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Effect of Chromium Supplementation on Performance, Mineral Retention and Tissue Mineral Accumulation in Layer Chickens

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ABSTRACT

Twenty two weeks old Babcock layer chickens (324) were randomly allocated to 9 treatment groups with 3 replicates of 12 birds in each to study and compare the effect of Cr from three sources (inorganic, organic or nano) at two different dosage level on performance, mineral retention and tissue mineral accumulation in layer chickens. The trial lasted for 12 weeks. The control group (T1) was given with the standard layer diet while the other groups were supplemented with Cr at 200 µg/kg (inorganic Cr chloride-T2, organic CrProp-T4, CrPic-T6 and nanoCr-T8) and 400 µg/kg (inorganic Cr chloride-T3, organic CrProp-T5, CrPic-T7 and nanoCr-T9) diets. Neither the source nor the level of Cr supplement had significant effect on body weight, feed consumption, egg production and egg weight during the study period. The organic and nanoCr supplementation significantly ($P<0.05$) increased the retention of Cr, Zn, Fe, Ca and P without influencing Cu retention. NanoCr and organic CrPic/CrProp (400 µg/kg) increased the concentration of Cr and Zn in plasma, liver and egg shell, Ca in liver and egg shell and Zn in egg yolk. It may be concluded that organic or nanoCr supplementation (400µg/kg) in the diet improved the retention and tissue accumulation of minerals in layer chickens.

Keywords: Chromium, mineral retention, tissue accumulation, layer

As the requirement to produce poultry meat and eggs increases, new methods of improvement need to be sought besides the genetic and nutritional manipulations. Controversies on the use of antibiotic growth promoters in livestock diets because of transmission of resistant bacterial strains and increased food safety concerns evoked interest in the search for safer alternative growth promoters (Wegener, 2006). Thus, the use of natural additives has been gaining importance in poultry feeds, such as the use of probiotics, enzymes, acidifiers, yeast culture and organic trace minerals like chromium.

Chromium (Cr) is one of the trace mineral elements and its supplementation brings about a new interest in trace mineral nutrition, because it improves nutritional quality of poultry meat and egg (Li *et al*., 2013). As the intake of Cr is usually low through feed ingredients and diets, attention of researchers is being focussed for exploring the possible beneficial aspects of Cr supplementation on biological activities, body composition and health of animals including birds and humans. The National Research Council (NRC, 1994) has not given any recommended level for Cr supplementation in chicken diet.

Many evidences have supported that Cr supplemented diets have many beneficial effects *viz.*, improving nutrient digestion and consumption, enhancing food metabolism, immune responses, positive effects on egg quality, potent antioxidant, hypocholesterimic activity and helping retention of other essential elements in the body (Khan *et al.*, 2014).

The present work was formulated to study and compare the effect of source and level of dietary chromium on performance, mineral retention and tissue mineral accumulation in layer chickens

MATERIALS AND METHODS

Birds and diets

The experiment was carried out on Babcock layer chickens (324) from 22 weeks of age. Birds were allocated into 9 treatment groups (three replicates/group; 12 birds/replicate) to study and compare the effect on performance (growth and production) and tissue mineral accumulation. The birds were fed either the standard layer diet (Table 1) or that supplemented with Cr from three sources as inorganic, organic and nanoform, each at two different dosage levels viz., 200 µg/kg (inorganic Cr chloride-T2, organic CrProp-T4, CrPic-T6 and nanoCr-T8) or 400 µg/kg (inorganic Cr chloride-T3, organic CrProp-T5, CrPic-T7 and nanoCr-T9) diet for 12 weeks (up to 34 weeks of age). The birds were raised in well ventilated elevated two tier cage system and were provided with *ad libitum* drinking water and feed.

Analysis of minerals in samples

The concentration of chromium, zinc, copper, iron, calcium and phosphorus were estimated in the feed, excreta, liver, muscle, egg yolk and egg shell samples of the experimental birds. For analysis of chromium and zinc, the methodology suggested by Perkin Elmer Inc. (Germany) was followed in the atomic absorption spectrophotometer using graphite and flame technique with slight modifications. Concentrations of Cr and Zn in plasma samples were analysed directly in the atomic absorption spectrophotometer following suitable dilution. Standard methods of AOAC (2005) were used to determine copper (method 947.03), iron (method 944.02), calcium (method 927.02) and phosphorous (method 964.06) in the samples.

Metabolic Trial

A metabolic trial of 5 days was conducted at the last week (34th week) of the study.

During the trial the total amount of feed consumed and total excreta voided from each chicken were recorded (DM Basis), oven dried at 60°C and sampled for mineral analysis.

