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Effect of organic chromium (chromium picolinate) supplementation on production parameters, egg quality attributes and serum biochemistry in Pearl guinea fowls during second laying cycle

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ABSTRACT

A biological experiment was carried out with adult Pearl guinea fowl variety (160) in second laying cycle to assess the performance, hemato-biochemical attributes, egg quality and stress levels. Each treatment consisted of four replicates with ten birds in each replicate. The birds were reared for 42 days under battery cage system and fed with *ad lib.* feed and water. The experiment groups were control group fed basal diet without any supplementation of chromium picolinate (CrP), T_1 fed basal diet with 500 ppb CrP, T_2 fed basal diet with 1,000 ppb CrP and T_3 fed basal diet with 1,500 ppb CrP. There was no significant change in body weight and feed intake due to supplementation of CrP. However, supplementation of CrP significantly reduced the erythrocytes osmotic fragility (%), serum glucose, total cholesterol and improved the egg internal and external quality traits. The supplementation of CrP significantly improved both the internal and external egg quality along with shell thickness. From this study, it can be concluded that supplementation of chromium as chromium picolinate @ 500 ppb improved the egg quality and reduced the stress in Pearl guinea fowl during second laying cycle.

Key words: Chromium picolinate, Egg quality, Laying cycle, Pearl guinea fowls, Production parameters

Chromium is an essential trace mineral in both the human and animals. It is the main component for glucose tolerance factor and it helps in activation of many enzymes for cellular proteins and nucleic acid stabilization. Organic form of chromium (chromium picolinate, chromium nicotinate and chromium rich yeast) had higher efficient absorption (25–30%) than the inorganic form of chromium (chromium chloride) which have less absorption of about 1–3% (Underwood and Shuttle 1999). Chromium acts as potential insulin activator and also acts as potent antioxidant in stressful condition (Mirfendereski and Jahanian 2015). Chromium helps to increase insulin level, it will useful to increase glucose uptake, in case if chromium in adequate in condition the insulin level will be low and its leads to less glucose utilization by the cells present in body which leads to fat deposition (Haq *et al.* 2016). In this situation, chromium act as antioxidant as it has the property of scavenging free radicals and depletes the fat cells in the body. Supplementation of chromium on some serum parameters (glucose, protein and cholesterol) have been appraise in different animals. The birds during their second egg laying cycle will be under physiological stress and the present study was conducted to evaluate the effect of chromium picolinate (CrP) supplementation on production parameters, blood attributes serum chemistry, egg quality and stress levels in guinea fowl.

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MATERIALS AND METHODS

Ethical approval: The biological experiment was carried out as per the Institute Animal Ethics Committee's (IAEC) approved schedule (Permission no.: 452/01/ab/CPCSEA).

Experimental birds, design and dietary treatments: Adult guinea fowls of Pearl variety in second egg laying cycle were used in the present study. A total of 160 adult healthy female birds of similar body weight were divided in four treatments. Each treatment consisted of four replicates with ten birds in each. The birds were reared for 42 days under battery cage system and offered *ad lib.* feed and water. The experiment groups were control group fed basal diet without supplementary chromium, T_1 fed basal diet with 500 ppb CrP, T_2 fed basal diet with 1,000 ppb CrP, T_3 fed basal diet with 1,500 ppb CrP. The birds were fed as per ICAR (2013) nutrient recommendations to meet their nutritional requirements. The CrP contained 12% chromium and was obtained from Himedia chemicals Ltd., Mumbai, India. The experimental feeds were assayed in duplicate (AOAC

Table 1. Ingredient and nutrient composition of the Guinea fowl breeder layer diet

Ingredient	Amount (kg)
Maize	59.50
De-oiled rice bran (DORB)	4.010
Soybean meal (CP 44%)	15.40
Gaur korma (CP 49%)	4.00
Rape seed meal (CP 38%)	2.00
Fish meal (CP 44%)	4.00
Oyster shell	3.00
Marble chips	4.00
Limestone powder	2.50
Di-Calcium phosphate	0.80
Salt	0.20
DL-Methionine	0.02
Trace mineral premix	0.15
Vitamin premix	0.15
B-Complex vitamins	0.015
Choline chloride	0.05
Toxin binder	0.075
Vitamin E + Selenium	0.02
Phytase (2500 FTU)	0.01
Liver tonic	0.025
B-complex vitamins	0.025
Vitamin C	0.005
Sodium bicarbonate	0.05
Total	100
Nutrient composition (*%*)	
Crude protein	16.24
Crude fibre	5.68
Calcium	3.84
Phosphorus	0.46
Metabolizable energy (kcal/kg)	2689

1995). The ingredient and chemical compositions of the experimental feeds are shown in Table 1. Change in body weight (initial and final stage of feeding), feed intake was recorded.

