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Effects of Dietary Supplementation of Zinc and α-Tocopheryl Acetate on Performance and Zinc Concentrations in Egg and Tissues of Japanese Quails

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Abstract
We investigated the effects of dietary supplementation of zinc (ZnO; 0, 40, 80, 120 and 160 mg/kg) and Vit E (α-tocopheryl acetate; 0 and 40 IU/kg) on egg production, egg quality and Zn content of egg fractions and tissues in Japanese quails. Using a 5 × 2 factorial design, a total of 960 Japanese quails (*Coturnix coturnix japonica*) at day 70 of age were housed in cages and randomly assigned into one of ten experimental treatments, each with four replicates of 24 birds (16 females and eight males per replicate). Egg production was greater ($P < 0.05$) in birds fed diets containing 160 mg/kg of zinc (Zn) than those fed basal diet (control diet), but vitamin E supplementation had no effect on egg production. Quails fed basal diet supplemented with 80 mg/kg Zn showed a significant improvement in their feed conversion ratio compared to the other birds. Birds supplemented with 80, 120 and 180 mg/kg Zn had stronger egg shells than those fed the control diet, while shell thickness was lower in birds supplemented with 0 and 40 mg/kg of Zn ($P < 0.05$). Enrichment of Zn in egg yolk increased when birds received diets supplemented with 80, 120 and 160 mg/kg Zn compare to control group ($P < 0.05$). Supplementation of diet with Zn increased serum concentration of Zn when fed to quails at 120 mg/kg ($P < 0.05$). Thigh muscle, thigh bone, and liver Zn concentrations increased with concentration of Zn supplementation ($P < 0.05$). Vitamin E supplementation had no effects on laying performance, egg shell quality, and Zn concentrations in egg fractions and tissues of Japanese quail.

Introduction
Quail production has steadily grown over the past few years because of its high profit and low initial investments (Prabakaran, 2003). Quail rearing, like all types of poultry production, could be profitability optimized with scientific feeding and understanding of quail nutrient requirements. Commercial producers have recently shown great interest in examining the effects of supplemental zinc and vitamin E on quail raising profitability. Such concerns are derived mainly from scarcity of information on quail zinc (Zn) requirements despite numerous reports confirming its promising effects (along with other minerals and vitamins) on growing broilers and laying hens. Zinc is a trace element necessary for normal growth, bone development, feathering and regulation of appetite in all avian species (Batal *et al.*, 2001). Zinc is also involved in many enzymatic and metabolic functions in the

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body (Prasad and Kucuk, 2002): activities of several metabolic enzymes drastically decrease in Zn-deficient animals (Prasad and Kucuk 2002). Scaling of skin, especially on feet, and increased mortality have also been observed in severe cases of Zn deficiency in stressed birds (Sahin *et al.*, 2009). In a 24-week trial, supplementing laying hen rations with 48 mg/kg of Zn had no effect on egg production (Supplee *et al.*, 1958). In contrast, Kienholz *et al.* (1961) found that laying hens fed a soy-based diet containing 10 mg/kg of Zn had lower egg production and hatchability than normal until diets were further supplemented with additional Zn. Since most feed ingredients are marginally deficient in Zn, it is of utmost importance to supplement poultry diets with an additional source of Zn. However, Stahl *et al.*, (1986) found that supplementing diets of laying hens with Zn had no effect on egg production, feed intake, and feed conversion ratio (FCR). Japanese quails are particularly sensitive to dietary Zn deficiency, and it has found that Zn is necessary for their normal growth, feathering and normal skeletal development (Koréneková *et al.*, 2005). Therefore, quails may benefit from with Zn supplements.

Poultry cannot synthesize vitamin E. Therefore, their requirements for vitamin E must be fulfilled *via* dietary sources (Chan *et al.*, 1994). Supplementing laying hen diets with vitamin E increased egg production and oxidative stability, and improved the quality of eggs (Cherian *et al.*, 1996a). Sahin *et al.*, (2006) found no effect on body weight, feed intake, and egg weight in Japanese quail supplemented with vitamin E, though egg production increased. Vitamin E, as a biological antioxidant, has been added to animal diets to improve feed efficiency and immune response. Vitamin E supplementation can also improve the quality of meat and eggs, and increase their vitamin E content for the consumers (Sunder *et al.*, 1997). Salgueiro *et al.* (2000) and Kim *et al.* (1998) demonstrated that Zn, which is involved in some biological antioxidant systems, interacts with vitamin E, because vitamin E status was impaired in Zn-deficient animals. Therefore, this study aimed to investigate the effects of dietary supplementation of zinc and vitamin E on production performance and egg quality of breeder Japanese quail.

