EFFECTS OF HIGHER LEVELS OF CHROMIUM AND COPPER ON SOME HAEMATOLOGICAL PARAMETERS AND SERUM PROTEINS IN BROILERS

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ABSTRACT

Effects of higher levels of chromium alone and in combination with copper were investigated in broiler chicks divided into seven equal groups viz. A, B, C, D, E, F and G. Group G served as control receiving no treatment. Groups A, B and F received chromium chloride at the rate of 2g/kg and nicotinic acid 150 mg/kg feed, while C, D and E received chromium chloride 8 g/kg and nicotinic acid 150 mg/kg. Broilers of groups A and C received copper sulfate at the rate of 200 mg/kg while groups B and D 400 mg/kg feed. Haematological parameters studied revealed non-significant difference between treatment groups and control in haemoglobin concentration and total erythrocyte count. However, only at 4th week, lower PCV was observed in birds fed higher levels of chromium chloride alone. Increase in TLC was observed in birds fed low chromium alone or with low levels of copper. Results of serum proteins including total protein, albumin and globulin during first three weeks showed significantly or relatively lower values in treatment groups than control. Serum globulins generally revealed non-significant difference between treatment groups and control.

Key words: Chromium, nicotinic acid, broiler, copper, serum proteins, albumin, globulins, haematology, erythrocyte, leukocyte, PCV.

INTRODUCTION

Studies have confirmed that chromium has a critical role in metabolism and is essential for carbohydrate and lipid metabolism both in mammals and birds. Researchers first identified trivalent chromium (Cr^{+3}) as a primary active component of glucose tolerance factor (GTF), which make the metabolic action of insulin more effective (Schwartz and Mertz, 1957). Low-molecular-weigh: chromium-binding protein, a biologically active form of chromium in mammals, potentiates the effect of insulin on the conversion of glucose into lipid and carbon dioxide in isolated adipocytes (Davis *et al.* 1996). Dietary chromium supplementation did not significantly influence other serum constituents, including insulin, serum lipoproteins, total protein, albumin, and gamma-globulin at 18 and 22 weeks aged chicken (Chen *et al.* 2001) although it improved the livability and decreased the mortality in birds and animals (Wallach, 1985). In another study in broilers Cr supplementation showed non-significant effect on serum albumin concentration (Kalaycioglu *et al.*, 1999). Studies on haematological parameters revealed increase in PCV in calves (Moonsie and Mowat, 1999) in addition haemoglobin and erythrocyte count in carp (Al-Akel and Shamsi, 1996).

The use of copper at 200 mg / Kg in white leghorn hens has shown a positive response, while levels of 400 mg / Kg and above has shown a progressively negative response (Chiou *et al.*, 1997) and has significant effect on various serum enzymes in broilers (Koh *et al.* 1996; Chen *et al.*, 1997) and reported to cause an increase in haemoglobin level and also has toxins binding capacity (Wu *et al.*, 1988). However, Ward *et al.* (1995) reported no effect of copper on PCV and Hb in chicken, while caused decrease in Hb in ewes (Eckert *et al.*, 1999). Studies in chicken showed no effect of copper on serum total protein (Chen *et al.*, 1997), however, showed significant increase in total protein and albumin in rabbits during heat stress period (Ayyat *et al.*, 1997).

Data about these compounds show quite variation in effects on different parameters in various species especially haematology and serum proteins. Present study was therefore planned to investigate effects of chromium and copper at much higher levels on these parameters in particular of chromium in broilers.

MATERIALS AND METHODS

A total of 175 day-old broiler chicks were purchased from local market (High Tech), were randomly divided into seven equal experimental groups from A to G and were kept under identical conditions of feeding and management in wire cages. After one week of age all the groups, were given experimental feed except group seven (G) which was kept as control and was given basal feed (Table 1). Basal feed was the commercial feed (National

Feed) purchased from local market and experimental feed was also prepared by using the same feed mixed with various levels of compounds under study. The study was carried out for five weeks and at the end of each week, five birds were humanely slaughtered from each group including the control for blood and serum collection. Chromium chloride ($CrCl_3.6H_2O$; molecular wt. 266.47; No. 27752, Xingtu, China), nicotinic acid (No. 72310; Fluka Chemika) with 99 percent purity and copper sulphate ($CuSO_4.5H_2O$; No. 7631, Fluka) were used in the study.

Table 1: Broilers receiving various treatments during experimental trials through feed.