Biological sample collection and analysis: The blood samples were collected (10 birds per treatment) and haematological attributes, viz. red blood corpuscles count, white blood corpuscles count, haemoglobin, packed cell volume, mean corpuscular volume and erythrocyte osmotic fragility per cent (EOF%) were studied. All the blood parameters were analysed using Abacus Junior vet 5 (Abacus, USA) automatic blood analyser except EOF%. The EOF% was determined as per the modified Buffenstein *et al.* (2001) method.

Erythrocyte osmotic fragility% (EOF%): EOF% was determined by Dacie's method (Buffenstein *et al.* 2001) with following modification: Fresh heparinized blood (10 ml) was added to tubes containing 5 ml of 0.1, 0.5 and 0.9% phosphate-buffered saline. The tubes were mixed and incubated at room temperature (24°C) for 30 min. After mixing, the suspension was centrifuged at 530 rpm for 5 min. The supernatant was measured at 540 nm with a spectrophotometer, using the blood in 0.9% saline as a blank.

EOF was expressed as:

$$\text{Haemolysis rate (\%)} = \frac{\text{OD value at 0.5\% saline}}{\text{OD value at 0.1\% saline}} \times 100$$

Biochemical profile: Separated serum was subjected to blood biochemical test, viz. serum total protein, total cholesterol, triglycerides, calcium, phosphorus and aspartate transaminase (AST) by using standard commercial kits (Cogent, SPAN diagnostic Ltd, India). The serum protein was determined as per Lowry *et al.* (1951). Serum cholesterol react with hot solution of ferric perchlorate, ethyl acetate and sulphuric acid (cholesterol reagent) and resultant colour was measured at 560 nm (Wybenga *et al.* 1970). Triglycerides was estimated as per Bucolo and David (1973) method and absorbance was recorded at 505 nm. Serum AST and ALT activities were estimated by Reitman and Frankel's method (1957) by using kits procured from Coral Clinical Systems. Alkaline phosphatase (ALP) was estimated by modified Kind and King's method (1954) by using kits procured from Coral Clinical Systems. Uric acid was estimated by Uricase / PAP method by using the kits obtained from Coral Clinical Systems. Glucose content was estimated by the principle that glucose oxidase oxidises glucose to gluconic acid and hydrogen peroxide. In presence of enzyme peroxidase, released hydrogen peroxide is coupled with phenol and 4-aminoantipyrine to form coloured quinonenimine dye. Absorbance of coloured dye is measured at 505 nm and is directly proportional to glucose concentration in the sample.

Egg quality traits: Eight eggs per treatment were collected for a continuous period of two days for measuring various egg quality traits, viz. egg weight (g), volume (ml), egg length and width (mm), albumen length (mm), albumen width (mm), albumen height (mm), yolk colour, egg shell colour, shell thickness and egg shell weight, shape index, albumen index, yolk index.

Specific gravity: The specific gravity of an egg is equal to the egg's density relative to water. The specific gravity of egg is calculated at equals the weight of its volume relative to the weight of an equal volume of water.

Albumen height and width: After breaking the egg on a smooth surface, the height (mm) of thick albumen was measured at three different points with the help of AMES spherometer and their average was worked out. Similarly, with the help of Vernier Calliper, the width of the thick albumen from three different places was measured and average width was calculated.

Yolk height and width: The spherical nature of yolk can be expressed as yolk index on the separation of yolk and white, keeping the yolk intact. After measuring the albumen height, the yolk was carefully separated from the albumen with a blunt knife and then the height of the yolk was recorded by AMES micrometer and the width of the yolk was recorded by Vernier calliper.

Egg shell thickness (*mm*)*:* The egg shell thickness (mm) was measured with the help of AMES micrometer gauge at three points, the narrow, middle and broad end of the egg.

Table 2. Effect of graded levels of chromium picolinate supplementation on haematological attributes in Pearl guinea fowls in second laying cycle

Parameter	Control	T_1	T_2	T_3	P value
Red blood cell ($\times10^6$)	01.19±0.04	01.27±0.07	01.19±0.06	01.16±0.06	0.578
Haemoglobin (g/dl)	14.65±0.58	12.25±0.67	13.89±0.39	13.73±0.33	0.682
Packet cell volume (%)	43.95±1.73	36.75±2.01	41.67±1.18	41.20±1.00	0.439
Mean corpuscular volume (fl)	134.42±2.95	141.00±1.46	138.67±1.93	136.50±1.29	0.131
White blood cell ($\times10^3$)	24.98±1.27	25.07±0.53	22.46±1.22	21.23±1.58	0.074
Lymphocytes ($\times10^3$)	21.30±1.36	22.68±1.63	19.60±1.07	20.93±1.44	0.453
Heterophils ($\times10^3$)	02.79±0.24	02.97±1.13	02.74±0.80	02.16±0.26	0.202
Heterophil: Lymphocyte ratio	0.14±0.02	0.13±0.11	0.13±0.05	0.12±0.03	0.240
Erythrocyte osmotic fragility %	57.98^{b}±5.08	25.62^{a}±2.05	30.81^{a}±1.62	25.12^{a}±1.74	0.000

^{a,b,c}Means within column bearing different superscript differ significantly (P<0.05).