Materials and Methods

Experimental flock and dietary treatments

All procedures used in this study were approved by Animal Care Committee of Ramin Agriculture and Natural Resources University, Khuzestan, Iran. Nine hundred and sixty 70-d old Japanese quail (*Coturnix coturnix japonica*) were purchased from a commercial breeder flock. The birds were housed in battery cages (60 cm wide × 100 cm long × 28 cm high) equipped with a raised wire floor, and acclimatized to experimental diets and cages for two weeks. The ambient temperature of hen house was kept between 18 to 26˚C. The experimental period lasted 70 d, and throughout this period, birds were subjected to a 16L:8D photoschedule. A corn-soybean basal diet was supplemented with Zn as zinc oxide with 74.5% zinc (0, 40, 80, 120 and 160 mg/kg) and vitamin E as DL- α-tocopheryl acetate (0 and 40 IU/kg), creating ten experimental treatments. The basal diet was formulated to meet or slightly exceed the nutrient requirements of layer Japanese quail recommended by the NRC (1994; Table 1).

The birds were randomly assigned to one of the ten experimental treatments with four replicates of 24 birds each (sixteen females and eight males). Diets (in mash form) and water were offered to the birds *ad libitum* throughout the experimental period.

Data Collection

Daily egg production and egg weight were recorded for each cage. Feed intake (FI) was measured weekly and was used to calculate feed conversion ratio (FCR; feed intake divided by weight of eggs produced). Egg mass was calculated as egg weight multiplied by percentage of egg production to estimate grams of egg produced per day. On Day 70 of experiment, one female quail from each group was slaughtered using HALAL procedures and liver and thigh muscles were taken for Zn analysis. On the tenth week of the experiment, two eggs were randomly selected from each group and Zn concentration was determined in the egg yolk, egg white and eggshell individually. To determine strength, thickness, and eggshell proportion, six eggs from each treatment were randomly selected on days 42 and 70 of the experiment and were weighed, broken, and proportional weights of yolks and whites were calculated. The residue of whites were wiped from eggshells, and yolk, white, and eggshell were dried for 48 hrs at 60^0C.

Table 1. Composition and nutrient content (as fed) of the basal diet

Ingredient	% of the diet
Maize	55.5
Soybean meal	24.5
Wheat	4.0
Fishmeal	3.0
Vegetable oil	3.2
Oyster	7.37
Dicalcium phosphate	1.3
Salt	0.35
DL- Methionine	0.23
Lysine	0.05
Vitamin premix[1]	0.25
Mineral premix[2]	0.25
Calculated nutrient composition	
ME (Kcal/kg)	2900
Protein (%)	18
Methionine (%)	0.54
Lysine (%)	1
Calcium (%)	3.1
Available phosphorus (%)	0.45
Methionine + Cystine	0.82
Zinc (mg/kg)	22.45

[1] Vitamin premix per kg contained: vitamin A, 8000 IU; vitamin D3, 2500 IU; vitamin E, 20 IU; Vitamin K, 2 IU, thiamin, 2 mg; riboflavin, 6 mg; pyridoxine, 3 mg; pantothenic acid, 10 mg; folic acid, 1 mg; biotin, 100 µg; niacin, 40 mg and vitamin B12, 10 µg.
[2] Mineral premix supplied the following per kg of diet: manganese, 70 mg; Iron, 40 mg; copper, 10 mg; choline, 200 mg; iodine, 0.4 mg and selenium, 0.3 mg.

To determine Zn content, samples of yolk, white, and eggshell were ashed in a muffle furnace at 450°C for 12 hrs. Ash was then dissolved in 3 *M* HCl and transferred to a volumetric flask. The Zn concentration in the HCl extract was determined using atomic spectrometry (Analytic Jena, Contra A [300], Germany) (Skřivan *et al.*, 2005). Zinc concentrations in liver and tight muscle samples were also determined according to procedures described by Skřivan *et al.* (2005).

