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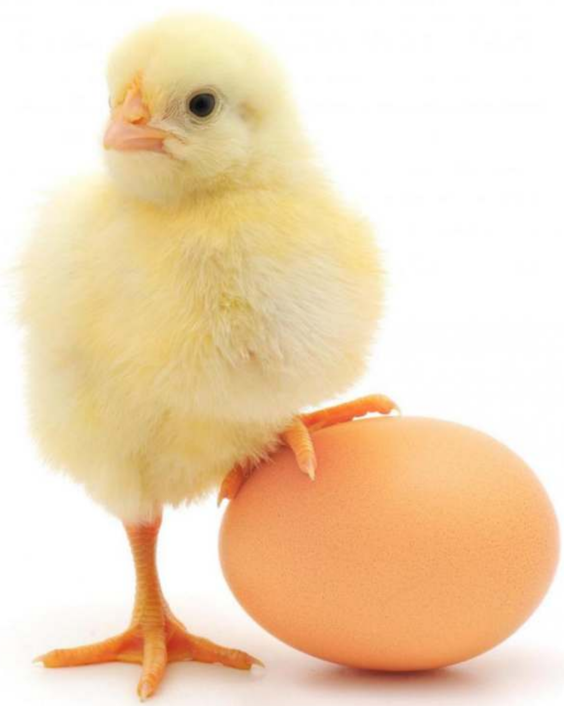

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Study of the Physiological Changes in Blood Chemistry, Humoral Immune Response and Performance of Quail Chicks Fed Supplemental Chromium

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Abstract: One hundred and fifty 2-week old Japanese quail chicks were allotted to 5 groups (30 chicks each) to investigate the effects of adding inorganic chromium (Cr) supplied from chromium chloride ($CrCl_3$, $6H_2O$) on some blood components, humoral immune responses and growth performance of growing quails. The dietary treatment was different dietary concentrations of Cr in the basal diet, to provide 0.0 (control), 125, 250, 375 and 500 µg / kg feed. Ten chicks per group, at 4 and 6 wk of age were injected intramuscularly with sheep red blood cells (SRBCs) to determining the primary and secondary antibody responses, respectively. Chromium supplementation significantly decreased the plasma levels of glucose, total cholesterol and total lipids, whereas total protein, globulin, AST and ALT increased. Plasma albumin concentration slightly but not significantly also increased. Dietary Cr caused a significantly increased in plasma level of Ca., but did not affect the plasma concentrate of P. In addition, supplementation with Cr significantly improved body weight, weight gain and feed conversion ratio compared to control. However, Cr administration did not affect the relative weights of any organ studied. With respect to humoral immune responses the results indicated that total antibody and IgG titers against SRBCs were significantly higher in chicks receiving Cr at low (125 µg/kg feed) and middle (250 µg/kg feed) doses compared with those of control at secondary immune responses. The results suggest that under condition of this experiment, Cr may play a part in carbohydrate; lipid and proteins metabolism in quail chicks and can used as additives in grower quail diets without side effects on the health of the bird.

