn levels significantly increased the relative weights of testes ($P < 0.01$), and thigh ($P < 0.05$). In this study, the optimal dietary Zn levels for body weight gain of Japanese quails were 90 mg/kg of diet for birds 10-20 days old, 70 mg/kg of diet for birds 20-30 days old, and 59 mg/kg of diet for birds 30-40 days old.

Introduction

Zinc (Zn) is an essential trace element that is required for normal growth, bone development, feathering, enzyme structure and function in avian species (Sahin *et al.*, 2009). Zinc plays substantial roles in the synthesis of DNA and RNA, as well as the activities of a variety of hormones such as glucagon, insulin, growth hormone, and sex hormones (Salim *et al.*, 2008; Sahraei *et al.*, 2013). Young Japanese quails are quite sensitive to dietary deficiencies of Zn (Shim and Vohra, 1984). Zn deficiency disrupts Zn homeostasis and affects growth, morphogenesis, and immune responses, as well as neurosensory and endocrine functions (Hara *et al.*, 2017). Nevertheless, a high concentration of Zn can affect the balance of other trace

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elements such as Ca, Cu, and Fe in the body (Sundaresan *et al*., 2008).

Based on NRC (1994), the Zn requirement in quails that are 1-42 days of age is 25 mg/kg of diet. However, this requirement was determined based on semi-purified diets, which have less anti-nutritional factors like fiber and phytic acid compared to practical diets (Zaghari *et al*., 2015). In chicks, dietary phytate can increase optimal dietary Zn (Ao *et al*., 2007; Linares *et al*., 2007) because zinc is susceptible to phytate compilation, thereby severely decreasing its availability (Cowieson *et al*., 2004). Therefore, the Zn requirement suggested by NRC (1994) would be too low to support the maximum growth potential of Japanese quails as Harland *et al*. (1975) showed that quail diets containing 75 mg Zn/kg significantly improved growth compared to those fed 25 mg Zn/kg. Zinc is currently added to poultry diets either as zinc oxide (ZnO) or zinc sulphate ($ZnSO_4$-H_2O). However, due to their high water solubility, zinc sulphates foster free radical formation from reactive metal ions and can therefore breakdown vitamins and reduce the nutrient value of the diet (Batal *et al*. 2001).

Consequently, zinc is primarily supplemented as ZnO (80-90%) despite its lower bioavailability in poultry than $ZnSO_4$ (Batal *et al*., 2001). Oxide forms of minerals are less reactive and less bioavailable. It would be useful to increase the bioavailability of ZnO. Nano zinc oxide has recently been produced and marketed using concepts of nanoscience and technologies (Song *et al*., 2010). Over the past 10 years, nanotechnology has been generally used in animal farming to increase the bioavailability of trace elements in the diets (Scott, 2005). Nanotechnology holds great promises for nutrition, since materials of this size have displayed properties unlike those isolated atoms and mass materials (Albrecht *et al*., 2006). In addition, the permeability of Nano-ZnO can also help prevent adverse gastrointestinal reactions and improve the absorption of medicine (Zhao *et al*., 2014). However, whether nano materials can improve animal performance is unclear, especially nano ZnO in Japanese quails. Therefore, the purpose of this study was to evaluate the effects of Zn levels and ZnO particle sizes on performance, serum enzyme activities, and carcass characteristics. We will also determine the optimal level of dietary Zn required for maximal performance in Japanese quails.

Materials and Methods

Birds and husbandry

All procedures and experiments were approved by the Ethics Committee of Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. A total of 576 day-old Japanese quails (mixed sex) were purchased from a local hatchery and housed in 36 deep litter floor pens (60 cm wide × 90 cm length). Each pen was equipped with one plastic pan feeder and one nipple drinker, and was covered with 5 cm of wood shaving materials. Birds drank tap water that contained 30 µg Zn/L (determined by polarography; Model VA 797 Metrohm). Initial ambient temperature was 34°C and gradually decreased by approximately 0.5°C per day until 26°C at the end of the experiment. The relative humidity was maintained at ~50% throughout the experiment. Birds were exposed to 24 hours of continuous lighting with an intensity of 30 Lux during the first three days and 10 Lux at the end of the experimental period.

Diets and ingredients

Quails received a basal corn–soybean meal diet that was deficient in zinc (27 mg zinc/kg) (Table 1) for the first 10 days post-hatching in order to deplete their zinc reserves. At the age of 10 d, birds were randomly divided into nine dietary treatments, which consisted of a control treatment (27 mg of Zn/kg), four levels of Zn (25, 50, 75, or 100 mg/kg of diet; i.e.: 52, 77, 102, and 127 mg/kg of total dietary Zn, respectively) under two levels of ZnO particle sizes (micro ZnO and nano ZnO). Four replicates of 16 birds were allocated to each treatment. The purity of both micro and nano zinc supplements (ZnO) was 78%.

The nano ZnO was provided by the US Research nano-materials, Inc. (Houston, USA). The sizes of the nano ZnO particle sizes were 10-30nm (determined using a laser particle analyzer; Mastersizer, 2000, Malvern), with the average size being about 18 nm. Birds were fed the experimental diets from 10 to 40 days of age with free access to water and feed. The basal diet was analysed to measure Zn and Ca (Atomic absorption spectroscopy method) as well as crud protein content (Kjeldahl method) as AOAC procedures (1995).