Left tibia samples were boiled in deionized water for 20 min, cleaned from all soft tissues, and dried at 60°C for 48 hrs. Fat was extracted with 96% ethanol in a glass container for 48 hrs. During the extraction period, ethanol was replaced several times until its color became clear. Thereafter, samples were dried for 48 hrs at 60°C before ashing at 600°C overnight in a muffle furnace. Zn concentrations from these samples were then determined as described by Skřivan *et al.* (2005).

Statistical analysis

Statistical analyses were carried out using the MIXED procedure of SAS 9.2 (2003). The model used was:

$$Y_{ijkl} = \mu + B_i + Z_j + E_k + (ZE)_{jk} + e_{ijkl}$$

Where Y_{ijkl} is the dependent variable under examination; µ is the population mean for the variable; B_i is the random effect of block; Z_j is the fixed Zn (j = 5; 0, 40, 80, 120 and 160 mg/kg of diet); E_k is the fixed effect of vitamin E (k = 2; 0 and 40 IU/kg of diet) and e_{ijkl} is the random error associated with the observation ijk.

The Fisher's protected least significant difference (LSD) test was used for multiple treatment comparisons using LSMEANS of SAS 9.2 (2003) with letter grouping obtained using SAS pdmix800 macro (Saxton, 1998). Residual analysis was carried out to test the model assumptions using the UNIVARIATE procedure of SAS 9.2 (2003) with NORMAL and PLOT options. For all statistical analyses, significance was declared at $P < 0.05$.

Results and Discussion

Performance and egg quality

Effects of dietary supplementation of Zn and vitamin E on performance parameters of Japanese quails are shown in Table 2. Egg production was greater in birds fed diets containing 160 mg/kg Zn compared to those fed 0 or 40 mg/kg Zn in diet ($P < 0.05$). Feed intake and egg weight were not affected by dietary treatments. Compared to control birds, quails supplemented with 80, 120 or 160 mg/kg Zn showed significant improvement in FCR. Supplee *et al.* (1958) found that supplementing diets with 48 mg/kg Zn had no effect on egg production in layers, but Bahakaim *et al.* (2014) observed an improved FCR when layers were fed diets supplemented with 50 or 100 mg/kg of Zn. Sahin and Kucuk (2003) also reported a linear increase in FI and egg production, and improved feed efficiency and egg quality in heat-stressed quails supplemented with 30 or 60 mg/kg of Zn. However, supplementing layer hens with 100 mg/kg Zn-methionine chelate decreased egg production (Lim *et al.*, 2003). Hermayer *et al.* (1977) found that high levels of Zn in diet reduced feed intake and egg production of laying hens. Broilers fed diets supplemented with different levels of Zn (40, 80 and 120 mg/kg diet) also reduced feed intake as levels of Zn increased (Refaie, 2009). In line with our findings, Shyam Sunder *et al.* (2008) also observed that supplementing broiler diet with Zn had no effect on feed intake. Kucuk *et al.* (2008) reported that FCR and egg production improved when both Zn (30 mg/kg) and pyridoxine (8 mg/kg) were included in a laying hen diet. Also, Kaya *et al.* (2001) observed an improved FCR when laying hens were fed different levels of supplemental Zn (0, 20, 50, 100 and 200 mg/kg diet), and that the best FCR was observed in birds received diets supplemented with 100 mg/kg of Zn.

Table 2. Laying performance and egg quality of Japanese quail supplemented with zinc (Zn) and vitamin E