Key words: Immunity, growth performance, quail, humoral response, chromium

Introduction

In spite of their long term practical impact on production, reproduction and immunity, a few trace elements have not received much attention in formulating animal diets (Prasad and Gowda, 2005). These elements are present in ultratrace concentration and their essentiality is not yet completely established. For example, poultry diets may deficient in chromium (Cr), because Cr concentration of plant products is low and it losses as a result of grinding of grains largely used in poultry diets (Uyanik *et al.*, 2002). No recommendation on the Cr requirement of poultry has been made (NRC, 1997). Chromium toxicity associated mainly with hexavalent chromium, however trivalent chromium such as chromium chloride ($CrCl_3$), is believed to be highly safe mineral (Barcelous, 1999). Trivalent chromium is in fact lower than the toxicity of other essential elements such as Cu., I., Zn., Mn. and Se. (Lindemann, 1996). It tends to accumulate in epidermal tissues, bone, liver, kidney, spleen, lungs and large intestine, while accumulation in other tissues especially muscles, seems to be limited or non existent (Whallach, 1985 and Jamal *et al.,* 1991). Previous studies revealed Cr essential role for regulation of carbohydrate, lipid, protein and nucleic acid metabolism via an enhancement of insulin action (Kroliczewska *et al.,* 2004 and Pechova and Pavlata, 2007). Chromium increases the number of insulin receptors on the cell surface and sensitivity of pancreatic β-cells simultaneously with increase of insulin-sensitivity (Anderson, 1997). So Cr act as glucose tolerance factor increases the uptake of glucose by cells. It is also improves amino acids uptake by muscle cells increasing the total protein deposition as well as decreases the total lipid and total cholesterol in blood results in increased proportion of muscles compared with fat (Kim *et al.,* 1996; Eren and Baspnar, 2004 and Yldz *et al.,* 2004). Chromium deficiency in diet may results in slow growth, impaired glucose tolerance, diabetes and coronary artery diseases (Abrham *et al.*, 1991). On the other hand, Cr supplementation support of the immune function by enhancement the cell-mediated and humoral immune responses (Lee *et al.*, 2003; Li *et al.*, 2004 and Lien *et al.*, 2005). Although Cr is believed to have immunomodulatory effect, the mechanism of action is less clear and the results of the studies were inconsistent (Uyanik *et al.,* 2002 and Pechova and Pavlata, 2007). Recently, and according to last review of literature investigated the role of Cr in animal livestock. Pechova and Pavlata (2007) illustrated that the chromium supplementation improved performance in some investigation (40 to 50% of

Table 1: Performance of quail chicks fed diets supplemented with different levels of chromium.

Cr levels µg/kg	Body weight (g) Initial	4 wk	7 wk	Weight gain (g) 2-4 wk	2-7 wk	FCR 2-7 wk
0	26.93 ± 0.60	112.84 ± 2.28^{b}	200.52 ± 3.99^{b}	85.91 ± 2.92^{b}	173.59 ± 3.91^{b}	4.39 ± 0.29^{a}
125	28.04 ± 0.29	152.25 ± 2.83^{a}	237.59 ± 6.43^{a}	124.21 ± 4.82^{a}	209.55 ± 6.42^{a}	3.84 ± 0.12^{b}
250	26.94 ± 0.78	149.34 ± 3.27^{a}	234.92 ± 5.26^{a}	122.40 ± 3.26^{a}	207.18 ± 6.56^{a}	4.16 ± 0.37^{ab}
375	27.75 ± 0.38	153.53 ± 2.79^{a}	240.52 ± 4.07^{a}	125.78 ± 2.79^{a}	213.17 ± 5.25^{a}	4.05 ± 0.39a^{ab}
500	26.28 ± 0.71	154.29 ± 2.71^{a}	228.65 ± 6.66^{ab}	128.01 ± 3.71^{a}	202.37 ± 4.07^{a}	4.28 ± 0.22^{a}
Sig.	NS	**	**	**	**	**

$^{a, b}$Means within column with no common superscript differ significantly (P<0.001). NS = not significant

Table 2: Relative percentage weights of dressing and some organs of quail chicks fed diet supplemented with chromium

Cr level µg/kg	Variable: Breast muscles Dressing	Major	Minor	Internal organs Liver	Kidney	Heart	Gizzard	Pancreas
0	69.25±1.24	7.66±0.61	2.31±0.33	2.44±0.21	0.62±0.03	0.82±0.03	2.51±0.15	0.34±0.07
125	69.19±2.72	7.44±0.29	2.11±0.21	2.67±0.23	0.72±0.05	0.89±0.03	2.30±0.13	0.30±0.03
250	70.18±3.41	8.36±0.40	2.32±0.17	2.84±0.24	0.60±0.05	0.84±0.04	2.76±0.23	0.27±0.03
375	67.74±3.39	7.57±0.41	2.14±0.42	2.81±0.33	0.60±0.06	0.83±0.06	2.13±0.31	0.33±0.03
500	66.89±2.39	6.98±0.46	2.08±0.39	2.85±0.24	0.68±0.04	0.79±0.01	2.38±0.01	0.30±0.02
Sig.	NS	NS	NS	NS	NS	NS	NS	NS

NS = not significant.

experiments), but did not in others. Because Cr can influence on carbohydrate, lipid and protein metabolism, which may be economically beneficial in poultry production, hence using Cr as a feed additives in poultry diets still need more investigation. This study was conducted to evaluate the effect of Cr supplementation in the form of chromium chloride ($CrCl_3$) on blood chemistry, immune responses and growth performance in quail chicks.