Table 1. Ingredients and chemical composition of the basal diet

Ingredients	Value (%)
Corn (CP = 8%)†	54.497
Soybean meal (CP = 42.78%)†	42.5
DCP (P = 18%, Ca= 21%)†	0.71
Oyster shell (Ca = 38%)†	1.25
NaCl	0.32
Vitamin supplement‡	0.25
Mineral supplement§	0.25
DL-Methionine	0.12
L-Theronine	0.09
Washed sand#	0.013
Chemical composition	----------%, unless mentioned----------
ME (Kcal/kg)	2773
Crude protein†	22.68
Calcium†	0.77
Available phosphor	0.29
Sodium	0.14
Lysine	1.28
Methionine	0.48
Methionine + Cystine	0.86
Threonine	0.98
Arginine	1.54
Zinc† (mg/kg)	27

†Analyzed value.

‡Vitamin premix provided the following per kilogram of diet: Vitamin A, 11000 IU; Cholecalciferol, 5000 IU; Vitamin E, 75 IU; Vitamin k3, 3mg; Vitamin B12, 0.016 mg; Biotin, 0.15 mg; Folacin , 2 mg; Niacin, 2 mg; Pantothenic acid, 15 mg; Pyridoxine, 4 mg; Riboflavin, 8 mg; Thiamine 3 mg.

§Mineral premix was free of zinc and provided the following per kilogram of diet: Copper (as cupric sulfate $5H_20$), 16mg; Iodine (as calcium iodate), 1.2mg; Iron (as ferrous sulfate 4H20), 40 mg; Manganese (as manganese oxide), 120 mg; Selenium (as sodium selenite), 0.3 mg.

#Experimental treatments were prepared by replacing appropriate amounts of Zinc oxide with washed sand as an inert.

Growth performance and carcass characteristics

Body weight and feed intake were measured for each pen at days 10, 20, 30, and 40. Feed conversion ratio (FCR) was calculated for each time point while accounting for mortality, which was recorded daily. At the end of the experiment, two male birds per replicate (eight birds per treatment) were chosen based on average body weight in each pen and slaughtered. After slaughtering, the carcass, breast, thigh, liver, heart, and testes were weighed individually. The carcass yields and internal organs were expressed as a percentage of live body weight.

Blood enzymes activities

Before slaughtering the birds, blood samples were collected from the jugular vein in plastic tubes. After samples were allowed to clot at room temperature for one hour, samples were centrifuged at 3500 × *g* for 14 minutes to isolate blood serum (Abbasi *et al*., 2015). The sera were stored at −20°C for further analysis. The activities of lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (AKP) were determined by a calorimetric method using an automated analyser (Prestige 24i, Tokyo Boeki Medical System, Japan) (Kang *et al*., 2007).

Statistical analysis

Data were analysed in a completely randomised design with 2 × 4 factorial arrangement using the General Linear Model (GLM) procedure of SAS software (2002). Two-way analyses of variance were carried out using the following model: $Y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + e_{ijk}$,

where Y_{ijk}, individual observation; μ, overall mean; a_i, effect of zinc level i, b_j effect of zinc particle size j, $(ab)_{ij}$ = interaction effects, e_{ijk} = random variation.

Table 2. Effect of dietary zinc levels and particle sizes on Japanese quails performance†