	Zn (mg/kg of diet)	Vitamin E (IU/kg of diet)	Egg production (%)	Feed intake (g/bird/day)	Egg weight (g)	FCR[1]	Egg mass (g/d)	Shell strength (kg/cm²)	Shell thickness (mm)	Egg shell proportion (%)
Main effects										
	0		89.08[c]	32.92	13.37	2.46[a]	11.91	0.42[b]	0.196[b]	12.70
	40		89.39[bc]	32.71	13.43	2.43[ab]	12.01	0.57[ab]	0.195[b]	12.84
	80		92.55[ab]	31.47	13.39	2.35[c]	12.39	0.85[a]	0.217[a]	13.38
	120		90.25[abc]	31.85	13.37	2.38[bc]	12.08	0.70[a]	0.210[a]	13.21
	160		93.17[a]	32.03	13.51	2.37[bc]	12.59	0.73[a]	0.209[a]	13.52
SEM			1.08	0.41	0.11	0.03	0.20	0.09	0.003	0.396
		0	90.87	32.31	13.41	2.41	12.19	0.70	0.203	13.13
		40	90.90	32.09	13.42	2.39	12.20	0.61	0.208	13.52
SEM			0.68	0.26	0.71	0.02	0.12	0.06	0.002	0.396
Zn × Vit E										
	0	0	89.14	32.98	13.35	2.47	11.90	0.45	0.198	12.75
	0	40	89.02	32.86	13.38	2.46	11.91	0.38	0.195	12.65
	40	0	90.28	33.35	13.41	2.49	12.10	0.69	0.194	12.83
	40	40	88.50	32.07	13.46	2.38	11.92	0.45	0.195	12.84
	80	0	92.04	31.51	13.42	2.35	12.35	0.95	0.212	13.51
	80	40	93.07	31.42	13.36	2.35	12.43	0.75	0.222	13.24
	120	0	90.03	31.69	13.20	2.40	11.88	0.79	0.206	13.18
	120	40	90.46	32.01	13.55	2.36	12.27	0.62	0.215	13.23
	160	0	92.87	31.99	13.67	2.34	12.70	0.64	0.205	13.38
	160	40	93.47	32.08	13.36	2.40	12.49	0.83	0.214	13.65
SEM			1.53	0.58	0.16	0.04	0.28	0.13	0.005	0.559
P-value										
Zn			0.03	0.10	0.87	0.02	0.10	0.02	0.002	13.13
Vitamin E			0.97	0.56	0.89	0.41	0.93	0.24	0.804	13.12
Zn × Vit E			0.90	0.69	0.36	0.27	0.82	0.48	0.507	0.250

[1] FCR, feed conversion ratio

[a,b,c] Lsmeans within a column with different superscripts differ ($P < 0.05$).

In the present study, the improved egg production and FCR in birds that received supplemental Zn could be partly attributed to its role in protection of pancreatic tissue against oxidative damage, which may facilitate proper functioning of the pancreas (e.g. secretion of digestive enzymes), thereby improving digestibility of nutrients. Furthermore, because Zn is involved in metabolism of carbohydrates, proteins, and lipids (MacDonald, 2000; Ibs and Rink, 2003), energy and protein utilization may have been improved in Zn supplemented birds.

However, in contrast to our findings, El-Latif (1999) reported that egg production in layers was not influenced by supplemental Zn. We found no influence of vitamin E supplementation on laying performance and eggshell quality of breeder quails. In contrast, Cherian *et al.* (1996b) showed that adding vitamin E to poultry diets improved oxidative stability, egg production, and egg quality. In this study, the lack of difference in laying performance in birds supplemented with vitamin E compared to control birds may be that the birds reared under the neutral temperature (21-26°C), and that they received adequate vitamin E (20 IU/kg diet) *via* the basal diet.

Birds supplemented with 80, 120 and 180 mg/kg Zn had stronger egg shells than those fed the control diet, while shell thickness was lower in the birds fed basal diet and basal diet supplemented with 40 mg/kg Zn compared with the other treatments ($P < 0.05$). The results of this study agree with the findings of Mabe *et al*. (2003) who reported that dietary supplementation of laying hens with 60 mg/kg Zn had no effect on percentage of eggshell and eggshell index, but improved egg shell strength. Zinc is directly involved in eggshell synthesis as a co-factor of carbonic anhydrase, which provides carbonate ions in magnum during the deposition of albumen and in isthmus for formation of eggshell membranes. Zn is also involved in eggshell formation pathways in uterine cells (Bahakaim *et al.,* 2014).