Materials and Methods

Experimental procedures: Two-week old (150 chicks) Japanese quail weighing 26 to 28 gm were randomly distributed into 5 equal groups (30 chicks each) and housed in a starter battery. Chicks in control group was fed on the basal diet (commercial quail chick starter diet) planned to meet the nutrient requirement of grower quail as recommended by NRC (1994). The basal diet was supplemented with 125, 250, 375 and 500 µg Cr/kg as a chromium chloride ($Cr\ Cl_3$. $6H_2O$) and fed to group 2, 3, 4 and 5, respectively tell the end of the study (at 7 wk of age). Feed and water were available *ad lib*. Chicks were maintained on a light cycle of 24 hr until 4 wk of age, at which time the light cycle was changed to 16 L:8D.

Data collection and chemical analysis: Body weight and feed consumption were measured weekly. Feed efficiency was calculated by dividing the weekly feed consumption by weight gain. Sheep red blood cells (SRBCs) were used as T-dependent antigens to quantify the antibody response. At 4 wk of age 10 chicks per group were injected intramuscularly with SRBCs (40% suspension in PBS, 0.5 ml/bird) followed by a booster injection of SRBCs suspension at 6 wk of age. Blood samples were collected at 7 day after each injection. The plasma from each sample was harvested and stored at -20°C until tested. Plasma was heat inactivated at 56°C for 30 min and then analyzed for total, mercaptoethanol-sensitive (MES) IgM and mercaptoethanol-resistant IgG anti–SRBC antibodies as previously described (Yamamato and Glick, 1982; Qureshi and Havenstein, 1994 and Fathi *et al.,* 2003). Titers were expressed as the log 2 of the reciprocal of the highest dilution giving complete agglutination. The difference between the total and IgG response was considered to be the IgM antibody level. At the end of the experiment, randomly 15 birds from each group were individually weighed then slaughtered by severing the carotid artery and jugular veins and necropside. Dressing, heart, liver, kidney, gizzard, pancreas, breast muscles, spleen and bursa were measured as a percentage of live body weight. Blood samples were collected at slaughtering in heparinized tubes. Plasma were separated by centrifugation at 4000 rpm. for 15 min and stored at -20°C until assay. Plasma total protein, albumin, total lipid, total cholesterol, aspartate aminotransferase (AST), alanine amino transferase (ALT), calcium, phosphorus and glucose were determined using commercial kits (Spinreact, S.A. Spain). Globulin was calculated by subtraction of plasma albumin from total protein.

Statistical analysis: Data were subjected to a one-way analysis of variance using the General Linear Models (GLM), procedure of SAS User's guide (2001). When significant difference among means was found, means were separated using Duncan's multiple range test.

Table 3: Effect of chromium chloride supplementation on immune responses and relative weights of bursa and spleen in quail chicks

Cr level	Variable							
	Lymphoid organs		Primary immune response			Secondary immune response		
μg/kg	Spleen	Bursa	Antibody titer	IgG	IgM	Antibody titer	IgG	IgM
0	0.07±0.01	0.08±0.09	3.00±0.55	1.40±0.25	1.60±0.40	3.40±0.25[b]	2.90±0.44[b]	0.50±0.05
125	0.08±0.02	0.08±0.01	3.75±0.48	1.25±0.34	2.50±0.29	5.45±0.50[a]	4.60±0.24[a]	0.85±0.02
250	0.08±0.03	0.09±0.02	3.40±0.25	1.00±0.32	2.40±0.51	4.80±0.37[a]	4.00±0.49[a b]	0.80±0.02
375	0.09±0.02	0.09±0.03	3.40±0.58	1.20±0.25	2.20±0.47	4.47±0.51[a b]	3.60±0.47[a b]	0.87±0.01
500	0.08±0.04	0.09±0.04	3.20±0.58	1.20±0.30	2.00±0.55	4.00±0.55[a b]	3.22±0.25[a b]	0.78±0.03
Sig.	NS	NS	NS	NS	NS	*	*	NS

[a, b]Means within column with no common superscript differ significantly (P<0.05). NS = not significant.