Treatments		10-20 d			20-30 d			30-40 d			10-40 d		
Zinc oxide particle size	Zinc levels (mg/kg)	BWG (g)	FI (g)	FCR	BWG (g)	FI (g)	FCR	BWG (g)	FI (g)	FCR	BWG (g)	FI (g)	FCR
	25	63.48	92.77	1.46	76.30^{ab}	227.30	2.98	53.83	281.67	5.23	191.07	598.45	3.14
	50	62.27	96.53	1.55	67.32^{b}	209.27	3.11	55.49	275.51	4.97	193.61	601.73	3.10
	75	60.56	92.59	1.53	81.34^{a}	220.32	2.71	48.16	285.57	5.93	185.08	581.31	3.14
	100	65.94	101.31	1.54	76.85^{ab}	219.93	2.86	49.59	291.48	5.88	190.06	598.47	3.15
	SEM‡	3.17	4.28	0.07	3.85	6.45	0.17	3.07	9.82	0.38	3.78	13.95	0.065
Micro		63.09	96.43	1.53	75.70	223.84	2.96	52.73	289.86	5.50	191.28	596.95	3.12
Nano		63.03	95.17	1.51	75.20	214.56	2.85	50.81	277.25	5.46	188.64	593.02	3.15
SEM‡		2.24	3.02	0.05	2.72	4.56	0.12	2.17	6.95	0.27	2.67	9.86	0.046
Micro	25	65.78	95.53	1.45	74.75	234.93	3.14	64.02^{a}	313.27^{a}	4.89^{bc}	204.55^{a}	643.73	3.14
	50	62.81	95.66	1.52	60.58	206.09	3.40	56.46^{ab}	263.61^{bc}	4.67^{c}	179.85^{b}	565.36	3.15
	75	56.59	88.47	1.56	85.76	229.94	2.68	47.77^{bc}	288.23^{abc}	6.03^{ab}	190.13^{ab}	606.64	3.20
	100	67.19	106.06	1.58	81.72	224.41	2.75	42.66^{c}	294.34^{ab}	6.90^{a}	191.56^{ab}	624.81	3.27
Nano	25	61.17	90.00	1.47	77.86	219.67	2.82	43.64^{c}	250.06^{c}	5.73^{abc}	182.67^{ab}	559.73	3.06
	50	61.72	97.41	1.58	74.06	212.44	2.87	54.53^{abc}	287.41^{abc}	5.27^{bc}	190.31^{ab}	597.25	3.14
	75	64.53	96.70	1.50	76.92	210.69	2.74	48.55^{bc}	282.91^{abc}	5.83^{abc}	190.00^{ab}	590.31	3.11
	100	64.69	96.56	1.49	71.98	215.45	2.99	56.52^{ab}	288.62^{abc}	5.11^{bc}	193.19^{ab}	600.64	3.11
SEM‡		4.48	6.05	0.09	5.44	9.15	0.24	4.34	13.90	0.54	5.36	19.73	0.092
	Control#	56.22	86.13	1.53	74.54	202.99	2.72	59.82	282.97	4.73	190.58	572.08	3.00
ANOVA							*P*-value						
Zinc level		0.681	0.447	0.656	0.041	0.288	0.321	0.308	0.708	0.085	0.554	0.663	0.232
Zinc Particle size		0.983	0.770	0.776	0.898	0.163	0.491	0.539	0.211	0.548	0.525	0.159	0.742
Zinc level × Particle size		0.528	0.484	0.801	0.134	0.528	0.367	0.006	0.032	0.049	0.0499	0.080	0.734
Polynomial contrast													
Control *vs.* zinc level		0.138	0.110	0.957	0.907	0.130	0.247	0.077	0.551	0.039	0.962	0.221	0.104
Control *vs.* Particle size		0.168	0.126	0.960	0.866	0.098	0.343	0.146	0.655	0.091	0.945	0.212	0.097
Linear zinc level		0.992	0.011	0.944	0.245	0.665	0.800	0.009	0.376	0.001	0.997	0.809	0.045
Quadratic zinc level		0.059	0.937	0.598	0.663	0.247	0.017	0.601	0.923	0.05	0.500	0.231	0.694
Linear Nano ZnO		0.141	0.144	0.895	0.414	0.645	0.218	0.003	0.225	0.21	0.359	0.108	0.253
Quadratic Nano ZnO		0.642	0.458	0.849	0.654	0.654	0.159	0.009	0.523	0.56	0.378	0.804	0.640
Linear Micro ZnO		0.195	0.002	0.482	0.216	0.944	0.159	0.0001	0.750	0.0004	0.844	0.487	0.048
Quadratic Micro ZnO		0.746	0.177	0.770	0.205	0.224	0.011	0.0034	0.651	0.007	0.484	0.523	0.795

†Means without common letters in each column differ significantly ($P < 0.05$).
‡Standard error of the mean
#Basal diet supplied 27 mg of Zn/kg.

To obtain estimates of ZnO particle sizes and Zn levels as main effects and interactions (sources of ZnO particle size × Zn level), the data were analysed without the control group. Pen was considered as the experimental unit. Differences between treatment means were tested using LSD test for main effects and Tukey's test for interactions. Linear and quadratic polynomial contrasts analyses were used to evaluate the effects of different levels of Zn sources, zinc levels and comparison of control group vs average levels of zinc and mean of particle sizes. Statistical significance was declared at $P < 0.05$. Regression analyses of broken-line (Robbins *et al*., 2006), quadratic and logistic models (Pesti *et al*., 2009) were performed to determine Zn requirement. Zn requirement in each rearing period was determined with the control group and micro ZnO treatments because the models could not be fitted for nano ZnO treatments.

Results

Growth performance

Effects of dietary treatments on growth performance are shown in Table 2. Dietary treatments had an insignificant effect on growth performance in quails aged 10 to 20 d ($P > 0.05$). However, feed intake between these days increased linearly as Zn level and micro ZnO supplementation increased. Effect of dietary Zn on body weight gain was significant between 20 to 30 d ($P < 0.05$), while the effect of ZnO particle sizes did not significantly affect growth performance. Whereas FCR increased linearly with dietary zinc levels and micro ZnO supplementation, quadratic body weight gain decreased between 30 to 40 days of age.

Table 3 shows various models that have been fitted to determine Zn requirements of Japanese quails for growth performance criteria. Based on broken line models, Zn requirements of quails were 90 mg/kg for body weight gain between 10-20 d (Figure 1), and 70 mg/kg between 20 to 30 d (Figure 2). Using quadratic models, zinc requirements of quails were 69 mg/kg for FCR between 10 to 20 d (Figure 3), and 53 mg/kg between 30 to 40 d (Figure 4). The two slopes of broken line-quadratic models showed that Zn requirement for quails was 44 mg/kg between 10 to 20 d (Figure 5), and 73 mg/kg between 30 to 40 d (Figure 6). Accordingly, two slope broken line models showed that 70 mg Zn/kg was required for optimal FCR between 10 to 20 d (Figure 7), and 59 mg/kg for body weight gain between 30 to 40 d in Japanese quails, respectively (Figure 8). The logistic model showed that Zn requirement for optimal FCR between 10 to 20 d was 114 mg/kg (Figure 9). There were significant interaction effects between Zn levels and ZnO particle sizes on body weight gain, feed intake, and feed conversion ratio during 30 to 40 d as well as on body weight gain through 10 to 40 d ($P < 0.05$).

