INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
16–208/2016/18–6–1103–1108
DOI: 10.17957/IJAB/15.0192
http://www.fspublishers.org

Full Length Article

Chromium Toxicity and Oxidative Stress in Broiler Chicks and its Amelioration with Vitamin E and Bentonite

Javaria Mashkoor[1], Ahrar Khan[1*], Muhammad Zargham Khan[1] and Iftikhar Hussain[2]
[1]*Department of Pathology, Faculty of Veterinary Science, University of Agriculture, Faisalabad-38040, Pakistan*
[2]*Institute of Microbiology, Faculty of Veterinary Science, University of Agriculture, Faisalabad-38040, Pakistan*
[*]For correspondence: ahrar1122@uaf.edu.pk; ahrar1122@yahoo.com

Abstract

This experiment utilized 140 one-day-old broiler chickens. Group 1 served as control. Birds of groups 2, 5, 6 and 7 were administered chromium (Potassium dichromate, $K_2Cr_2O_7$; Cr^{6+}) @ 270 mg/kg through the feed. Groups 3, 5 and 7 received vitamin E (150 mg/kg) and groups 4, 6 and 7 received bentonite (5% of feed), respectively. Cr-treated birds were dull and depressed and gained significantly less body weight gain and increased feed conversion ratio (FCR). All the birds treated with Cr (group 2) showed a significant decrease in hematological parameters at day 21 and 42. Biochemical parameters i.e. total protein, albumin and globulin were decreased while ALT, AST, urea and creatinie were increased at day 21 and 42. Birds treated with Cr (group 2) showed a significant decrease in total antioxidant activity and catalase, while malondialdehyde (MDA) and total oxidant status levels were increased at days 21 and 42. Co-administration of bentonite along with Cr resulted in partial amelioration (group 6) as compared to groups 5 and 7 administered chromium + vitamin E and chromium + vitamin E + bentonite, respectively. Highest concentration of Cr was determined in Cr treated birds (group 2) followed by groups 2, 6, 5 and 7 at 21 and 42 experimental days. It was concluded that Cr leads to oxidative stress in broilers. Vitamin E and bentonite partially ameliorated Cr toxicity. The combination of vitamin E and bentonite is promising in combating Cr toxicity effects.

Keywords: Broilers; Chromium; Oxidative stress; Vitamin E; Bentonite

Introduction

Pakistan poultry industry is one of the most important sectors and is expanding very rapidly. This sector has played a significant role in poverty alleviation predominantly in rural areas by providing employment. Poultry meat is contributing about 19% of the total meat production in the country and is providing affordable and good quality animal protein (Bachaya *et al.*, 2015; Shahzad *et al.*, 2015). However, the growth of poultry is being hampered by infectious and non-infectious causes. Among the latter, heavy metals coming in poultry water/feed from various sources badly affecting the poultry health and productivity.

Hexavalent chromium (Cr^{6+}) is the most dangerous form of chromium (Cr) used in chrome plating and causes cancer and mutagenesis (Gomez and Callao, 2006). Anthropogenic activities like tannery effluents, smelting, electroplating, metallurgy, industrial and agricultural practices have increased Cr level beyond the assimilation capacity of biota in the ecosystem (Vlizlo *et al*., 2014) which ultimately bioaccumulate in different organisms i.e. fish and poultry. Therefore, it has become the most toxic substance in marine and terrestrial ecosystems (Costa, 2003; Iftikhar *et al*., 2015; Bouaziz *et al*., 2015).

Free radical injury is responsible for many pathological conditions. Free radicals can exist independently and they bear one or two unpaired electrons in valance shell. They are highly reactive to cell organelles e.g. polyunsaturated fatty acids, proteins and nucleic acid (Sánchez-Mendoza *et al*., 2015). Metals like Cr are very reactive and induce oxidative damage by generating reactive oxygen species (ROS) (Zhang *et al*., 2014). It interferes with metabolic process and biological reactions i.e. change in enzyme activity, membrane fluidity, ion transport and protein synthesis (Javed, 2015). Chromium (Cr^{6+}) once entered in a cell is converted into its lower oxidation states i.e. Cr^{3+}, which is a reactive intermediate of Cr^{6+} responsible for the generation of ROS (Shati *et al*., 2014).

Vitamin E has chromanol ring in its structure which donates hydrogen to free radicals thus making them unreactive (Kumari *et al*., 2013). It is lipid soluble antioxidant and biologically crucial for reducing heavy metal toxicity. In broiler feed, it is used to prevent different stress related diseases occurring due to intensive rearing (Murakami *et al*., 2007; Mashkoor *et al*., 2013).

To cite this paper: Mashkoor, J., A. Khan, M.Z. Khan and I. Hussain, 2016. Chromium toxicity and oxidative stress in broiler chicks and its amelioration with vitamin E and bentonite. *Int. J. Agric. Biol.*, 18: 1103–1108

Bentonite (a clay) have been used as a preventive medicine in clinical therapies to remove the heavy metals accumulated in different organs of the body (Moosavi *et al*., 2015). In poultry feed, it is used as an adsorbent of heavy metal toxin, bacteria and virus, moreover, also used as pellet binder in poultry feed (Araba, 1992). It is due to its high absorptive nature that now a day is being used as a detox in acute heavy metal poisoning in birds as well as animals (Moosavi *et al*., 2015).

