Journal of Agricultural Science; Vol. 5, No. 2; 2013
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Effect of Different Levels of Nanoparticles Chromium Picolinate Supplementation on Performance, Egg Quality, Mineral Retention, and Tissues Minerals Accumulation in Layer Chickens

Nattapon Sirirat[1], Jin-Jenn Lu[1], Alex Tsubg-Yu Hung[1] & Tu-Fa Lien[1]

[1] National Chiayi University, Department of Animal Science, Chiayi, Taiwan

Correspondence: Tu-Fa Lien, National Chiayi University, Department of Animal Science, Chiayi, Taiwan. E-mail: tflien@mail.ncyu.edu.tw

Received: November 6, 2012 Accepted: November 21, 2012 Online Published: January 15, 2013

doi:10.5539/jas.v5n2p150 URL: http://dx.doi.org/10.5539/jas.v5n2p150

Abstract

This study was conducted to investigate the effects of various levels of nanoparticles chromium picolinate on performance, egg quality, minerals retention, and tissues accumulation of layer chickens. This study used 54 seventy-week old post-molt laying hens randomly allocated into 0 (control), 500 ppb (μg kg^{-1}) Cr and 3000 ppb Cr groups for a 60-day experiment. The chromium was nanosize (80.8 ± 2.7 nm) chromium picolinate (NanoCrPic) and each treatment was undertaken with six replicates. In the meantime, a total of 18 birds (1 bird/replicate) were used for metabolic experimentation. The results of the experiment indicated that there were no significant effects on body weight, feed intake, feed efficiency, and egg production of layers. Supplemental NanoCrPic could significantly ($p < 0.05$) improve egg quality, or retention of chromium and zinc, but decrease shell ratio in the 60^{th} day eggs. The addition of NanoCrPic resulted in increased minerals accumulation in tissues such as Cr, Ca, and P concentration in the liver, Cr concentration in the yolk and Ca concentration in the eggshell. In conclusion, supplemental NanoCrPic improved Cr and Ca accumulation in the liver and egg, improved Zn and Mn retention in layer chickens.

Keywords: nanoparticles, chromium picolinate, mineral retention, laying hens

1. Introduction

Chromium is an essential mineral element in humans and domestic animals (Lukaski, 1999). Trivalent chromium (Cr (III)) is associated with the metabolism of carbohydrates, lipids and proteins in animals, it is also term as "glucose tolerance factor (GTF)", which regulates the metabolic action of insulin (Schwarz & Mertz, 1957; Chen et al., 2009). Wang and Xu (2004) suggested that the absorption and utilization of Cr may be dependent on its status in the intestinal tract. However, different Cr (III) forms have diverse rates of absorption. Organic Cr (III) has greater biological availability than inorganic Cr (III) (NRC, 1997; Lukaski, 1999). Inorganic Cr (e.g. $CrCl_3$) is in the range of 0.5-2% (Mertz, 1969); while organic Cr (e.g. CrPic) is in the range of 10-25% (Seerley, 1993).

In poultry, supplemental dietary CrPic increases production at low temperatures and increased CrPic results in an increase in egg production as well as resulting in an improved feed conversion ratio (Sahin et al., 2001; Yildiz et al., 2004). Moreover, organic Cr supplementation, particularly at 1200 ppb, increased the performance criteria, egg quality and serum insulin concentrations in Japanese quails (Sahin et al., 2002).

The nanoparticle, which is at least one dimension reduced to a nanometric size, exhibits new electrical, magnetic, mechanical, and biological properties (Gref et al., 1994), which have been determined as critical factors influencing particle uptake (Delie, 1998). Therefore, the new phenomena and properties of nanoparticles may have unique potential applications. In previous work, chromium nanoparticles were shown to produce beneficial effects on growth performance, body composition, resulting in increases in tissues Cr concentration in selected muscles. Additionally, they have been shown to enhance Cr digestibility and absorption in rats when supplemented with 200 ppb NanoCr as well as altering certain blood metabolite concentrations (Zha et al., 2007a; Lien et al., 2009). Wang and Xu (2004) found that supplemented 200 μg kg^{-1} chromium nanoparticles (NanoCr) produce beneficial effects on carcass characteristics, pork quality and individual skeletal muscle weight, with approximately two- to three-fold higher tissue chromium deposition in selected muscle and organs compared to

the control group, which implicated higher absorption and bioavailability of nanosize chromium. However, there are no existing studies about nano-sized chromium supplementation in poultry.