Results and Discussion

The effect of chromium chloride supplementation on growth performance of quail chicks was determined on the basis of live body weight, weight gain and feed conversion ratio. Data in (Table 1) illustrated that Cr supplementation was associated with a significantly (P<0.001) increase in body weight and weight gain as well improved feed conversion ratio than in the control group. Improvement of feed conversion ratio was only significant in chicks receiving Cr at low (125 μg/kg feed) dose compared with the control. These results are consistent with previous studies (Chen *et al*., 2001; Eren and Baspnar, 2004; and Kroliczewska *et al*., 2004). in which it has been reported that Cr supplementation to various poultry diet improved body weight, body weight gain and feed conversion ratio. This improvement in growth performance might be due to that the activity of Cr is mediated by the anabolic action of insulin. Evans and Bowman (1992) have demonstrated that Cr supplementation increased amino acids and glucose uptake by skeletal muscles. The potential improvement of amino acids uptake by muscle cells is beneficial to the total protein deposition in meat (Kim *et al*., 1996 and Pechova and Pavlata, 2007).

Although Cr promoted growth rate, no significant differences were observed among the groups fed on different levels of Cr and the control one with respect to the relative weights of dressing or the other organs (Table 2). Slight but not significant increases were observed in chicks receiving 250 μg/kg feed Cr in the relative weights of dressing and breast muscles. The present data is agreed with the finding of (Lu *et al*., 2002 and Fiaz *et al*., 2004). However (Mizanul *et al*., 2002 and Uyanik *et al*., 2002). indicated that Cr supplementation increased the ratio of liver and kidney to body weight of broiler chicks. The variation between the results may be due to differences in the Cr supplementation or due to the different species. A slight but not significant increase also was observed with supplemental Cr in the relative weights of spleen and bursa (Table 3). Similar results have been reported by Uyanik *et al*. (2002) who found that chromium chloride increased the ratio of bursa and spleen to body weight of broiler. Mizanul *et al*. (2002) found that the ratio of bursa and spleen to body weight of hexavalent Cr-treated birds was lower than those of control (the lower weight could be attributed to the highly toxicity of hexavalent Cr).

With respect to humoral immune responses, the results (Table 3) indicated that total antibody titers against SRBCs were increased in chicks receiving different level of Cr as compared to the control. These effects were more pronounced (P<0.05) in chicks received low and middle Cr (125 and 250 μg/kg feed) dose at the secondary immune responses. Similarly to our results, Cao *et al*. (2004) and Lee *et al*. (2003) investigated that total antibody tended to be higher (P<0.05) in Cr added groups. Immunoglobulin, IgG and IgM titers to SRBCs also tended to increase with adding Cr to the basal diet and the effect was more marked (P<0.05) in chick receiving the low (125 μg Cr/kg) dose at secondary immune response. In the present study, it appeared that adding Cr to quail chick diet have immunomodulatory effects supported by the increase in the total antibody, IgG and IgM titers (improving primary and secondary immune responses) as well as the slight increases in the relative weights of bursa and spleen.

With regard to blood constituents (Table 4) plasma glucose, total lipids and total cholesterol decreased (P<0.01), while total protein and globulin concentration increased (P<0.01) linearly as dietary Cr levels increased. However, plasma albumin value slightly but not significantly increased in all Cr groups compared with control. These results are consistent with previous studies (Kroliczewska *et al*., 2004 and Wang *et al*., 2003) who reported that plasma glucose and cholesterol values decreased whereas plasma total protein increased by Cr supplementation. Uyanik *et al*. (2002) obtained comparable results. They found that Cr did not affect serum cholesterol, but reduced serum glucose and increased serum protein, albumin and globulin. Other studies using broilers have reported either that Cr supplementation increased serum cholesterol (Lee *et al*., 2003) or has no effect on cholesterol and protein (Chwen *et al*., 2002). According to Prasad and Gowda (2005) Cr act as glucose tolerance factor increased

Table 4: Effects of chromium chloride supplementation on some blood constituents of quail chicks