Table 3. The estimated requirement of Japanese quails

Response	Model	Equation	R^2	*P*- value	Requirement (mg/kg)§
BWG (g) 10-20	Broken-line†	$Y = 64.57 - 0.1318 \times (89.35 - X)$	0.2606	0.103	89.35
FCR 10-20	quadratic equation‡	$Y = 1.62 - 0.00426x + 0.000031x^2$	0.2044	0.143	68.93
	Broken-line#	$Y = 1.46 + 0.004 \times (43.84 - X) + 0.000015 \times (X - 43.84)^2$	0.2213	0.218	43.83
BWG (g) 20-30	Broken-line†	$Y = 78.61 - 0.2148 \times (69.97 - X)$	0.0728	0.567	69.97
FCR 20-30	Broken-line#	$Y = 3.42 - 0.015 \times (70.3 - X) - 0.014 \times (X - 70.3)$	0.29	0.134	70.30
BWG (g) 30-40	Broken-line#	$Y = 65.17 - 0.17 \times (58.87 - X) - 0.39 \times (X - 58.87)$	0.715	0.0004	58.87
FCR 30-40	quadratic equation‡	$Y = 5.95 - 0.050x - 0.000476x^2$	0.5638	0.0013	53.26
	Broken-line#	$Y = 4.76 + 0.0016 \times (72.91 - X) + 0.0008 \times (X - 72.91)^2$	0.5878	0.0034	72.91
	Logistic¥	$Y = 4.73/(1 + ((4.73 - 4.75)/4.75) \times e^{[(0.0421x)]})$	0.9780	0.0001	114

† $Y = L + U(R - X)$ Where Y = performance parameter (e.g., Body weight gain, Feed conversion ratio, etc.), L = the ordinate of the breakpoint in the curve; R = the abscissa of the breakpoint in the curve (requirement estimate); X = value of x less than R and (R−X) = z1 is defined as zero when X<R; and U = slope of the line for X less.

‡$Y = a + bx + cx^2$ X = value required to achieve maximal Y.

$Y = L + U(R - x) + V(x - R)$ Y = performance parameter (e.g., Body weight gain, Feed conversion ratio, etc.), L = the ordinate of the breakpoint in the curve; R = the abscissa of the breakpoint in the curve (requirement estimate), (R−X) =z1 is defined as zero at values of X<R, and (X−R) =z2 is defined as zero when X<R. We define parameters for the breakpoint x value (R), an asymptote for the first segment (L), and slopes for the 2 line segments (U, V).

¥$Y = a/(1 + ((a - b)/b) \times e^{[(-cx)]})$Y= performance (e.g., Body weight gain, Feed conversion ratio, etc.), a to c = are constants, x = dietary nutrient concentration and e = base of natural logarithms.

§Zn Requirement= optimal supplemental Zn level + Zn in the basal diet (27mg/kg).

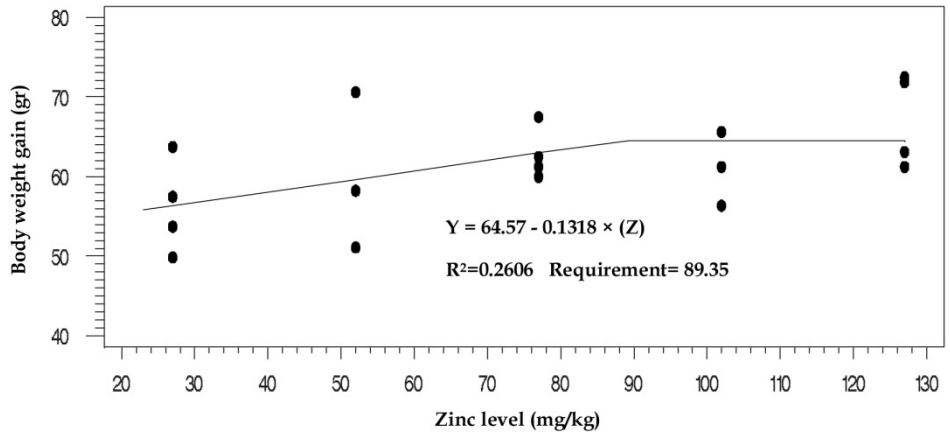

Figure 1. Body weight gain from 10 to 20 days of age to dietary zinc levels based on one slope broken-line model.

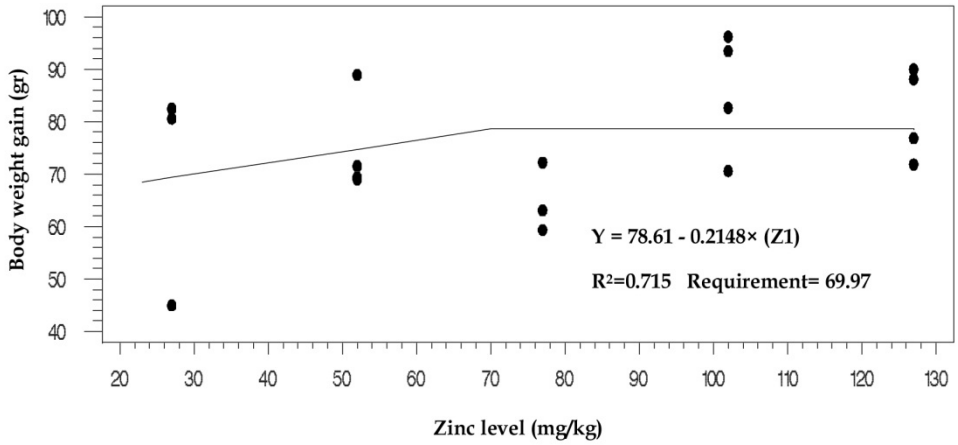

Figure 2. Body weight gain from 20 to 30 days of age to dietary zinc levels based on one slope broken-line model.