It is well known that mineral intake at high levels will have antagonist effects on other minerals. However, knowledge about this antagonist in Cr still is in its early stage. Some reports have indicated that iron-binding proteins are involved in chromium binding, transport, and storage (Feng et al., 2003). $CaCO_3$ reduces Cr uptake and retention in rats (Seaborn & Stoecker, 1990). It has also been reported that in stressed mice, loss of zinc, copper, iron, and manganese was reduced by supplemental chromium (Schrauzer et al., 1986; N. Sahin & K. Sahin, 2002). Sahin and Sahin (2002) reported that the utilization of nitrogen and Ca, P, Zn, and Fe, is improved by supplemental chromium and ascorbic acid.

Our hypothesis is that the nanoparticles chromium may also have an antagonistic effect on other minerals utilization and excretion, and consequently influence the animal performance. Therefore, this study was designed to compare the effects of dietary nanoparticles of chromium picolinate supplementation on the production performance, egg quality, minerals retention, and tissue minerals accumulation in layer chickens.

2. Materials and Methods

2.1 Nanoparticle of Chromium Picolinate Determination

The NanoCrPic was supplied by the Industrial Technology Research Institute of Taiwan (Shintsu, Taiwan). The NanoCrPic was measured by transmission electric microscopy (TEM). The average particle size of NanoCrPic was 80.8 ± 2.7 nm (Figure 1).

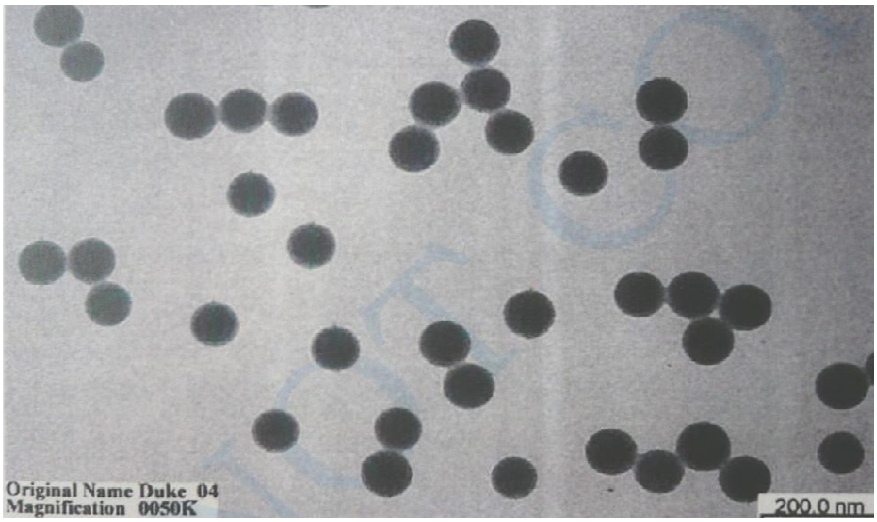

Figure 1. The image of nanoCr particles measured by TEM. Average particle size was 80.8 ± 2.7 nm

2.2 Experimental Design

A total of fifty-four 70-week old post molt laying hens (hy-line) were randomly selected for three dietary treatments with six replicates (three birds per replicate), with one hen per cage, (a cage length and width of 35 cm×20 cm, respectively) in a completely randomized design. The three treatments used in the present study consisted of the following: (1) basal diet was formulated based on NRC (1994)(Table 1); (2) basal diet with 500 ppb (μg kg^{-1}) Cr as nanoparticles CrPic (NanoCrPic) and (3) basal diet with 3000 ppb Cr as NanoCrPic for a 60 day experiment. Feed and water were *ad libitum* with floor feeding. The average room temperature at day time was 30.2 ± 2.5℃, lighting was about 15 hours. Egg production was recorded every day. 2 eggs from each bird was collected at middle (30^{th} day) and final (60^{th} day) period of the experiment for egg quality analysis, and the eggs at final period were also used for egg yolk and shell mineral content analysis. At the final of the experiment, 6 layers in each group (1 bird/replicate) were sacrificed and liver samples were taken for mineral analysis. The animals were reared according to the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching*, and this study was approved by the University's Animal Care and Use Committee.