Zn concentration in tissues

Effects of dietary supplementation of Zn and vitamin E on Zn concentration in serum, muscle, bone, liver and egg fractions are shown in Table 3. Serum concentrations of Zn increased when quails were fed 120 mg/kg Zn. Thigh muscle, thigh bone, and liver Zn concentrations improved only when birds were supplemented with 80 mg/kg Zn or greater. Supplementation of diet with Zn in concentrations beyond 40 mg/kg increased yolk concentration of Zn ($P < 0.05$) which agreed with the results of Plaimast *et al.* (2008) who found that Zn deposition in egg increased linearly as dietary Zn levels increased. Henry *et al*. (1987) showed tissue concentration of Zn, especially bone, increased with dietary Zn content. Furthermore, as the levels of dietary zinc increased in turkey, zinc content of tibia, beaks, kidneys, liver, testes and feathers also increased (Vohra *et al.,* 1968). Tissue uptake of Zn in chicken is related to dietary Zn intake (Sandoval *et al.,* 1997). In agreement with our results, it has been observed that feeding chicken with dietary zinc at levels greater than growth requirements increased Zn concentration in plasma and tibia (Pimentel *et al.,* 1991).

In the present study, Zn supplementation had no effect on eggshell and egg white concentrations of Zn. Yang *et al.,* (2004) indicated that Zn content of egg increased by 55.7% and 70.2% when laying hens were fed diets containing 240 and 840 mg/kg Zn, respectively. Mabe *et al.* (2003) also found a significant increase in Zn content of egg yolk when birds were supplemented with 60 mg/kg Zn. Stahl *et al.,* (1988) reported that hens fed diets containing high levels of zinc (1762 or 1861 mg/kg diet) produced eggs was containing 57-95% more zinc than those fed a diet containing 26 mg/kg Zn. In the current study, dietary inclusion of 80 mg Zn/kg of diet increased Zn content of egg yolk by approximately 20% compared with the basal diet, indicating a positive correlation between dietary Zn content and that of the yolk content. Unlike egg yolk, dietary Zn did not change Zn contents of eggshell and egg white most likely due to naturally low content of Zn in these fractions. In contrast to our results, Skřivan *et al.* (2005) found no significant differences in Zn content of egg yolk when 80 mg/kg Zn was added to a basal diet fed of laying hens.

When Zn and vitamin E were supplemented together, there were no significant differences in production performance nor Zn concentration in egg, thigh muscle, bone, liver and serum (Tables 2 and 3). Kim *et al.,* (1998) reported that intestinal absorption of vitamin E was influenced by the Zn status in rats. Zn is involved in certain anti-oxidative enzymatic systems which may interact with vitamin E in stressed birds. Vitamin E status can be impaired in Zn-deficient animals (Salgueiro *et al*., 2000; Kim *et al.*, 1998). Sahin *et al.* (2006) observed a significant interaction between vitamin E and Zn in improved final body weight and feed intake of growing heat-stressed quails. In this experiment, the lack of interaction between Zn and vitamin E supplementation may be attributed to the fact that birds were raised under neutral temperatures (21-26 ^{0}C) and therefore, may not have been exposed to oxidative stress.

Table 3. Concentrations of Zn in serum (mg/dL), egg, liver and thigh muscle (mg/kg dry matter) of Japanese quail supplement with zinc and vitamin E

	Zn (mg/kg of diet)	Vitamin E (IU/kg of diet)	Zn concentration						
			egg shell	egg yolk	egg white	Thigh muscle	Thigh bone	liver	Serum
Main effects									
	0		4.30	41.65^{c}	8.23	57.51^{b}	115.75^{c}	66.10^{c}	338.5^{b}
	40		4.24	45.31^{bc}	8.96	59.16^{b}	133.92^{bc}	69.28^{bc}	359.75^{b}
	80		4.80	50.05^{ab}	9.86	64.1b^{ab}	153.23^{ab}	73.98^{abc}	397.25^{ab}
	120		4.65	54.17^{a}	8.77	63.42^{ab}	161.52^{a}	80.37^{ab}	406.13^{ab}
	160		4.97	54.11^{a}	9.17	69.69^{a}	173.47^{a}	85.46^{a}	467.63^{a}
SEM			0.25	2.59	0.58	2.51	8.19	3.90	24.85
		0	4.63	48.74	8.88	60.99	149.04	73.55	397.80
		40	4.54	49.38	9.11	64.59	146.12	76.52	389.90
SEM			0.16	1.64	0.37	1.59	5.18	2.47	15.72
Zn × Vit E									
	0	0	3.94	41.91	8.23	57.47	121.44	66.74	339.00
	0	40	4.65	41.39	8.24	57.55	121.44	65.46	337.50
	40	0	4.62	45.82	8.96	55.27	125.61	71.65	340.75
	40	40	3.85	44.81	8.96	63.05	142.24	66.92	378.75
	80	0	4.97	47.41	9.32	64.46	149.90	65.81	401.50
	80	40	4.63	52.69	10.40	63.85	156.55	82.14	393.00
	120	0	4.82	51.84	8.98	58.57	170.25	79.76	429.75
	120	40	4.48	56.50	8.57	68.26	152.79	80.98	382.50
	160	0	4.82	56.71	8.96	69.15	177.98	83.79	477.50
	160	40	5.11	51.52	9.39	70.23	168.97	87.12	457.75
SEM			0.35	3.66	0.82	3.55	11.58	5.51	35.15
P-value									
Zn			0.198	0.006	0.398	0.018	0.001	0.010	0.012
Vitamin E			0.691	0.783	0.668	0.120	0.694	0.401	0.725
Zn × Vit E			0.272	0.594	0.913	0.477	0.573	0.393	0.816

