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Original Article

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# Effects of different levels of nano chromium chloride in diet on egg quality and blood chromium content of layingjapanese quail

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## Abstract

The goal of this experiment was to investigating of effects of different levels of nano chromium (N-Cr) in diet on egg quality and blood chromium content of japanese quail. Two hundred and forty laying japanese quail (45 day old) were randomly distributed to 20 experimental unit with 12 birds in cage pens of 40 × 80 cm. Experimental treatments include: 1) control national research council (NRC, 1994) based diet (with no N-Cr), 2) 200 ppb, 3) 400 ppb, 4) 600 ppb and 800 ppb of N-Cr. Egg weight, egg internal quality (Huagh unit) and blood chromium were

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determined. Treatments influenced egg weight, yolk weight, albumen height, albumen weight, shell weight and thickness and Haugh unit ( $p \leq 0.05$ ). But blood chromium content was not affected by levels of N-Cr ( $p \geq 0.05$ ). In conclusion, it seems that N-Cr level of 800 ppb had further effects on Japanese quail egg quality.

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**Keywords:** Japanese quail, Nano chromium, Egg quality, Haugh unit, blood chromium.

## 1. Introduction

Chromium (Cr) is an essential mineral element for livestock (Lukaski, 1999). Notwithstanding, national research council (NRC, 1994) had no recommendation for Cr requirement of poultry especially for Japanese quail. But, in recent years, more researches in using of Cr and its useful effects on poultry egg and meat quality were done (Sahin et al., 2005; Sahin et al., 2002; Lien et al., 1999). Supplemental Cr in the form of Cr-picolinate (1.2 mg/kg) resulted in further egg weight of quail (Sahin et al., 2001). Derya et al., (2009) demonstrated that supplemental Cr-picolinate had increasing effect on shell thickness but no effect on egg weight of Japanese quail. In addition, with adding supplemental Cr to diet, egg weight, shell thickness and Haugh unit were linearly increased (Sahin et al., 2002). Today, nanotechnology prepared new Cr resources named N-Cr that have further bioavailability than organic and inorganic Cr supplements (Zha Ly et al., 2008). Positive effects of N-Cr on performance, egg quality and tissues mineral accumulation in layer chickens were shown by Sirirat et al., (2013). They use three levels of nanoparticles Cr-picolinate (0, 500 and 3000 ppb) in their experiment. Chromium chloride ( $\text{CrCl}_3$ ) used in poultry nutrition (Eren and Baspinar, 2004) but not in nano form. Nano chromium (N-Cr) in chemical form of chromium chloride were not used in laying Japanese quail diet, yet. Therefore, the goal of this experiment was to investigate the effects of different levels of nano chromium (N-Cr) in diet on egg quality and blood chromium content of laying Japanese quail.

## 2. Materials and methods

Two hundred and forty laying Japanese quail (45 day old) were randomly distributed to 20 experimental units with 12 birds in cage experimental units of  $40 \times 80$  cm, in a completely randomized design for 7 weeks. The five treatments used in the present experiment included the following: (1) basal diet was formulated based on national research council (NRC, 1994) (Table 1); (2) basal diet plus 200 ppb Cr as nanoparticles chromium chloride (N-Cr); (3) basal diet plus 400 ppb Cr as N-Cr; (4) basal diet plus 600 ppb Cr as N-Cr and (5) basal diet plus 800 ppb Cr as N-Cr. The nano chromium chloride was prepared by Industrial Research Institute of Iran (Tehran, Iran). The mean particle size of N-Cr chloride was  $80.73 \pm 2.96$  nm. Feed and water were *ad libitum*. Lighting program was about 16 hours. The quails were reared according to commercial quail production conditions.

### 2.1. Egg parameters measurement

Total egg produced were daily numbered and weighed with an electronic scale with 0.01 g accuracy. At the end of the experiment four eggs per experimental unit were randomly selected for egg traits measurements. Yolk weight, albumen weight and shell weight of each egg was measured with an electronic scale with 0.001 g accuracy. Thickness of egg shell in the equator, blunt end, and pointed end of the eggs (excluding the membrane of shell) was measured with a micrometer (OSK-13469, OGAWA SEIKI CO., LTD. JAPAN). For measuring of Haugh unit, after weighing of each egg, the egg was broken and albumen height was measured by an albumen height determiner (OSK-13471, OGAWA SEIKI CO., LTD. JAPAN). The Haugh unit (HU) was measured:  
$$HU = 100 \log (H + 7.57 - 1.7^{0.37})$$

### 2.2. Blood chromium

On day 93 of trial, blood samples from the quails were collected and placed into trace-mineral-free tubes with sodium heparin, and then centrifuged for 15 min at  $3000 \times g$  at  $4^\circ \text{C}$  (SIGMA 4-15 Lab Centrifuge, Germany).