Table 1. The basal diets of laying hen

Ingredients	kg
Yellow Corn	511
Soybean meal	250
Full fat soybean meal	100
Soybean oil	20
Salt	4
Chloline chloride (50%)	1
Limestone (35%)	100
Dicalcium phosphate (18%)	11
Vitamin premix[1]	1
Mineral premix[2]	1
Total	1000
Calculated value	
Crude protein (%)	19
ME, kcal/kg	2900
Calcium (%)	3.86
Available phosphorus (%)	0.25
Copper (mg)	6
Zinc (mg)	44
Analyzed value	
Gross energy (%)	3860
Crude protein (%)	21.57
Chromium (ppb)	961

[1] Vitamin premix (content per kg): vitamin A 1,250,000 IU; vitamin D_3 25,000 ICU; vitamin E 2,000 IU; vitamin K_3 250 mg; vitamin B_1 200 mg; vitamin B_6 300 mg; vitamin B_{12} 12 mg; pantothenate 120 mg; niacin 350 mg; biotin 200 μg; folic acid 100 mg.

[2] Mineral premix (content per kg): Fe ($Fe_2(SO_4)_3$) 0.2005 g; Mn ($MnSO_4$) 0.076 g; Cu ($CuSO_4$) 0.024 g; Zn(ZnO) 0.055 g; I (KI) 0.0057 mg; Se (Na_2SeO_3) 0.0175 mg.

2.3 Metabolic Trial

Eighteen chickens (1 bird replicate^{-1}) were selected in last week of the experiment for the metabolic trial and conducted in duplicates, using the individual cages (35 cm×20 cm) and a plastic bag for collection of total excreta. The metabolic trial lasted five days. During the metabolic trial, the total amount of feed consumed (about 110 g day^{-1} bird^{-1}) and total excreta voided (dry matter basis) from each chicken was recorded and sampled for components analysis. Minerals retention ratio was calculated using the following equation:

$$\text{Mineral retention ratio (\%)} = \frac{(\text{WFI} \times \text{EF}) - (\text{WEV} \times \text{EE})}{(\text{WFI} \times \text{EF})} \times 100$$

WFI = weight of feed intake (110 g)

EF = concentration of element in feed

WEV = weight of total excreta voided

EE = concentration of element in total excreta

2.4 Chromium Analysis

Chromium analysis was base on the method of Anderson et al. (1985). Weighting 0.2 g of ground feed and feces, egg shell, yolk, and liver samples in duplicates were placed into a crystal beaker, in which 10 mL of 70% nitric acid was added and rested for 8 h before starting heat digestion at 80°C for 8 h, left for cooling and after that the samples were diluted with 50 mL of deionized water. The filtered solution was kept in a polypolene bottle. Then, a polarized Zeeman atomic absorption spectrometer (Hitachi, Japan) equipped with a graphite furnace atomization was used for analysis.

2.5 Copper, Zinc, Manganese, and Iron Analysis

According to AOAC (2000) methodology (NO. 2.109), the steps are as follows: weighting 1 g of feed, feces, liver, egg shell and yolk samples in duplicates crushed under the crucible. Following this, samples were subjected to 550℃ for 5 h in a furnace to be converted to ash. The crucible was subjected to 10 mL 3 N HCl under a heating plate and heated until the solution became clear. It was then allowed to cool (added to 50 mL with 0.1 N HCl). An atomic absorption spectrometer (Perkin Elmer, Atomic Analyst 100, USA) was used for copper, zinc, manganese and iron contents analysis.

2.6 Calcium and Phosphorous Analysis

According to AOAC (2000) methodology (Ca: No. 7.096, P: No. 7.119) the steps are as follows: weighting 1 g of feed, feces, and liver samples in duplicates crushed under the crucible. Following this, samples were subjected to 550℃ for 5 h in a furnace to be converted to ash. The crucible was subjected to 10 mL 3 N HCl under a heating plate and heated until the solution became clear. It was then allowed to cool (added to 50 mL with 0.1 N HCl).