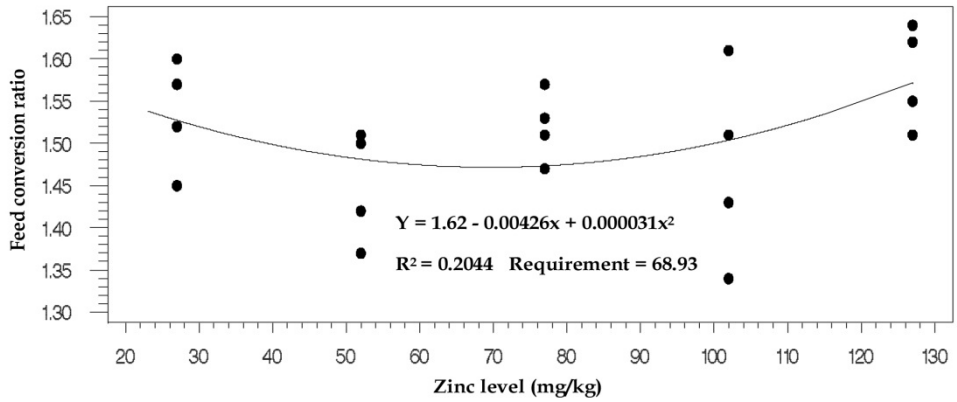

Figure 3. Feed conversion ratio from 10 to 20 days of age to dietary zinc levels based on quadratic model.

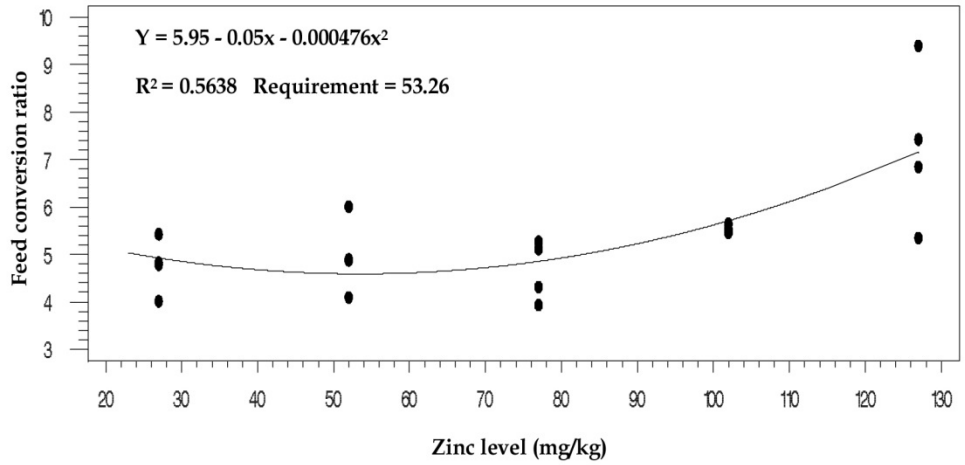

Figure 4. Feed conversion ratio from 30 to 40 days of age to dietary zinc levels based on quadratic model.

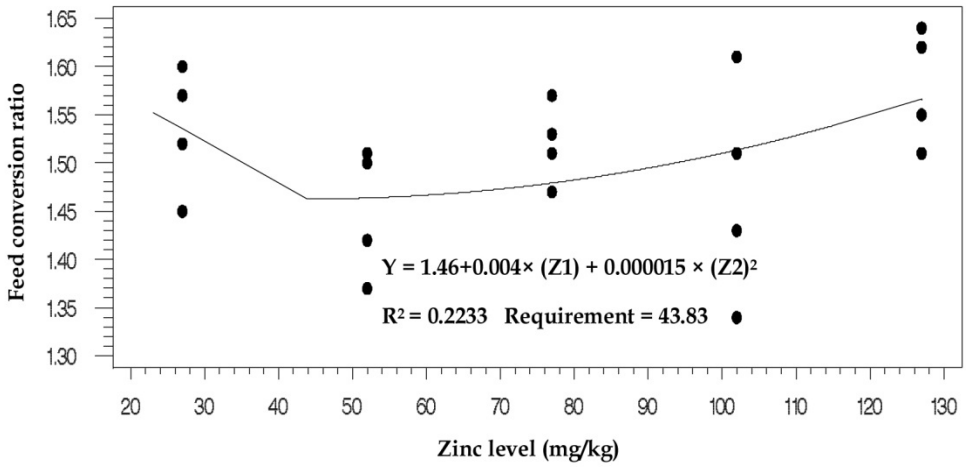

Figure 5. Feed conversion ratio from 10 to 20 days of age to dietary zinc levels based on two slope broken-line-quadratic model.