After centrifugation, the fresh plasma was collected and analyzed for chromium. Plasma chromium levels were analyzed by using atomic absorption spectrophotometer (PerkinElmer, AA 600, USA).

### 2.3. Statistical analysis

Data were subjected to statistical analysis using analysis of variance (ANOVA) appropriate for a completely randomized design. When significant effects were detected by ANOVA, treatment means were compared using Duncan's multiple range tests. All statistical analyses were performed with SAS (SAS, 2001). Differences were considered significant at  $P < 0.05$ . All of parameters were analyzed as follows:  $Y_{ij} = \mu + T_i + e_{ij}$ , where  $Y_{ij}$ =individual observation,  $\mu$ =overall mean,  $T_i$ =effect of treatment and  $e_{ij}$  represents the random error.

**Table 1**  
Composition of basal diet.

Ingredients	% Diet
Corn	50.48
Soybean meal	32.36
Vegetable oil	1.92
Meat meal	7
Ca CO <sub>3</sub>	6.89
DCP	0.19
Na <sub>2</sub> CO <sub>3</sub>	0.14
Salt	0.17
dl-methionine	0.29
Lysine hydro chloride	0.06
Premixe(vitamin <sup>1</sup> +mineral <sup>2</sup> )	0.5
Total	100
<b>Calculated nutrients</b>	
Metabolizable energy (kcal/kg diet)	3000
Crud protein	20
Ca	2.5
P	0.35
Methionine	0.45
Lysine	0.85

<sup>1</sup>Supplied per kg of vitamin mixture: Vitamin A: 7.2gr; Vitamin D: 7. gr; Vitamin E: 14.4gr; Vitamin K3: 1.6gr; Vitamin B1: 0.72gr; Riboflavin: 3.3gr, Pantothenic acid: 12gr, niacin: 12160 mg; Vitamin B6: 6.2 mg; Biotin: 0.2 gr; Vitamin B12 - 0.6 gr; choline chloride 440. <sup>2</sup> Supplied per kg of mineral mixture: manganese (oxide): 64 gr; iron (FeSO<sub>4</sub>) -100 gr; zinc (oxide): 44 gr; copper (CuSO<sub>4</sub>):16 gr; iodine (calcium iodate): 64 gr; selenium (1%): 8 gr; cobalt :0.2 gr.

## 3. Results

### 3.1. Egg traits

Mean egg traits of laying japanese quail were shown in Table 2. Over all egg traits were influenced by the N-Cr levels of diet. Quails receiving 800 ppb of N-Cr had the greatest significant egg weight than 0, 200 and 400 ppb receiving birds (13.52 vs. 10.60, 11.20 and 11.30 g, respectively) ( $p \leq 0.05$ ). Treatment 5 had the highest yolk weight than treatments 1 and 3 (4.31 vs. 3.73 and 3.80 g, respectively) ( $p \leq 0.05$ ). Albumen height in 800 ppb group was significantly higher than control and 200 ppb group (2.54 vs. 1.58 and 1.66 mm, respectively) ( $p \leq 0.05$ ). Quails receiving 800 ppb of N-Cr had the greatest significant albumen weight than control (7.09 vs. 6.24 g, respectively) ( $p \leq 0.05$ ). Quails in treatment 5 (800 ppb N-Cr) had significant greatest shell thickness and HU than control (387 vs. 310  $\mu$ m, for shell thickness and 75.92 vs. 68.59, for HU, respectively).

### 3.2. Blood chromium

Treatments had no effects on blood chromium. Notwithstanding, quails in 800 ppb group had the highest blood chromium.