Measurements

The body weight was recorded at the beginning and end of the study period. Egg production was recorded every day and eggs at the final period were used for egg yolk and shell mineral content analysis. At the last week (34th week) of the study 6 layers from each group was selected randomly and sacrificed. Prior to sacrifice, the birds were kept off fed and blood sample was collected from jugular vein in heparinised vials. The plasma was separated; liver samples were taken and stored at -20°C up to analysis.

Statistical analysis

The experimental data were analysed as randomised complete block design (Snedecor and Cochran, 1994). The results were presented as means ± standard error. Statistical differences were analyzed by one way ANOVA wherein differences were considered to be significant at P<0.05.

RESULTS AND DISCUSSION

Neither the source nor the level of Cr supplement had any significant effect on body weight, feed consumption, egg production and egg weight in layer birds (Fig. 1).

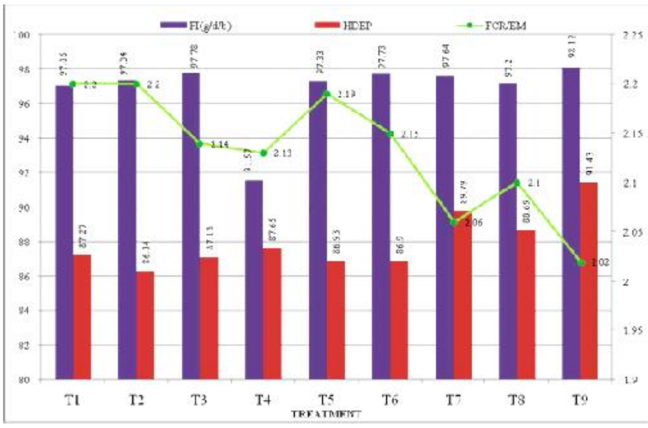

Fig. 1: Mean production performance of layer chickens (22-34 weeks) fed inorganic, organic and nanochromium

However the performance was better (P>0.05) in nanoCr (400 µg/kg) supplemented groups as compared to other Cr supplemented groups during the 12 weeks study period. Supporting to the results of the current study, Sirirat *et al.* (2013) observed that nanoCr at 500 and 3000 ppb

Table 1: Mean (±SE) plasma minerals content of layer chickens (22-34 weeks) fed inorganic, organic and nanochromium

Treatment	Chromium (μg/dl)	Copper (μg/dl)	Zinc (μg/dl)	Iron (μg/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)
T1-Standard diet	0.021^{a} ± 0.081	31.41^{c} ± 1.20	75.35^{a} ± 2.67	223.05 ± 13.15	7.923 ± 0.31	4.68 ± 0.56
T2-Standard diet+200μg inorganic Cr /kg	0.023^{a} ± 0.071	29.38^{c} ± 2.25	76.10^{a} ± 4.25	220.54 ± 21.32	7.871 ± 0.43	4.25 ± 0.31
T3- Standard diet+400μg inorganic Cr /kg	0.027^{a} ± 0.120	28.79^{c} ± 1.32	78.67^{b} ± 2.22	201.67 ± 11.25	8.003 ± 0.52	4.54 ± 0.47
T4- Standard diet+200μg organic CrProp /kg	0.030^{b} ± 0.052	26.10^{ab} ± 0.74	78.30^{b} ± 1.91	219.10 ± 17.87	8.007 ± 0.23	4.58 ± 0.54
T5- Standard diet+400μg organic CrProp /kg	0.036^{bc} ± 0.025	25.82^{ab} ± 1.47	81.26^{bc} ± 2.65	218.54 ± 14.37	8.011 ± 0.51	4.60 ± 0.52
T6- Standard diet+200μg organic CrPic /kg	0.034^{b} ± 0.034	25.71^{ab} ± 2.67	78.54^{b} ± 3.82	214.25 ± 8.49	8.005 ± 0.23	4.54 ± 0.47
T7- Standard diet+400μg organic CrPic /kg	0.040^{bc} ± 0.134	23.41^{a} ± 1.27	82.66^{bc} ± 1.84	215.48 ± 11.83	8.014 ± 0.41	4.71 ± 0.62
T8- Standard diet+200μg nanoCr /kg	0.041^{bc} ± 0.042	24.68^{ab} ± 1.32	80.85^{bc} ± 6.23	217.26 ± 15.50	8.018 ± 0.50	4.63 ± 0.58
T9- Standard diet+400μg nanoCr /kg	0.046^{c} ± 0.023	22.80^{a} ± 2.65	86.22^{c} ± 2.52	218.05 ± 22.54	8.023 ± 0.22	4.69 ± 0.71

Each value is mean of six observations. Means with different superscripts in a column differ significantly (P<0.05).