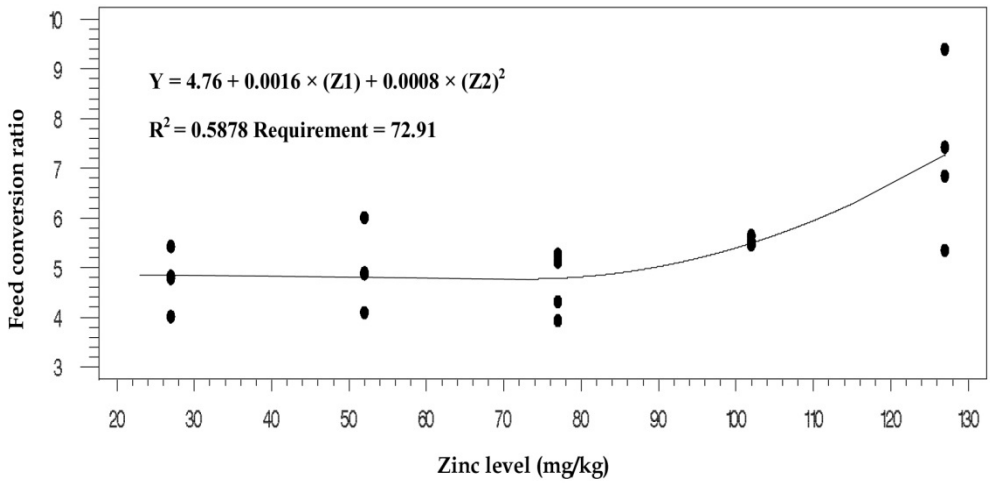

Figure 6. Feed conversion ratio from 30 to 40 days of age to dietary zinc levels based on two slop broken-line-quadratic model.

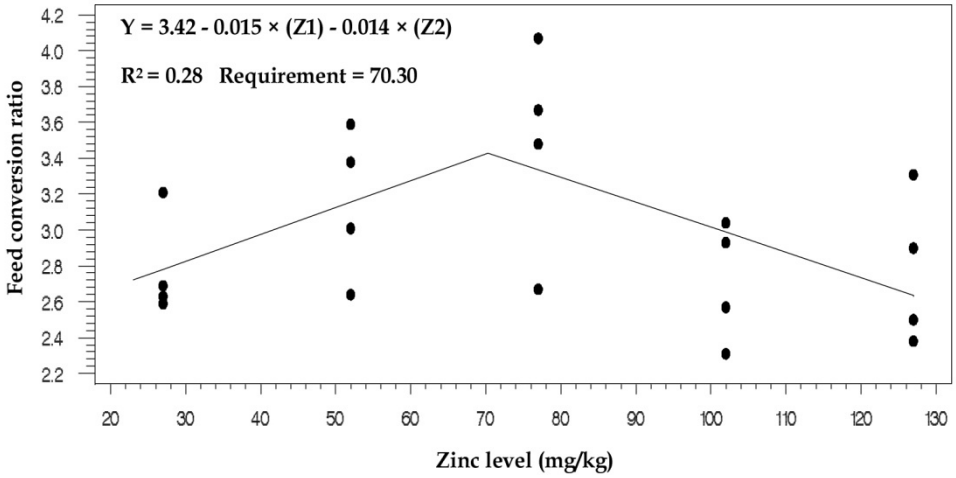

Figure 7. Feed conversion ratio from 20 to 30 days of age to dietary zinc levels based on two slop broken-line model.

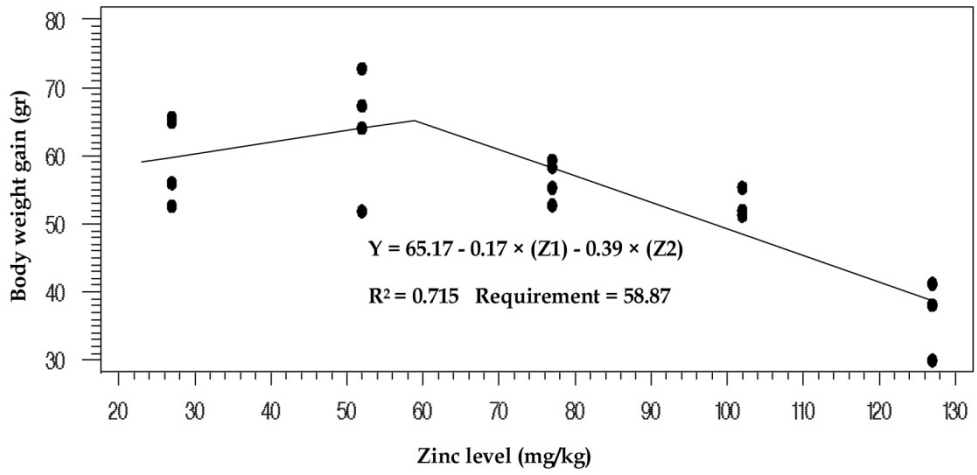

Figure 8. Body weight gain from 30-40 days of age to dietary zinc levels based on two slope broken-line model.

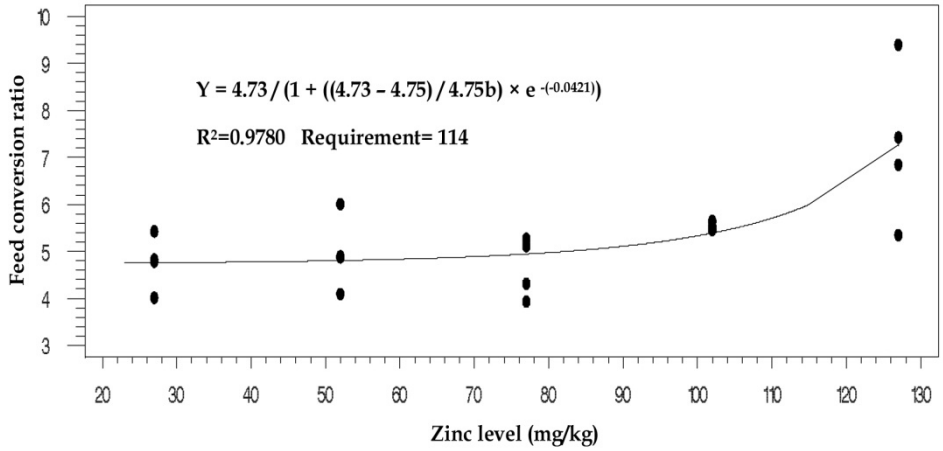

Figure 9. Feed conversion ratio from 30 to 40 days of age to dietary zinc levels based on logistic model.