**Table 2**  
Effects of different levels of nano chromium in diet on egg quality.

Egg traits	Levels of nano chromium in diet (ppb)					Significance
	0	200	400	600	800	
Egg weight (g)	10.60 <sup>b</sup> ±0.19	11.20 <sup>b</sup> ±0.32	11.30 <sup>b</sup> ±0.41	11.74 <sup>ab</sup> ±0.37	13.52 <sup>a</sup> ±1.20	**
Yolk weight (g)	3.73 <sup>b</sup> ±0.18	3.90 <sup>ab</sup> ±0.13	3.80 <sup>b</sup> ±0.15	4.10 <sup>ab</sup> ±0.09	4.31 <sup>a</sup> ±0.10	*
Albumen height (mm)	1.58 <sup>b</sup> ±0.08	1.66 <sup>b</sup> ±0.09	2.01 <sup>ab</sup> ±0.30	2.05 <sup>ab</sup> ±0.21	2.54 <sup>a</sup> ±0.18	**
Albumen weight (g)	6.24 <sup>b</sup> ±0.32	6.74 <sup>ab</sup> ±0.22	6.63 <sup>ab</sup> ±0.25	6.74 <sup>ab</sup> ±0.26	7.09 <sup>a</sup> ±0.16	*
Shell weight (g)	1.28 <sup>b</sup> ±0.03	1.42 <sup>ab</sup> ±0.04	1.37 <sup>b</sup> ±0.07	1.44 <sup>a</sup> ±0.08	1.56 <sup>a</sup> ±0.02	*
Shell thickness (µm)	310 <sup>b</sup> ± 20	344 <sup>ab</sup> ±14	341 <sup>ab</sup> ±19	355 <sup>ab</sup> ±24	387 <sup>a</sup> ±22	*
Huagh unit (HU)	68.59 <sup>b</sup> ±0.47	71.50 <sup>ab</sup> ±1.14	71.59 <sup>ab</sup> ±2.26	71.21 <sup>ab</sup> ±1.54	75.92 <sup>a</sup> ±1.53	*

Data are mean ± SD. a-b: In each raw means with different superscript had significant differences (p≤0.05).

**Table 3**  
Effects of different levels of nano chromium in diet on plasma chromium.

Trait	Levels of nano chromium (ppb)					Significance
	0	200	400	600	800	
Plasma chromium (mg/dl)	221.47±2.54	224.40±2.57	222.15±2.41	222.78±2.10	226.14±3.26	Ns

Ns: not significant. Data are mean ± SD.

### 4. Discussion

There is no report using nanoparticle chromium in laying quails. Perhaps our experiment is the first one that used nanoparticle chromium chloride. Chromium is a keypoint of glucose tolerance factor (GTF) then it can influencing insulin performance that is a most important anabolic hormones (Anderson et al., 1997; Holdsworth and Neville, 1990). Due to insulin, body cells can use glucose and sufficient amino acids enter to cells, so performance increases (Hossain et al., 1998). Perhaps, with this reason, in our experiment egg weight increased. Our results are parallel to Kim et al., (1997). They used Cr-picolinate in their experiment and concluded 800 ppb of Cr in the diet resulting in further egg weight of laying hens. Level of 800 ppb of N-Cr chloride had highest effect on yolk weight. These conclusions are similar to Sahin et al., (2001) and Yildiz et al., (2004) in laying quails and Uyanick et al., (2002) in laying hens but not to Sirirat et al., (2013). In our experiment the 800 ppb of N-Cr chloride had significant beneficial effects on albumen weight, albumen height and HU. Sahin et al., (2001) and Yildiz et al., (2004) in laying quails and Uyanick et al., (2002) in laying hens were taken same results but Sirirat et al., (2013) was

taken similar result in HU, only. Jensen et al., (1987) reported that Cr had useful effects on the internal quality of egg. It seems, there is a linear effect between supplemental Cr levels in the diet and significant increasing of HU, Sahin et al., (2002) reported. In the experiment of Sirirat et al., (2013), two levels of nano chromium picolinate (500 and 3000 ppb) had beneficial significant effect on HU of post-molt laying hens' eggs. Of course, in another experiment, organic or inorganic chromium did not affect HU, egg and yolk weight (Eseceli, et al., 2010; Piva et al., 2003). Shell thickness was influenced by the level of 800 ppb N-Cr chloride than control (0 ppb N-CR chloride). In other experiment indicated that supplemental Cr in diet had increasing effects on quail egg shell thickness (Derya et al., 2009; Sahin et al., 2002; Sahin et al., 2001). But, based on investigations of Sirirat et al., (2013), Huseyin et al., (2010) and Yildiz et al., (2004) different supplemental Cr forms did not affect laying quail egg shell thickness.

Chromium is presence in most of tissues (Anderson, 1987). In our experiment, there were not significant effects of N-Cr chloride on blood plasma chromium content among treatments. However, quails in treatment 5 (800 ppb N-Cr chloride) had higher Cr concentration in their blood plasma. This finding is not similar to the investigation of Sirirat et al., (2013). They reported increasing levels of nano chromium picolinate resulted in further retention of Cr in liver, egg yolk and egg shell.

## 5. Conclusion

In conclusion, it seems that N-Cr level of 800 ppb had further effects on Japanese quail egg internal quality. But there was no effect on Cr content of blood plasma.

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