Table 2: Mean (±SE) liver minerals (μg/g dry mater) content of layer chickens (22-34 weeks) fed inorganic, organic and nano chromium

Treatment	Chromium	Copper	Zinc	Iron	Calcium	Phosphorus
T1-Standard diet	0.153^{a} ± 0.013	3.162 ± 0.47	44.919^{a} ± 1.87	109.980 ± 6.87	0.011^{a} ± 0.64	0.166 ± 0.84
T2-Standard diet+200μg inorganic Cr /kg	0.234^{ab} ± 0.044	3.142 ± 0.32	47.522^{a} ± 2.89	110.315 ± 4.32	0.012^{a} ± 0.71	0.171 ± 0.60
T3- Standard diet+400μg inorganic Cr /kg	0.274^{ab} ± 0.024	3.218 ± 0.62	51.976^{ab} ± 1.96	107.070 ± 2.38	0.012^{a} ± 0.34	0.171 ± 0.82
T4- Standard diet+200μg organic CrProp/kg	0.315^{b} ± 0.033	3.215 ± 0.54	52.364^{ab} ± 1.91	107.222 ± 1.59	0.016^{b} ± 0.27	0.176 ± 0.44
T5- Standard diet+400μg organic CrProp/kg	0.334^{bc} ± 0.051	3.235 ± 0.35	57.638^{bc} ± 2.17	109.546 ± 3.21	0.019^{bc} ± 0.15	0.177 ± 0.52
T6- Standard diet+200μg organic CrPic /kg	0.327^{b} ± 0.009	3.228 ± 0.53	53.494^{ab} ± 2.64	108.679 ± 5.71	0.017^{b} ± 0.47	0.172 ± 0.24
T7- Standard diet+400μg organic CrPic /kg	0.378^{c} ± 0.028	3.231 ± 0.46	55.821^{bc} ± 1.73	110.804 ± 4.37	0.020^{bc} ± 0.22	0.181 ± 0.20
T8- Standard diet+200μg nanoCr /kg	0.348^{bc} ± 0.011	3.134 ± 0.41	55.130^{bc} ± 2.69	106.503 ± 4.27	0.018^{bc} ± 0.65	0.177 ± 0.18
T9- Standard diet+400μg nanoCr /kg	0.407^{c} ± 0.022	3.263 ± 0.37	58.926^{c} ± 2.27	111.780 ± 2.67	0.024^{c} ± 0.57	0.179 ± 0.62

Each value is mean of six observations. Means with different superscripts in a column differ significantly (P<0.05).

Table 3: Mean (±SE) yolk minerals (μg/g dry mater) content of layer chickens (22-34 weeks) fed inorganic, organic and nanochromium

Treatment	Chromium	Copper	Zinc	Iron	Calcium	Phosphorus
T1-Standard diet	0.151 ± 0.031	0.912 ± 0.67	18.39^{a} ± 1.30	29.34 ± 1.54	0.153 ± 0.51	0.514 ± 0.60
T2-Standard diet+200μg inorganic Cr /kg	0.154 ± 0.010	0.907 ± 0.58	18.26^{a} ± 1.27	28.70 ± 1.48	0.151 ± 0.31	0.481 ± 0.24
T3- Standard diet+400μg inorganic Cr /kg	0.153 ± 0.018	0.912 ± 0.53	18.81^{a} ± 0.89	28.80 ± 1.31	0.146 ± 0.42	0.504 ± 0.43
T4- Standard diet+200μg organic CrProp/kg	0.160 ± 0.036	0.906 ± 0.34	20.13^{ab} ± 0.91	29.92 ± 2.22	0.143 ± 0.47	0.491 ± 0.54
T5- Standard diet+400μg organic CrProp/kg	0.165 ± 0.027	0.908 ± 0.31	21.74^{b} ± 0.93	30.13 ± 2.54	0.152 ± 0.66	0.514 ± 0.23
T6- Standard diet+200μg organic CrPic /kg	0.163 ± 0.017	0.910 ± 0.64	20.74^{ab} ± 0.78	30.24 ± 1.37	0.150 ± 0.48	0.513 ± 0.51
T7- Standard diet+400μg organic CrPic /kg	0.164 ± 0.011	0.913 ± 0.81	21.88^{b} ± 0.32	31.47 ± 1.34	0.152 ± 0.39	0.530 ± 0.37
T8- Standard diet+200μg nanoCr /kg	0.164 ± 0.022	0.911 ± 0.53	20.82^{ab} ± 0.87	30.57 ± 0.98	0.147 ± 0.57	0.521 ± 0.25
T9- Standard diet+400μg nanoCr /kg	0.170 ± 0.008	0.909 ± 0.91	22.31^{b} ± 0.90	32.73 ± 1.73	0.153 ± 0.26	0.526 ± 0.60