The calcium content analysis used a sample solution of 6 mL with added lanthanum 185.4 μL 50000 mg kg^{-1}. Then, analysis was performed using an atomic absorption spectrometer (Perkin Elmer, Atomic Analyst 100, USA).

For phosphorous determination, a sample of 1 mL plus 1 mL Vanadium-molybenum acid and 3 mL de-ionized water (a total of 5 mL) were combined and left standing for 10 min. An automatic scanning sub-ray spectrophotometer was then used for analysis (Beckman, DRR 640i, USA), with the wavelength set at 400 nm.

2.7 Determination Traits of Egg

Egg albumen weight, yolk weight, and egg shell weight of each egg was measured (Fujihira Kogyo Inc., Tokyo). Egg shell thickness in the equator, blunt end, and pointed end of the eggs (excluding the shell membrane) was examined with a micrometer.

Egg shell strength was mechanically measured (Fujihira Kogyo Inc., Tokyo) to record the pressure used to rupture the shell.

Egg freshness was examined as follows: (1) egg weight was recorded; (2) eggs were broken and the heights of albumen and yolk, (short and long diameters of the albumen and the diameter of the yolks) were measured with a digital caliper; (3) yolk was separated from albumen and recorded; (4) the ratios of egg weight and thickness as well as albumen height were calculated; (5) the Haugh unit (HU) was examined (Nesheim et al., 1979):

Haugh unit (HU) = $100 \times \log (H - 1.7 \times W^{0.37} + 7.57)$

H=albumen height (mm).

W=egg weight (g).

2.8 Statistical Analysis

The experiment data were subjected to statistical analysis using SAS software (version 9.1, SAS 1998). The general linear model procedure (GLM procedure) was used based on the completely randomized design (CRD) model. Tukey's tests (SAS, 1998) were adopted in the model to determine the significant difference, according to the following model, treatment (T) was the main effect.

$$Y=\mu + T_i + P_j + e_{ijk}$$

Where Y is the dependent variable, μ represents the mean, P is the pen (replicate, the experiment unit) effect and e is the random residual error term. The level of significantly different was set at $P < 0.05$.

3. Results and Discussion

3.1 The Effects of Various Levels of NanoCrPic on the Performance of Laying Hens

The effect of dietary NanoCrPic supplementat on laying hens' performance parameters are shown in Table 2. During the experiment intervals at 30 and 60 days, the body weight, feed intake, and egg production were similar ($p > 0.05$) among treatments. Supplemental NanoCrPic 500 ppb either for 0-30 or 30-60 days increased egg weight ($p = 0.09$) compared with NanoCrPic 3000 ppb diet.

Table 2. The effect of different level of nano-chromium on performance of laying hen

Items	Supplemented dietary NanoCrPic (ppb)			SEM	P value
	0	500	3000		
			0-30 days		
Body weight (g)	1452	1479	1458	38.8	0.87
Feed intake (g bird^{-1} day^{-1})	107.8	105.6	105.5	1.02	0.28
Egg weight (g)	69.73^{ab}	71.27^{a}	67.24^{b}	0.60	0.09
Egg production (%)	80.62	84.89	84.47	1.70	0.22
Feed intake Egg mass^{-1}	1.83^{ab}	1.75^{b}	1.95^{a}	0.05	0.06
			30-60 days		
Body weight (g)	1460	1482	1498	10.7	0.11
Feed intake (g bird^{-1} day^{-1})	107.1	108.4	107.2	4.76	0.97
Egg weight (g)	67.45^{ab}	69.83^{a}	65.32^{b}	1.10	0.07
Egg production (%)	82.40	84.53	83.43	1.26	0.52
Feed intake Egg mass^{-1}	1.90	1.84	1.98	0.06	0.32

^{a,b} Means within the same row without the same superscripts differ significantly ($p < 0.05$).

SEM: standard error of mean.

n=7.

In the egg quality parameters (Table 3) at the 30 day interval, no significant differences ($p > 0.05$) in egg strength, egg shell thickness, yolk weight, albumen ratio, yolk ratio, egg shell ratio, and yolk index were detected among groups. However, albumen index and Haugh unit of NanoCrPic groups were significantly better compared to the control group ($p < 0.05$).