	Cr level (µg/kg)					
Variable	0	125	250	375	500	Significance
Total protein (g/dl)	3.33±0.15[b]	5.25±0.49[a]	5.75±0.40[a]	6.40±0.73[a]	6.70±0.60[a]	**
Albumin (g/dL)	1.26±0.03	1.44±0.16	1.69±0.16	1.42±0.10	1.54±0.15	NS
Globulin (g/dl)	2.07±0.13[b]	3.81±0.39[a b]	4.06±0.42[a]	5.17±0.66[a]	5.15±0.62[a]	**
Total lipid (mg/dl)	1.19±0.04[a]	0.91±0.04[b]	0.76±0.01[c]	0.67±0.05[c d]	0.55±0.03[d]	**
Cholesterol (mg/dl)	251.42±4.26[a]	231.94±3.61[b]	227.85±4.31[b]	220.99±7.90[b]	196.67±6.30[c]	**
AST (U/l)	36.57±1.20c	41.68±1.99[bc]	44.10±1.62[bc]	48.64±3.53[a,b]	54.08±3.07[a]	**
ALT (U/l)	21.02±0.26[b]	28.85±0.85[a]	28.90±0.84[a]	31.61±0.45[a]	32.37±1.86[a]	**
Glucose (mg/dl)	217.31±5.49[a]	188.63±7.28[b]	182.79±5.26[b]	159.89±6.74[c]	147.88±4.81[c]	**
Phosphorus (mg/dl)	6.66±0.46	5.82±0.85	5.30±0.44	5.39±0.73	5.12±0.39	NS
Calcium (mg/dl)	14.91±0.34[b]	15.43±0.62[b]	18.60±0.53[a]	18.39±0.25[a]	19.48±0.99[a]	**

[a, b, c, d] Means within row with no common superscript differ significantly ($P<0.001$). NS= not significant

the uptake of glucose by cells and potentiated insulin action. The reduction effect of Cr on plasma glucose in the present study may be support this suggestion. On the other hand Uyanik *et al.* (2002) attributed the positive effects of Cr on plasma protein and its fractions to the anabolic action of insulin mediated through increasing the amino acids synthesis by liver, enhancement the incorporation of several amino acids into protein. However, Mc Namara and Valdez (2005) suggested that effect of Cr on lipid metabolism may be due to that Cr increased the synthesis of fat in the adipose tissue and decreased the release of it. This might be acting through increased glucose flux into the adipocytes.

Generally, the results showed that adding Cr had better regulative effects on glucose, lipids and proteins metabolism. Data for AST and ALT enzymes as indicator of liver function are presented in (Table 4). Dietary Cr supplementation significantly ($P<0.01$) increased plasma levels of AST and ALT. The highest value for AST was obtained when 500 µg/kg feed Cr was adding. It is suggested that excessive Cr intake may be affect liver functions. These results are in harmony with those obtained by Eren and Baspnar (2004) who found that chromiune chloride administration significantly increased serum AST.

Increasing dietary Cr levels caused an increase in plasma concentration of Ca ($P<0.01$), but a decrease in plasma concentration of P (not significant). This agreed with the finding of Lee *et al.* (2003) who reported that serum Ca was higher and P was lower as affected by Cr supplement. Sahin and Onderci (2002) found that adding Cr to broiler diet increased either serum Ca and P. A possible explanation for the effect of Cr supplementation on Ca metabolism may be due to that these two minerals (Cr and P) compete for the same binding sites, so increased Cr concentration causing a decrease in freeing of binding sites on the transferring, competed for by the individual minerals.

In conclusion, and based on these results, $CrCl_3$. $6H_2O$ supplementation improves body weight gain and feed conversion ratio, but have no effects on the relative weights of any organ studied. Cr supplementation also supports of the immune function through enhancement the humoral immune responses. Furthermore, Cr administration may influence the metabolism of glucose, lipids, proteins, Ca and P. Data revealed no obvious influence of Cr on the health of quail chicks, except that the highest values (375 and 500 µg/kg feed) of it increased the plasma levels of AST and ALT. Because Cr have additives effects on growth performance and improves immunity, diet supplemented with Cr up to 250 µg/kg feed may be economically beneficial to grower quails but still need more investigation. Further researches should focus primarily on determining daily requirements of Cr in different poultry species.

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