Each value is mean of six observations. Means with different superscripts in a column differ significantly (P<0.05).

supplementation had no significant effect on body weight, feed intake and egg production of post moult 70 weeks old laying hens. Similarly addition of inorganic Cr (Yesilbag and Eren, 2009) or organic Cr (Yıldız *et al.*, 2004) in Japanese quails and organic Cr (Torki *et al.*, 2014; Yenice *et al.*, 2015a) in layers did not affect the performance (growth and production).

In the metabolic trial (Fig. 2) Mean mineral retention (%) of layer chickens (22-34 weeks) fed inorganic, organic and nanochromium) conducted at the end of the study period, the retention of Cr, Zn and P were increased significantly ($P<0.05$) by organic and nanoCr than inorganic Cr and control groups. The Fe and Ca retention was increased by nanoCr followed by both organic Cr source at 400 µg/kg and nanoCr (200 µg/kg). Dietary Cr supplements did not influence the Cu retention.

With respect to the retention of Cr, Zn, Ca and P, the results of this study concurred with the findings of Sahin and Sahin (2002) and Sahin *et al.* (2002) in cold stressed layers supplemented CrPic (400 µg/kg) with 250 mg/kg ascorbic acid and Sirirat *et al.* (2013) in layers fed nanoCr at 500 ppb. The present study revealed that the nanoCr supplementation retained the minerals to a higher level followed by organic Cr sources which might be due to higher absorption efficiency of nanoCr than CrPic, CrProp and Cr chloride (Zha *et al.*, 2007).

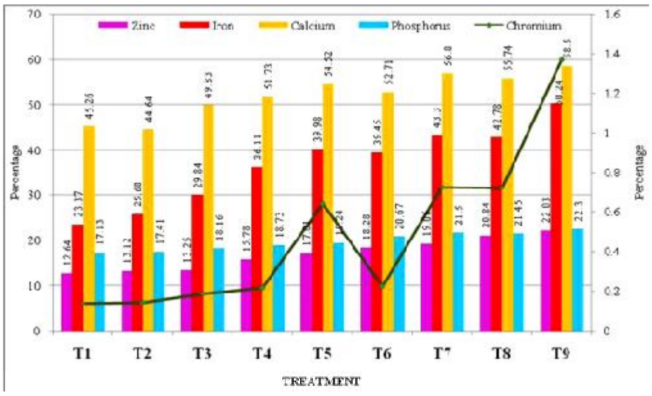

Fig. 2: Mean mineral retention (%) of layer chickens (22-34 weeks) fed inorganic, organic and nanochromium

It has also been reported that in stressed mice, loss of Zn and Fe was reduced by CrPic supplementation (Schrauzer *et al.*, 1986). The present result not only corroborated with the earlier finding but also indicated that Cr supplementation might help in reducing the level of supplementation of Zn, Fe, Ca and P in diet which might lead to better economic returns.

The concentration of Cr and Zn in plasma (Table 1) and liver (Table 2) were significantly ($P<0.05$) higher by nanoCr supplementation which was closely followed by organic Cr source compared to other sources and in liver the accumulation of Cr and Zn by organic Cr (200 µg/kg) and inorganic Cr at 400 µg/kg was similar whereas in plasma inorganic Cr source did not influence the Cr level.

Previous reports indicated that Cr supplementation led to increased Cr in serum of stressed laying hens (Sahin and Sahin, 2002), Japanese quails (Sahin *et al.*, 2004) and in pregnant rabbits (Sahin *et al.*, 2001). As Cr is involved in insulin function and Zn is a component of insulin, these two might interact with each other and hence increase in Cr concentration could have been accompanied by the increase in Zn levels.

The Ca level in liver was increased by nanoCr and organic Cr supplementation than inorganic Cr supplemented groups which concurred with the earlier studies of Sirirat *et al.* (2013) in layers.