At the 60 day interval, the egg strength, egg shell thickness, albumen weight, and albumen ratio of laying hens did not differ significantly ($p > 0.05$) between the treatment groups. The Haugh unit, albumen index and yolk index of layers was significantly greater ($p < 0.05$) in NanoCrPic supplemented groups as compared to the control group. However, the yolk weight, yolk ratio, and egg shell ratio were lower ($p < 0.05$) in supplemental NanoCrPic groups.

Table 3. The effect of different level of nano-chromium on egg quality of laying hens

Parameter	Supplemented dietary NanoCrPic (ppb)			SEM	P value
	0	500	3000		
	30 days				
Egg strength (kg cm^{-2})	3.37	3.43	3.19	0.23	0.76
Egg shell thickness (mm)	0.36	0.38	0.37	0.02	0.94
Yolk weight (g)	19.9	19.1	18.5	0.46	0.18
Albumen weight (g)	43.3^{ab}	44.3^{a}	41.6^{b}	0.55	0.03
Albumen ratio (%)	62.0	61.9	61.8	0.73	0.98
Yolk ratio (%)	28.5	26.8	27.5	0.55	0.17
Egg shell ratio (%)	9.39	9.69	9.67	0.52	0.90
Haugh unit (HU)	63.9^{b}	83.4^{a}	84.8^{a}	1.97	0.0005
Albumen index (%)	4.54^{b}	8.07^{a}	8.15^{a}	0.29	0.0002
Yolk index (%)	38.6	42.1	49.1	7.33	0.61
	60 days				
Egg strength (kg cm^{-2})	3.23	2.78	3.14	0.30	0.57
Egg shell thickness (mm)	0.29	0.33	0.33	0.02	0.24
Yolk weight (g)	19.7^{a}	17.9^{b}	17.7^{b}	0.40	0.02
Albumen weight (g)	40.4	42.3	40.1	0.93	0.28
Albumen ratio (%)	59.7	60.4	58.1	0.87	0.23
Yolk ratio (%)	29.4^{a}	25.8^{c}	27.2^{b}	0.42	0.002
Egg shell ratio (%)	10.4^{a}	8.2^{b}	8.7^{b}	0.51	0.044
Haugh unit (HU)	69.9^{b}	83.2^{a}	79.9^{a}	2.07	0.009
Albumen index (%)	3.33^{b}	7.97^{a}	7.25^{a}	0.54	0.001
Yolk index (%)	35.5^{b}	42.1^{a}	41.8^{a}	0.84	0.002

$^{a, b}$Means within the same row without the same superscripts differ significantly ($p < 0.05$).

SEM: standard error of mean. n=7.

There is no report using nanosize chromium in laying hens. However, Sahin et al. (2002*a;* 2003) stated that higher doses of supplemental chromium increased egg production, improved feed efficiency, increased egg weight, egg specific gravity, egg shell thickness, and Haugh unit in laying hens kept in low temperatures. Moreover, Kim et al. (1997) reported that feeding 800 ppb CrPic to hens resulted in higher egg production, egg weight and egg mass. However, a later study reported that the same amount of organic or inorganic chromium did not influence the hen production performances (Lin & Lin, 1999) and, in another experiment, organic or inorganic chromium fed at five weeks did not affect feed intake, egg production, egg and yolk weight, and Haugh unit (Piva et al., 2003). Lien et al. (1996) also indicated that the shell thickness was not affected by chromium picolinate supplementation (400 and 800 ppb) under thermally neutral conditions.

3.2 The Effects of Various Levels of NanoCrpic on the Minerals Retention in Laying Hens

Chromium retention and other minerals, namely Cu, Zn, Fe, Mn, Ca, and P in layers are presented in Table 4. Cr retention in the 500 ppb and 3000 ppb NanoCrPic groups were significantly higher ($p < 0.0001$) than the control group. When compared the control group, increased retention of Zn ($p = 0.0002$) was observed in the 500 ppb and 3000 ppb NanoCrPic groups and Mn in the 3000 ppb group. However, retention of Cu, Fe, Ca, and P show no difference when the chickens were fed with diets containing 500 ppb and 3000 ppb NanoCrPic.