Groups	Chromium Chloride g/kg	Nicotinic Acid mg/kg	Cooper sulphate mg/kg
A	2	150	200
B	2	150	400
C	8	150	200
D	8	150	400
E	8	150	---
F	2	150	---
G	---	---	---

All the experimental groups received treatment for four weeks, while during 5th week all the groups were given basal diet to study the withdrawal effect. The experiment was conducted during summer season (June and July) with maximum temperature range from 40-45°C and lowest between 35-38°C which can easily be stated as heat stress period under tropical conditions.

Blood samples were collected with and without anticoagulant after slaughtering birds. Blood collected with anticoagulant was used for haematology including Hb (Spectrophotometrically, Benjamin, 1978), PCV (haematocrit tube method), TLC and total erythrocyte count (Nat and Herrick, 1952) and that collected without anticoagulant was used for separation of serum for studies including serum total proteins, albumin and globulins. Serum total proteins were determined by biurette method (Oser, 1976) and albumin by bromocresol green dye binding method (Varley *et al.*, 1980). Serum globulins were determined by subtracting albumin from total protein values.

Data thus obtained was analysed by using General Linear Model Procedure and means were compared by LSD on personal computer by using SAS statistical software package (SAS, 1996).

RESULTS AND DISCUSSION

Haematology

Haematological parameters studied included haematocrit (PCV), haemoglobin (Hb), total erythrocyte count and total leukocyte count (Table 2,3). There was non-significant difference observed between treatment groups and control group in haemoglobin concentration and total erythrocyte count. However, only at 4th week, lower PCV was observed in birds fed higher levels of chromium chloride alone. This suggests that chromium alone or in combination with copper at the studied levels has no or negligible effect on erythrocyte, haemoglobin concentration and haematocrit. These findings are highly related with organ weight and macro or micropathology as no change in various organs was observed at both levels (unpublished data). Previous studies in sheep also showed no effect of copper on haematocrit and other parameters (Eckert *et al.*, 1999; Canton *et al.*, 1994; Ward *et al.*, 1995). However, Cr supplementation has been reported to cause increase in haematocrit in calves (Moonsie and Mowat, 1993) and haemoglobin, total erythrocyte count and haematocrit in carp (Al-Akel and Shamsi, 1996). Thus it can be concluded from these results that chromium alone and in combination with copper at the experimental dose levels are not toxic in broilers and also the results of total leukocyte count suggest no involvement of leukocytes in any inflammatory or repair process in the body which can occur due to toxicity or toxic damage in the body tissues. As only at week two of treatment, increase in TLC was observed in birds fed low chromium alone or with two levels of copper that may not be considered of significance.

Serum Proteins

Results of serum proteins including total protein, albumin and globulin during first three weeks showed significantly or relatively lower values in treatment groups than control (Tables 4,5). There appears that lower levels of serum proteins were not due to the depressing effects of these compounds on synthesis of protein as the liver weight along with gross and histopathology does not suggest any damage (unpublished data). Similarly, gross and histological studies revealed kidney to be normal thus the lowering of serum total proteins were also not associated with leakage of these from kidneys. The most probable reason of lower level of albumin leading to lower level of serum total proteins might be that albumin was engaged in transport of chromium and copper (Goyer, 1986). It may be possible that bound albumin has not reacted with agents used for determination of serum albumin and serum total proteins. However, further studies are needed to know the real reasons of lower level of serum albumin and total proteins. Serum globulins generally revealed non-significant difference between treatment and control groups. Thus strengthened the viewpoint that the decrease in other serum proteins was not due to any pathological reasons. Previous studies on Cr in broilers revealed no effect on albumin levels in blood (Kalaycioglu *et al.*, 1999). Copper was found to be associated with increase in serum total proteins and albumin in rabbits during heat stress period (Ayyat *et al.*, 1997), while no effect on these was noted in chicken (Chen *et al.*, 1997).

Table 2: **Comparison of means (± SEM) between groups of PCV and Hb at each week.of treatment with 5th week as withdrawal period.**