Table 3. Effect of graded levels of chromium picolinate supplementation on serum biochemistry in Pearl guinea fowls in second laying cycle

Parameter	Control	T_1	T_2	T_3	P value
Plasma glucose (mg/dl)	243.60^{b}±9.84	204.28^{a}±24.02	197.77^{a}±26.14	221.07^{a}±17.50	0.025
Plasma cholesterol (mg/dl)	223.00^{b}±16.64	191.79^{a}±9.48	194.95^{a}±14.08	189.61^{a}±8.94	0.000
Plasma triglycerides (mg/dl)	194.18±8.26	159.82±22.74	217.30±25.50	146.98±37.73	0.215
Plasma total protein (mg/dl)	04.74±0.12	04.60±0.13	04.85±0.05	04.59±0.08	0.242
Plasma calcium (mg/dl)	09.21±0.32	08.64±0.36	08.57±0.43	08.63±0.54	0.676
Plasma phosphorus (mg/dl)	04.34±0.37	04.27±0.17	04.43±0.23	04.83±0.37	0.549
Plasma alanine transferase (U/l)	178.28^{c}±7.70	108.48^{a}±2.17	107.36^{a}±2.03	138.32^{b}±4.13	0.000
Plasma asparatate transferase (U/l)	161.49^{b}±2.62	148.09^{ab}±5.72	142.60^{ab}±10.76	131.42^{a}±6.37	0.033
Plasma alkaline phosphatase (KA units)	04.24^{ab}±0.14	04.52^{b}±0.27	04.56^{b}±0.26	03.61^{a}±0.14	0.009
Plasma uric acid (mg/kg)	05.37^{c}±0.07	02.71^{ab}±0.24	02.29^{a}±0.24	03.35^{b}±0.20	

^{a,b,c}Means within column bearing different superscript differ significantly (P<0.05).

Table 4. Effect of graded levels of chromium picolinate supplementation on external and internal egg qualities in Pearl guinea fowls

Parameter	Control	T_1	T_2	T_3	P value
Egg weight (g)	42.83±0.41	43.63±0.41	43.24±0.59	44.44±0.59	0.155
Specific gravity	35.00±1.51	40.00±1.51	38.33±0.71	39.17±1.72	0.081
Albumin length (mm)	69.13±1.51	70.93±1.51	71.92±1.83	73.02±1.83	0.418
Albumin width (mm)	52.18^{a}±2.07	54.28^{ab}±2.07	59.34^{bc}±1.10	61.14^{c}±1.10	0.001
Yolk diameter	41.28±2.52	42.18±2.52	39.72±0.59	40.93±1.71	0.853
Albumen height (mm)	03.33^{ab}±0.47	04.53^{b}±0.47	02.13^{a}±0.12	02.93^{a}±0.12	0.000
Yolk height	13.92^{b}±0.07	16.22^{c}±0.07	13.80^{b}±0.18	12.79^{a}±0.18	0.000
Yolk colour	08.25^{b}±0.25	08.17^{b}±0.22	07.37^{a}±0.11	07.25^{a}±0.18	0.000
Shell weight (g)	05.27^{a}±0.21	06.30^{b}±0.23	06.15^{b}±0.20	06.44^{b}±0.22	0.004
Shell thickness (mm)	0.42^{a}±0.01	0.61^{b}±0.01	0.64^{b}±0.01	0.62^{b}±0.01	0.000
Egg cholesterol (mg/dl)	272.99^{b}±26.13	257.95^{a}±14.53	255.26^{a}±12.95	258.95^{a}±13.01	0.048

^{a,b,c}Means within column bearing different superscript differ significantly (P<0.05).

Yolk colour: Roche yolk colour fan with numerals was used to determine the yolk colour.

Egg cholesterol content: The total lipid was extracted from egg samples as per the method suggested by Folch *et al.* (1957). The extracted egg cholesterol was estimated by one-step method of Wybenga *et al.* (1970).

Statistical analysis: Data emanated from different treatments were analyzed for statistical significance using completely randomized design (CRD) by following standard methods (Snedecor and Cochran 1989). Variables having unequal observations were analysed following least square design method and the Tukey's test.