The Cu concentration in plasma was significantly reduced ($P<0.05$) by nanoCr and organic Cr as compared to inorganic Cr but in liver, egg yolk and shell the level was not affected

($P>0.05$) by Cr source or level. Sahin *et al.* (2001) reported that Cr supplementation to pregnant rabbits decreased the Cu level in serum of pregnant does and their offspring and in stressed layers, fed Cr as CrPic (400 µg/kg) along with either 250 mg of vitamin C/kg (Sahin *et al.*, 2002), or 30 mg of zinc/kg (Onderci *et al.*, 2003). High levels of dietary Zn can impair the intestinal absorption of Cu (Starcher, 1969) as indicated by decreased plasma Cu level without any change in liver Cu levels (Table 4) and also Cu retention.

The Cr, Cu and Ca contents (Table 3) of yolk were not influenced by level and source of Cr supplementation. The observed effect of Cr supplementation on yolk minerals content concurred with those of Mabe *et al.* (2003) who reported that hen diets containing trace minerals, namely Cr, Zn, and Cu and the source (organic or inorganic) of trace mineral supplementation did not affect the yolk mineral contents. The Zn content in yolk was higher in

Table 4: Mean (±SE) shell minerals (µg/g dry mater) content of layer chickens (22-34 weeks) fed inorganic, organic and nanochromium

Treatment	Chromium	Copper	Zinc	Iron	Calcium	Phosphorus
T1-Standard diet	0.157[a] ± 0.072	3.20 ± 0.41	3.89[a] ± 0.69	50.34 ± 1.37	28.23[a] ± 1.15	0.048 ± 0.51
T2-Standard diet+200µg inorganic Cr /kg	0.169[a] ± 0.091	2.95 ± 0.35	4.19[a] ± 1.87	50.11 ± 1.24	28.50[a] ± 0.44	0.048 ± 0.42
T3- Standard diet+400µg inorganic Cr /kg	0.180[a] ± 0.084	3.29 ± 0.55	4.21[a] ± 0.64	51.16 ± 1.80	28.67[a] ± 0.27	0.052 ± 0.83
T4- Standard diet+200µg organic CrProp /kg	0.194[b] ± 0.102	3.21 ± 0.22	5.13[b] ± 0.89	51.26 ± 2.22	27.33[a] ± 0.51	0.050 ± 1.21
T5- Standard diet+400µg organic CrProp /kg	0.204[b] ± 0.090	3.24 ± 0.84	5.20[b] ± 1.17	52.89 ± 1.52	30.03[b] ± 0.28	0.052 ± 0.28
T6- Standard diet+200µg organic CrPic /kg	0.196[b] ± 0.041	3.27 ± 0.53	5.37[b] + 0.91	51.54 ± 2.25	28.17[a] ± 1.01	0.051 ± 0.81
T7- Standard diet+400µg organic CrPic /kg	0.207[b] ± 0.065	3.29 ± 0.32	5.56[bc] ± 0.73	52.59 ± 2.44	29.87[b] ± 0.31	0.053 ± 0.91
T8- Standard diet+200µg nanoCr/kg	0.212[b] ± 0.081	3.22 ± 0.65	5.18[b] ± 1.96	52.14 ± 0.89	29.67[b] ± 0.82	0.051 ± 0.95
T9- Standard diet+400µg nanoCr/kg	0.254[c] ± 0.112	3.30 ± 1.02	5.81[c] ± 1.27	53.91 ± 1.53	30.63[b] ± 0.37	0.053 ± 0.64

Each value is mean of six observations. Means with different superscripts in a column differ significantly (P<0.05).

nanoCr and organic Cr (400 µg/kg) supplemented group followed by nanoCr and organic Cr (200 µg/kg). The increased Zn concentration observed in the present work established with Sahin *et al.* (2004) who reported higher concentration of Zn together with Fe and Cr in yolk of quails that received CrPic at 8 mg/kg diet.

The Cr and Ca in egg shell (Table 4) were significantly (P<0.05) increased by supplementation of nanoCr and organic Cr compared to the inorganic Cr while the Zn content in shell was higher in nanoCr followed by organic CrPic (400 µg/kg) than other Cr supplements. Yenice *et al*. (2015b) reported that Cr and Zn concentrations were increased by the addition of organic than inorganic Cr source and Sirirat *et al.* (2013) reported only Ca accumulation in shell of laying hens that received trace mineral mixture and nanoCr respectively. The Fe and P levels in plasma, liver, egg yolk and shell were not influenced by Cr supplements.

Based on these results, it was concluded that supplementation of nanoCr at 400 µg/kg improved the retention of Cr, Zn, Fe, Ca and P while organic CrPic/CrProp at 400 µg/kg improved the retention of Cr and Zn in layer chickens. NanoCr and organic CrPic/CrProp (400 µg/kg) increased the concentration of Cr and Zn in plasma, liver and egg shell, Ca in liver and egg shell and Zn in egg yolk.

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