Groups	Weeks 1st	2nd	3rd	4th	5th
PCV (%)					
A (Cr 2g + Cu 200mg)	24.00 ± 0.32	22.40 ± 1.21	27.20 ± 1.24	23.60 ± 1.29	27.80 ± 1.12
B (Cr 2g + Cu 400mg)	24.00 ± 1.05	23.80 ± 1.80	21.60 ± 1.91	25.80 ± 1.56	25.20 ± 1.06
C (Cr 8g + Cu 200mg)	22.20 ± 1.20	24.20 ± 2.00	21.60 ± 1.12	24.20 ± 1.71	23.80 ± 1.15*
D(Cr 8g + Cu 400mg)	21.40 ± 0.93	24.40 ± 1.44	25.60 ± 1.63	24.60 ± 1.53	26.20 ± 1.24
E (Cr 8g)	20.20 ± 1.24	23.80 ± 0.97	20.40 ± 1.86	21.40 ± 1.16*	24.60 ± 1.03
F (Cr 2g)	22.80 ± 1.07	24.00 ± 1.92	23.40 ± 1.20	24.40 ± 1.20	25.20 ± 0.97
G (Control)	21.67 ± 2.33	22.50 ± 2.32	23.40 ± 1.83	26.75 ± 1.49	27.80 ± 1.07
Hb (g/dl)					
A (Cr 2g + Cu 200mg)	8.24 ± 0.64	8.42 ± 0.32	7.64 ± 1.18	9.82 ± 0.89	9.21 ± 0.85
B (Cr 2g + Cu 400mg)	6.49 ± 0.94	9.72 ± 0.50	8.65 ± 0.36	8.43 ± 0.24	8.37 ± 0.72
C (Cr 8g + Cu 200mg)	6.86 ± 0.56	9.00 ± 1.19	8.78 ± 0.92	8.77 ± 0.60	9.14 ± 0.40
D(Cr 8g + Cu 400mg)	7.44 ± 0.97	8.96 ± 0.86	10.44 ± 1.18	8.39 ± 0.64	9.24 ± 0.34
E (Cr 8g)	8.66 ± 1.49	9.70 ± 1.31	8.31 ± 0.27	7.79 ± 0.35	8.66 ± 0.30
F (Cr 2g)	8.79 ± 0.75	8.15 ± 0.67	10.41 ± 0.59	8.05 ± 0.58	8.00 ± 0.43
G (Control)	8.32 ± 0.67	10.34 ± 0.49	9.64 ± 0.74	9.59 ± 1.03	10.79 ± 1.21

* Significant difference from control in a column at P< 0.05

Table 3: **Comparison of means (± SEM) between groups of Total erythrocyte count and total leukocyte count at each week of treatment with 5th week as withdrawal period.**

Groups	Weeks 1st	2nd	3rd	4th	5th
Total Erythrocyte Count (10^6/µl)					
A (Cr 2g + Cu 200mg)	3.37 ± 0.30	3.66 ± 0.33	3.21 ± 0.21	3.55 ± 0.20	3.87 ± 0.23
B (Cr 2g + Cu 400mg)	2.98 ± 0.45	3.65 ± 0.16	3.07 ± 0.45	3.55 ± 0.26	3.32 ± 0.06
C (Cr 8g + Cu 200mg)	2.84 ± 0.24	3.52 ± 0.10	2.64 ± 0.42	4.23 ± 0.91	3.13 ± 0.16
D(Cr 8g + Cu 400mg)	2.74 ± 0.10	2.96 ± 0.30	2.92 ± 0.28	3.33 ± 0.75	3.33 ± 0.12
E (Cr 8g)	2.55 ± 0.05	3.23 ± 0.50	3.24 ± 0.17	3.81 ± 0.53	3.38 ± 0.27
F (Cr 2g)	2.83 ± 0.05	3.23 ± 0.50	3.24 ± 0.17	3.91 ± 0.53	3.38 ± 0.27
G (Control)	2.68 ± 0.08	3.18 ± 0.23	3.25 ± 0.27	3.50 ± 0.40	3.71 ± 0.29
Total Leukocyte Count (/µl)					
A (Cr 2g + Cu 200mg)	39600.0 ± 2993.33	42333.3 * ± 1452.97	37666.67 ± 5044.25	35666.6 ± 2027.5	39400.0 ± 2315.17
B (Cr 2g + Cu 400mg)	33500.0 ± 2101.59	44000.0 * ± 577.35	38666.6 ± 2905.93	34666.6 ± 1763.83	42250.0 ± 2954.52
C (Cr 8g + Cu 200mg)	39500.0 ± 2101.59	33666.6 ± 881.92	34000.0 ± 2000.0	34750.0 ± 3837.8	33333.3 ± 5925.46
D(Cr 8g + Cu 400mg)	36500.0 ± 2986.08	40000.0 ± 2073.64	32333.3 ± 1452.97	36250.0 ± 6799.20	40333.33 ± 3382.96
E (Cr 8g)	41666.67 ± 1763.83	37250.0 ± 2056.49	41666.67 ± 8819.17	36333.3 ± 4630.81	39666.6 ± 2962.73
F (Cr 2g)	36333.3 ± 3179.80	42750.0 * ± 2096.62	44000.0 ± 2081.67	37666.6 ± 7333.3	50666.67 * ± 8810.92
G (Control)	33000.0 ± 3605.5	35000.0 ± 3055.05	36666.67 ± 4055.18	30666.6 ± 2333.3	38000.0 ± 110.54

* Significant difference from control in a column at P< 0.05

Table 4: Comparison of means (± SEM) between groups of serum total proteins and albumin at each week of treatment with 5th week as withdrawal period.