a,b,c Lsmeans within a column with different superscripts differ ($P < 0.05$).

Conclusion

Dietary supplementation with Zn beyond 40 mg/kg of diet improved egg production and eggshell strength and thickness in laying Japanese quail. Zn supplementation also enriched Zn content of egg yolk. Zn contents of thigh muscle and thigh bone, liver, and serum increased only when supplementation concentration was beyond 80 mg/kg of diet. Supplementary vitamin E had no effect on laying performance of Japanese quail.

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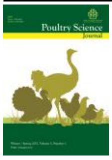

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تاثیر مکمل عنصر روی و α-توکوفرول استات بر عملکرد و غلظت عنصر روی در تخم و بافت‌های بدن بلدرچین ژاپنی

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چکیده

به‌منظور بررسی تأثیر افزودن مکمل عنصر روی (در پنج سطح صفر، ۴۰، ۸۰، ۱۲۰، ۱۶۰ میلی‌گرم در کیلوگرم جیره) و ویتامین E (آلفا- توکوفرول استات در دو سطح صفر و ۴۰ واحد بین‌المللی در کیلوگرم جیره) بر تولید و کیفیت تخم و غلظت عنصر روی در بخش‌های مختلف تخم و بافت‌های بدن، ۹۶۰ قطعه بلدرچین ژاپنی مادر در سن ۷۰ روزگی، برای آزمایشی فاکتوریل ۵ × ۲، در قالب طرح بلوک‌های کامل تصادفی با ۱۰ تیمار و چهار تکرار و ۲۴ قطعه پرنده مولد (۱۶ قطعه ماده و هشت قطعه نر) در هر تکرار مورد استفاده قرار گرفت. تولید تخم در پرنده‌هایی که ۱۶۰ میلی‌گرم بر کیلوگرم عنصر روی دریافت کردند نسبت به گروه شاهد بالاتر بود ($P < ۰/۰۵$). مکمل ویتامین E بر تولید تخم تاثیری نداشت. بلدرچین‌های دریافت کننده ۸۰ میلی‌گرم بر کیلوگرم عنصر روی ضریب تبدیل غذایی بهتری نسبت به تیمارهای دیگر داشتند ($P < ۰/۰۵$). ضخامت پوسته تخم پرندگان تغذیه شده با جیره‌های شاهد و حاوی ۴۰ میلی‌گرم بر کیلوگرم عنصر روی نسبت به بقیه پرندگان کمتر بود ($P < ۰/۰۵$). غلظت عنصر روی در زرده تخم پرندگان دریافت کننده جیره حاوی ۸۰، ۱۲۰ و ۱۶۰ میلی‌گرم بر کیلوگرم نسبت به گروه شاهد افزایش داشت ($P < ۰/۰۵$). مکمل عنصر روی در سطح ۱۲۰ میلی‌گرم بر کیلو گرم میزان عنصر روی سرم خون را نسبت به گروه شاهد افزایش داد. غلظت عنصر روی در عضله ران، استخوان درشت نی و کبد با تغذیه مکمل عنصر روی افزایش یافت ($P < ۰/۰۵$). مکمل ویتامین E تاثیری بر درصد تولید تخم، کیفیت پوسته تخم و غلظت عنصر روی در بخش‌های تخم و بافت‌ها در بلدرچین‌های ژاپنی نداشت.

کلمات کلیدی
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