Carcass characteristics

Effects of Zn levels and ZnO particle size on carcass characteristics are shown in Table 4. Quadratic analysis showed that supplemental Zn levels significantly affected testes yield (P < 0.01). Zn levels above 25 mg/kg significantly increased thigh yield (P < 0.05). Compared to control treatment, adding zinc to basal diet decreased liver yield (P < 0.05). Micro-ZnO supplementation linearly (P < 0.01) reduced liver weight and quadratically decreased the heart and testes weights. There were significant interaction between Zn levels and ZnO particle sizes for heart and testes yields (P < 0.05).

Table 4. Effect of dietary zinc levels and particle sizes on carcass yields of male Japanese quails (% of live body weight)†

Treatments							
Zinc oxide particle size	Zinc levels (mg/kg)	Carcass (%)	Thigh (%)	Breast (%)	Liver (%)	Heart (%)	Testes (%)
	25	68.19	15.13^{b}	26.93	2.18	0.80	1.36^{b}
	50	70.04	15.63^{ab}	27.45	2.26	0.83	1.45^{b}
	75	69.66	16.65^{a}	26.21	2.28	0.93	1.55^{ab}
	100	68.95	15.95^{ab}	27.11	2.02	0.87	2.11^{a}
	SEM‡	0.85	0.44	0.56	0.09	0.055	0.15
Micro		69.24	15.48	26.94	2.18	0.88	1.53
Nano		69.17	16.16	26.92	2.19	0.83	1.70
SEM‡		0.61	0.31	0.40	0.06	0.04	0.10
Micro	25	67.82	15.01	26.24	2.35	0.85^{ab}	1.15^{b}
	50	70.30	15.72	27.37	2.29	0.79^{b}	1.38^{b}
	75	70.43	15.72	26.76	2.11	1.10^{a}	1.52^{ab}
	100	68.55	15.51	27.30	1.98	0.80^{b}	2.08^{a}
Nano	25	68.56	15.24	27.53	2.04	0.74^{b}	1.58^{ab}
	50	69.78	15.54	27.52	2.22	0.88^{ab}	1.52^{ab}
	75	68.99	17.46	25.72	2.43	0.78^{b}	1.57^{ab}
	100	69.35	16.39	26.91	2.06	0.94^{a}	2.14^{a}
SEM‡		0.68	0.62	0.78	0.13	0.08	0.22
	Control#	67.73	15.21	26.69	2.45	0.90	1.76
ANOVA		*P*-value					
Zinc level		0.466	0.04	0.501	0.969	0.377	0.005
Zinc Particle size		0.833	0.121	0.551	0.462	0.673	0.286
Zinc level × Particle size		0.796	0.489	0.317	0.237	0.003	0.028
Polynomial contrast							
Control *vs.* zinc level		0.133	0.169	0.699	0.0308	0.218	0.555
Control *vs.* Particle size		0.182	0.432	0.850	0.067	0.272	0.389
Linear zinc level		0.072	0.889	0.565	0.058	0.959	0.165
Quadratic zinc level		0.272	0.844	0.738	0.945	0.142	0.007
Linear Nano ZnO		0.076	0.371	0.827	0.361	0.289	0.732
Quadratic Nano ZnO		0.492	0.102	0.419	0.821	0.0013	0.004
Linear Micro ZnO		0.206	0.162	0.245	0.002	0.502	0.215
Quadratic Micro ZnO		0.302	0.522	0.802	0.697	0.972	0.021

†Means within the same column without common letters differ significantly (P < 0.05).
‡Standard error of the mean
#Basal diet supplied 27 mg of Zn/kg.

Serum enzymes activities

Effects of dietary Zn levels and ZnO particle sizes on serum enzyme activities are shown in Table 5. Dietary Zn levels and ZnO particle size had insignificant effects on serum enzymes activities, but had significant interactions for APK and LDH activities (P < 0.05). However, LDH levels linearly increased as the levels of Nano-ZnO increased. Increasing Zn levels caused increased LDH activities only for nano-ZnO but not micro-ZnO.

Table 5. Effect of dietary zinc levels and particle size on blood serum enzyme activities of male Japanese quails†

Treatments					
Zinc oxide particle size	Zinc levels (mg/kg)	AST§ (U/L)	ALT§ (U/L)	AKP§ (U/L)	LDH§ (U/L)
	25	333.62	7.20	1342.50	1381.20
	50	297.87	7.38	1430.13	1503.29
	75	293.25	8.00	1562.25	1598.13
	100	314.63	8.88	1409.70	1976.12
	SEM‡	30.04	1.14	90.37	204.19
Micro		298.68	7.48	1353.06	1451.73
Nano		321.00	8.25	1515.73	1777.64
SEM‡		21.24	0.81	62.50	223.85
Micro	25	353.00	6.66	1253.00^{b}	1372.66^{b}
	50	266.00	6.50	1219.50^{b}	1356.25^{b}
	75	261.25	7.75	1636.75^{a}	1502.50^{ab}
	100	314.50	9.00	1302.66^{ab}	1575.50^{ab}
Nano	25	314.25	7.75	1431.66^{ab}	1389.75^{b}
	50	329.75	8.25	1640.75^{a}	1650.33^{ab}
	75	325.25	8.25	1487.75^{ab}	1693.75^{ab}
	100	314.75	8.75	1502.75^{ab}	2376.75^{a}
SEM‡		42.48	1.61	120.72	348.50
	control#	305.75	8.00	1383.50	1562.25
ANOVA		*P*-value			
Zinc level		0.774	0.739	0.086	0.147
Zinc Particle size		0.464	0.514	0.309	0.267
Zinc level × Particle size		0.556	0.936	0.022	0.013
Polynomial contrast					
Control *vs.* zinc level		0.713	0.774	0.941	0.777
Control *vs.* Particle size		0.138	0.769	0.837	0.750
Linear zinc level		0.415	0.903	0.535	0.086
Quadratic zinc level		0.701	0.339	0.410	0.106
Linear Nano ZnO		0.573	0.758	0.685	0.048
Quadratic Nano ZnO		0.488	0.604	0.863	0.216
Linear Micro ZnO		0.543	0.939	0.533	0.855
Quadratic Micro ZnO		0.918	0.177	0.266	0.484