Groups	Weeks 1st	2nd	3rd	4th	5th
Serum Total Proteins (g/100 ml)					
A (Cr 2g + Cu 200mg)	2.50 ± 0.23	1.51 ± 0.15	2.09 ± 0.12*	5.19 ± 0.63	3.49 ± 0.17
B (Cr 2g + Cu 400mg)	1.64 ± 0.16	1.42 ± 0.12	2.50 ± 0.26	4.72 ± 0.42	3.85 ± 0.54
C (Cr 8g + Cu 200mg)	1.56 ± 0.07	1.48 ± 0.28	2.36 ± 0.22*	4.86 ± 0.51	3.40 ± 0.25
D(Cr 8g + Cu 400mg)	1.52 ± 0.07	1.82 ± 0.20	2.25 ± 0.19*	5.42 ± 0.69	3.36 ± 0.54
E (Cr 8g)	2.21 ± 0.46	2.36 ± 0.32	1.99 ± 0.19*	2.79 ± 0.14	2.91 ± 0.36
F (Cr 2g)	2.18 ± 0.54	1.98 ± 0.11	2.10 ± 0.24*	3.75 ± 0.42	3.63 ± 0.57
G (Control)	1.59 ± 0.10	2.09 ± 0.12	3.60 ± 0.86	4.21 ± 0.42	3.65 ± 0.54
Serum Albumin (g/100 ml)					
A (Cr 2g + Cu 200mg)	1.51 ± 0.09	1.11 ± 0.12*	1.51 ± 0.13*	2.96 ± 0.67	2.35 ± 0.17
B (Cr 2g + Cu 400mg)	1.25 ± 0.10	1.05 ± 0.13*	1.35 ± 0.09*	2.92 ± 0.32	2.67 ± 0.22
C (Cr 8g + Cu 200mg)	1.38 ± 0.083	1.30 ± 0.11*	1.56 ± 0.11	2.43 ± 0.15	2.48 ± 0.20
D(Cr 8g + Cu 400mg)	1.29 ± 0.01	0.99 ± 0.10*	1.44 ± 0.24*	2.72 ± 0.54	1.84 ± 0.06
E (Cr 8g)	1.29 ± 0.04	1.23 ± 0.10*	1.30 ± 0.03	2.38 ± 0.16	2.10 ± 0.11
F (Cr 2g)	1.12 ± 0.15	1.13 ± 0.04*	1.20 ± 0.08*	2.74 ± 0.21	2.32 ± 0.08
G (Control)	1.28 ± 0.02	1.77 ± 0.06	1.80 ± 0.35	2.84 ± 0.40	2.35 ± 0.11

* Significant difference from control in a column at P< 0.05

Table 5: Comparison of means (± SEM) between groups of serum globulins (g/100 ml) at each week of treatment with 5th week as withdrawal period.

Groups	Weeks 1st	2nd	3rd	4th	5th
A (Cr 2g + Cu 200mg)	0.98 ± 0.24	0.40 ± 0.18	0.58 ± 0.17*	2.22 ± 1.03	1.14 ± 0.21
B (Cr 2g + Cu 400mg)	0.39 ± 0.18	0.37 ± 0.04	1.15 ± 0.33	1.79 ± 0.44	1.18 ± 0.50
C (Cr 8g + Cu 200mg)	0.17 ± 0.04	0.62 ± 0.13	0.68 ± 0.11	2.43 ± 0.46	0.92 ± 0.33
D(Cr 8g + Cu 400mg)	0.23 ± 0.08	0.48 ± 0.25	0.81 ± 0.24	2.69 ± 0.94	1.51 ± 0.52
E (Cr 8g)	0.92 ± 0.42	0.59 ± 0.14	0.69 ± 0.18	0.40 ± 0.03	0.80 ± 0.30
F (Cr 2g)	1.06 ± 0.57	1.23 ± 0.35	0.87 ± 0.41	1.19 ± 0.61	1.31 ± 0.54
G (Control)	0.30 ± 0.09	0.20 ± 0.05	1.80 ± 0.67	1.37 ± 0.46	1.29 ± 0.44

* Significant difference from control in a column at P< 0.05

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