RESULTS AND DISCUSSION

The overall body weight and feed intake did not show any significant difference (P>0.05) between control and treatment groups. Similar to the present findings, Debski *et al.* (2004) reported no significant difference in body weight and feed intake among broilers fed up to 1g Cr rich yeast per kg feed. This also in accordance with the findings

of other researchers that supplementation of CrP @ 250, 500, 1,000 and 1,500 ppb did not have any effect on the body weight, body weight gain and feed consumption in broilers (Hanafy 2011), laying hens (Eseceli *et al.* 2010) and laying quail hens and broiler chicken (Moeini *et al.* 2011, Rama Rao *et al*. 2012). On the contrary, supplementation of both the form of Cr (organic and inorganic) at different doses (500, 1,000 and 1,500 ppb) to broiler chicken increased body weight gain and feed consumption particularly higher dose of chromium (1,500 ppb) in heat stressed condition (Toghyani *et al.* 2012, Norain *et al.* 2013). This indicated that supplementation of CrP to heat stressed birds reduced the negative effects of heat stress. However, in the present study, the birds were maintained under normal condition and were not under heat stress.

In blood metabolites, the results revealed significant reduction in erythrocyte osmotic fragility (EOF%) in all the three levels of chromium supplementation than that of control (25.62, 30.81, 25.12 vs. 57.98%). However, no significant difference was observed in erythrocyte count (1.16 to 1.27 × 10^6/ml), haemoglobin (12.25 to 14.65 g/dl) level, packed cell volume (36.75 to 43.95%), white blood cells, lymphocytes, heterophils and heterophil: lymphocyte ratio. These results were in accordance with findings of Toghyani *et al.* (2012) who reported that supplementation of different sources (organic and inorganic) and different levels (500, 1000 and 1500 ppb) of chromium showed no significant difference on blood parameters (white blood cell count, red blood cell count, hematocrit percentage, hemoglobin concentration and blood indices MCV, MCH and MCHC). The parameter EOF% reveals the degree of erythrocyte membrane extensibility and cell geometry (Gharaibah *et al.* 1993). A lower EOF% value indicates that cellular membrane and structures were stable to oxidative stress. As the bird's age increases the fragility of erythrocytes membrane do show a linear increase. The dietary supplementation of Cr as CrP reduces the osmotic fragility of erythrocytes due to their antioxidant activity.

In plasma, there was significant reduction in the glucose (204.28, 197.77, 221.07 vs 243.60 mg/dl), cholesterol (191.79, 194.95, 189.61 vs 223.01 mg/dl), alanine, aspartate transferase, alkaline phosphatase and uric acid. Reduction in serum glucose might be due to increased insulin activity. Supplementation of Cr is helpful in improving the lipid and carbohydrate metabolism (Nam *et al.* 1995). But there was no significant difference in total protein, triglycerides, calcium and phosphorus. This result was in accordance with findings of Hanafy (2011), Moeini *et al.* (2011) and Attia *et al.* (2015) who fed different concentration of chromium (0, 250, 500, 1,000 and 1,500 ppb) to broiler chicken and observed that the glucose and total cholesterol concentrations decreased (Sahin and Onderci (2002) and Lee *et al.* (2003)) but in contrary to our results, reported significantly increased total protein and calcium concentration.

The results also revealed significant increase in albumin width (54.28, 59.34, 61.14 vs. 52.18 mm), height, egg shell weight (6.30, 6.15, 6.44 vs. 5.27 g) and thickness (0.61, 0.64, 0.62 vs. 0.42 mm). There was significant reduction in egg yolk cholesterol (257.95, 255.26, 258.95 vs. 272.99 mg/dl) and yolk colour (8.17, 7.37, 7.25 vs. 8.25). But no significant difference was observed in egg weight (42.83 to 44.44 g), specific gravity and albumin length and yolk diameter. The results were similar to finding of Hanafy (2011) who reported that supplementation of graded levels of Cr (250, 500, 1,000 and 1,500 ppb) to laying hens, significantly increased the albumen percentage, egg shell percentage, egg shell thickness, haugh unit and yolk index at higher level. This results was in agreement with the finding of Kucukersan *et al*. (2005) in laying hens. Cr maintain normal physical state of albumin in such a way it is necessary for the synthesis of ovomucin for albumin gel like structure and linking proteins as well as reduces the yolk cholesterol which could be attributed to presence of lower level of yolk in egg. It increases the insulin activity, which is helpful in ascorbic acid transportation at the time of egg shell formation. This also explained the mechanism behind increase in egg shell thickness due to CrP supplementation and especially to older birds where the calcium deposition on egg shell is inversely correlated with the age. The supplementation of chromium as chromium picolinate @ 500 ppb improved the performance, blood biochemical characteristics and reduced the stress during second laying cycle in Pearl variety guinea fowls.

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