Table 4. The effect of different level of nano-chromium on minerals retention ratio of laying hens

Items (%)	Supplemented dietary NanoCrPic (ppb)			SEM	P value
	0	500	3000		
Cr	3.58[c]	16.67[b]	21.21[a]	2.57	0.0001
Cu	29.63	36.61	38.66	3.54	0.24
Zn	18.70[c]	28.70[a]	25.28[b]	0.72	0.0002
Fe	26.04	33.54	29.18	2.09	0.11
Mn	39.81[b]	41.30[ab]	42.23[a]	0.58	0.06
Ca	54.72	60.40	56.46	1.85	0.16
P	27.48	29.11	28.30	0.56	0.19

[a,b,c] Means within a row with no common superscripts are significantly different ($p < 0.05$).

SEM: standard error of mean.

Retention ratio = (intake - excretion) ÷ intake × 100%

n=3.

The administration of Fe inhibits the absorption of Cr, indicating that Cr and Fe also share a common gastrointestinal transport mechanism, binding with transferring (Gomes et al., 2005). Antacid ($CaCO_3$) reduces Cr uptake and retention in rats (Seaborn & Stoecker, 1990). Another research reported that the utilization of nitrogen and Ca, P, Zn, Fe as well as Cr, is improved by supplemental chromium and ascorbic acid (X. L. Sahin & F. P. Sahin, 2002). It has also been reported that in stressed mice, loss of zinc, copper, iron and manganese was reduced by supplemental chromium (X. L. Sahin & F. P. Sahin, 2002; Schrauzer et al., 1986). Our results are in agreement with the results of Sahin and Sahin (2002), who reported that the retention of Zn was improved by supplemental Cr and ascorbic acid. In our previous study with broiler also indicated that supplemental NanoCrPic improved the retention of Zn (Sirirat et al., 2012), which may be owing to the Zn excretion being reduced in NanoCrPic group. Amatya et al. (2004) also reported that supplemental Cr had effect on the retention of Zn and Mn in broilers. However, retention of Cu, Fe, Ca, and P were not different when the chickens were fed with diets containing 500 ppb and 3000 ppb NanoCrPic.

The experiment results confirm the theory that nanoparticle size could increase the ability of the molecules to pass through the intestinal mucosa for better absorption (Gilligan & Po, 1991). Apart from particle size, surface charge and hydrophobicity can also affect the gastrointestinal uptake of nanoparticles (Po et al., 1995). Win and Feng (2005) also found that nanoparticles increased cellular uptake. Other studies have indicated that the transport of NanoCr exhibited considerably higher absorption efficiency than CrPic and $CrCl_3$, respectively (Zha et al., 2007a). Lien et al. (2009) also reported the NanoCrPic enhances chromium digestibility and

absorption in rats, as well as pigs (unpublished data). All of these reports agree with the results of the present study.

3.3 The Effects of Various Levels of NanoCrpic on the Liver and Egg Minerals Accumulation in Laying Hens

Liver minerals, namely Cr, Cu, Zn, Fe, Mn, Ca, and P in the layers are presented in Table 5. Cr, Ca, and P show significant increase ($p < 0.05$) in the 500 ppb and 3000 ppb NanoCrPic groups. Other minerals such as Cu, Zn and Fe were not affected by dietary NanoCrPic supplementation.

Table 5. The effect of different level of nano-chromium on liver minerals content of laying hens

Items	Supplemented dietary NanoCrPic (ppb)			SEM	P value
	0	500	3000		
Cr (μg kg^{-1})	618[b]	644[b]	798[a]	0.04	0.03
Cu (mg kg^{-1})	6.71	7.56	7.13	0.68	0.68
Zn (mg kg^{-1})	50.3	55.3	56.7	3.17	0.34
Fe (mg kg^{-1})	109	105	101	6.84	0.70
Mn (mg kg^{-1})	3.71	3.51	3.65	0.12	0.11
Ca (%)	0.017[b]	0.026[a]	0.023[a]	0.001	0.0003
P (%)	0.169[b]	0.189[a]	0.184[a]	0.004	0.01

[a,b] Means within a row with no common superscripts are significantly different ($p < 0.05$).