†Means within the same column without common letters differ significantly ($P < 0.05$).
‡Standard error of the mean
#Basal diet supplied 27 mg of Zn/kg.
§AST= Aspartate aminotransferase, ALT = Alanine aminotransferase, AKP = Alkaline phosphatase, LDH = lactate dehydrogenase.

Discussion

Zinc is involved in many diverse biological functions including metabolism of protein, nucleic acid, carbohydrate, and lipid (Salim *et al*., 2008; Sahraei *et al*., 2013). In this experiment, we show that supplementing basal diet with Zn promoted growth between 20 to 30 days of age and that 27 mg Zn/kg in the basal diet is insufficient for maximum body weight gain in Japanese quails. Fitted one slope and two slope broken line models showed that body weight gain reached its maximum at points 62.35, 42.97 and 31.87 mg Zn/kg or about 89.35, 69.67 and 58.87 mg/kg of total dietary of Zn, respectively, for 10 to 20 d, 20 to 30 d, and 30 to 40 days of age. These findings clearly show that Zn requirement differs for each rearing stage of Japanese quails. These estimations are higher than the NRC (1994) recommendations (25 mg Zn/kg) but are similar to those recommended by Harland *et al*. (1975) (75 mg/kg of diet for young Japanese quails). The value recommended by NRC (1994) is based on research with semi-purified diets, which have less anti-nutritional factors such as fiber and phytate compared to practical diets. In this study, adding more than 45 mg Zn/kg (about 72 mg/kg of total dietary Zn) to the basal diet throughout the last rearing period decreased body weight gain and increased feed conversion ratio, showing that Zn requirements differ in each stage of Japanese quail rearing period.

In this study, thigh yield significantly increased in birds fed diets supplemented with Zn. Zinc is an essential element for stimulating differentiation of chondrocytes, osteoblasts, and fibroblasts. In addition, Zn is involved in the synthesis of the somatomedin-C, a hormone that stimulates cartilage proliferation and linear growth of the skeleton, and is therefore an essential component of bone maturation. Zn deficiency can result in reduced bone collagen synthesis (Starcher *et al*., 1980). Odutuga (1982) showed that in rats, Zn deficiency reduces bone length and bone weight. High thigh yielded in this study may be related to the higher amount of bone ash of birds that received 102 mg Zn/kg. In this study, testes yield was affected by supplemental Zn levels and showed quadratic responses. Deficiency of zinc is associated with hypogonadism, insufficient development of secondary sex characteristics, and can cause atrophy of the seminiferous tubules. As a result, failure in spermatogenesis can occur (Endre *et al*., 1990). Our results suggest that a lower testes weight may be related to the gonadal dysfunction of male Japanese quails that were fed with zinc deficient diet. Similar decreases in the relative weight of testes have been previously reported (Namra *et al*., 2009; Amem and Al-Daraji, 2011).

Despite some studies reporting a relationship between Zn and enzymes (Meftah *et al*., 1991; Cepelak *et al*., 2002), Bao and Choct (2009) did not find correlations between loss of enzyme activity and Zn deficiency. Zinc metalloenzymes provide important functional indices of Zn status and possibly act as surrogate biomarkers for dietary Zn. The present study showed that supplemental Zn did not affect serum enzyme activities, which supports the important function of Zn metalloenzymes in indicating Zn status. The lack of responses we saw may be due to the extent of dietary Zn deficiency, which may not have been severe enough to cause a response to Zn supplementation in a practical corn–soybean basal diet. Zinc-containing enzymes have an extremely high affinity for their metal ligand and thus may retain Zn even in the face of low concentrations (Liao *et al*., 2013). Ahmadi *et al*. (2014) reported that nano-ZnO had no significant effects on ALT and AST activities in serum of broilers. In this study, AKP and LDH activities decreased when low Zn diets were offered as microparticles. It has been reported that Zn supplementation increases AKP activity (Levengood *et al*., 2000) and in severe Zn deficiency, activities of plasma AKP as well as LDH may be depressed (McDowell, 1992).

Conclusion

Zn requirements of Japanese quails reported by NRC (1994) should be modified. The optimal dietary Zn levels for body weight gain in Japanese quails were 90 mg/kg between 10 to 20 days, 70 mg/kg between 20 to 30 days, and 59 mg/kg between 30 to 40 days. We show that Zn requirements differ across Japanese quail age. We also show that testes yield was affected by supplemental Zn levels, suggesting that zinc may have a crucial role in male reproduction.

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