SEM: standard error of mean.

n = 6.

There were no differences in minerals namely Cr, Cu, Zn, Fe, Mn, and P as regards to the yolks among the dietary treatments (Table 6). Ca in the yolk was significantly decreased in the 500 ppb and 3000 ppb NanoCrPic groups as compared to the control group ($p = 0.01$).

Table 6. The effect of different level of nano-chromium on yolk minerals content of laying hens

Items	Supplemented dietary NanoCrPic (ppb)			SEM	P value
	0	500	3000		
Cr (μg kg^{-1})	420	499	478	34.5	0.27
Cu (mg kg^{-1})	25.0	20.5	20.7	1.74	0.15
Zn (mg kg^{-1})	51.9	54.0	52.6	3.48	0.90
Fe (mg kg^{-1})	89.9	100	108	6.58	0.16
Mn (mg kg^{-1})	3.81	4.46	4.84	0.47	0.32
Ca (%)	0.17[a]	0.16[a]	0.14[b]	0.005	0.01
P (%)	0.19	0.19	0.20	0.006	0.53

[a,b] Means within a row with no common superscripts are significantly different ($p < 0.05$).

SEM: standard error of mean.

n = 6.

The minerals of egg shell, i.e., Zn was significantly increased ($p = 0.0008$) by NanoCrPic supplementation and also Cr in 3000 ppb NanoCrPic groups ($p = 0.003$); whereas Cu, Fe, Mn, Ca, and P showed no differences among the dietary treatments (Table 7).

Table 7. The effect of different level of nano-chromium on egg shell minerals content of laying hens

Items	Supplemented dietary NanoCrPic (ppb)			SEM	P value
	0	500	3000		
Cr (μg kg^{-1})	254[b]	273[b]	355[a]	18.0	0.003
Cu (mg kg^{-1})	8.18	8.62	7.76	0.71	0.70
Zn (mg kg^{-1})	4.04[b]	6.14[a]	6.21[a]	0.36	0.0008
Fe (mg kg^{-1})	79.4	88.8	74.7	6.55	0.32
Mn (mg kg^{-1})	2.44	2.23	3.20	0.32	0.11
Ca (%)	37.5	38.5	40.4	1.42	0.35
P (%)	0.78	0.94	0.96	0.11	0.47

[a,b,c] Means within a row with no common superscripts are significantly different ($p < 0.05$).

SEM: standard error of mean.

n = 6.

In mouse, Anderson et al. (1989) reported that supplemental chromium resulted in an accumulation of Cr in the liver. Zha et al. (2007c) reported that nanoparticles chromium supplementation shown effects on primary tissues such as kidney, liver and hind leg muscle in rats. In addition, Cr has been shown that supplemental chromium led to increased Cr and Zn in serum, liver, kidney and longissimus muscle of laying hens and Japanese quails (Sahin et al., 2002a; Sahin et al., 2002b), but Fe concentrations were not affected in Japanese quails. Our previous study also indicated that NanoCrPic supplementation in broilers diet increased the concentration of Cr and Ca in the liver (Sirirat et al., 2012). Those reports were agreed with our results. From the result of this study it indicated that chromium accumulated major in liver.

Chemical analysis has shown the presence of other minerals (Cr, Co, Cu, Mn, Ni, and Zn) in eggs (Dauwe et al., 2005). Butcher and Miles (2002) suggested that the dry eggshell is approximately 95% calcium carbonate, 0.3% P, 0.3% Mg, with traces of Na, K, Zn, Mn, and Cu, which is consistent with our results.

The results pertaining to values of minerals in the yolk are in agreement with those of Mabe et al. (2003) and Piva et al. (2003) who reported that hen diets containing trace minerals, namely Cr, Zn, and Cu; and Cr supplementation did not affect yolk mineral content. From the present study, it is indicated that chromium supplementation did not affect yolk and egg shell minerals composition except as regards to calcium in the yolk.

4. Conclusion

This study found that chickens fed with NanoCrPic showed improved Cr and Ca accumulation in the liver and egg; NanoCrPic supplementation was also shown to improve the Zn and Mn retention of laying chickens.

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