In literature, there is scanty information available regarding the use of bentonite as ameliorative agent in Cr intoxicated broilers alone or in combination with vitamin E, therefore, the following study was designed to determine the Cr induced oxidative stress with various tests and its amelioration with vitamin E and bentonite. Moreover, this study will provide information whether co-administration of bentonite and vitamin E behave synergistically or antagonistically in Cr intoxicated broilers.

Materials and Methods

Experimental Birds and Management

One-day-old broiler chicks (n=140) of Hubbard breed were purchased from a local hatchery and kept under standard management and housing conditions i.e., in wire cages, temperature (24–35°C) and humidity (60–65%). The light was provided for 24 h during the experiment. The birds were kept on basal diet, i.e. chick starter crumbs having 21% total protein (Abu-Akkada and Awad, 2015) and provided clean water *ad libitum*. They were vaccinated against Newcastle disease (Nobilis® ND Lasota, Intervet S.A. (Pty) Ltd) on day 2nd and 23rd and infectious bursal disease (Nobilis Gumboro 228E, Intervet S.A. (Pty) Ltd) on 8th and 21st and hydropericardium syndrome (BioAngara Plus, Sana Lab) on day 19th.

Experimental Procedure

Experiment was executed using 2-factor factorial design under CRD. After two days acclimatization, birds were randomly divided into seven equal groups. From each group, 10 randomly selected birds were killed humanely on experimental days 21 and 42 to collect blood/serum for various studies, in this way there were 10 replicates in each group as each bird is considered as a replicate. Treatments (Table 1) started at 3rd day and continued till 42 days. All the treatments were given through feed on daily basis. Group 1 served as control. Group 2, 3 and 4 were fed Cr (Potassium dichromate, $K_2Cr_2O_7$; Cr^{6+}), vitamin E and bentonite @ 270 and 150 mg/kg, and 5% of feed, respectively. Group 5 received Cr along with vitamin E, group 6 received Cr along with bentonite 5% and group 7 received Cr along with bentonite and vitamin E. All the birds were monitored for clinical signs twice daily. Each bird was weighed weekly. Feed intake was measured on daily basis. FCR was calculated at day 21 and 42.

Hematobiochemical Parameters

From each group, 10 birds were selected randomly and killed humanely on experimental days 21 and 42 to collect blood for hematological studies i.e., RBC and TLC (Benjamin, 1978). Hematocrit was dogged by microhematocrit capillary tubes and hemoglobin concentration by spectrophotometer. Erythrocyte indices i.e., MCHC and MCV were calculated (Ghaffar *et al*., 2015). Biochemical studies i.e. Total serum proteins (Saleemi *et al*., 2014), albumin (Varley *et al*., 1980), globulin (Benjamin, 1978), ALT and AST (Randox Company Lot # 086580 and 128011, respectively) were performed.

Oxidation Stress and Residue Analysis

Randomly picked 10 birds from each group were killed humanly on day 21 and 42 of the experiment and blood sample without anticoagulant was collected for the procurement of serum. Serum level of the enzyme catalase was determined by the method of Goth (1991). In this method, free radical formed i.e. hydrogen per oxide reacts with molybdate forming a yellowish complex which is determined using spectrophotometer. The total anti-oxidant capacity in the serum samples was measured by using method of Erel (2004). In this method antioxidants present in the sample reduce dark blue-green colored ABTS radial to colorless reduced ABTS form. Total oxidant status was determined using novel automated measuring method developed by Erel (2005). In this method, ferrous-o-dianisidine is oxidized by oxidants present in the sample to the ferric ion which then reacts with glycerol giving color. Malondialdehyde was determined according to the method developed by Ohkawa (1979). MDA reacts with deoxyadenosine and deoxyguanosine in DNA, forming DNA MDA and other thiobarbituric acid reactive substances (TBARS) condense with two equivalents of thiobarbituric acid to give a fluorescent red derivative.

Residues of Cr were determined in serum by atomic absorption spectrophotometric method (Richards, 1968). For this purpose, serum was digested concentrated HNO_3 and $HClO_4$. Then the contents were heated vigorously and diluted with de-ionized water. The digested and diluted samples were subjected to Cr determination with double-beam atomic absorption spectrophotometer (AAS), model PG-990 equipped with computer atomic absorption AA Win 2 software (PG instruments Ltd., UK).

Statistical Analysis

The data collected were analyzed statistically by applying 2-factor factorial under CRD keeping two factors i.e., group and time as factors. Different group means were compared by Duncan multiple range test using M-Stat computer statistical package.

Table 1: Experimental groups and treatments

Groups	Treatments		
	Chromium (ppm)	Vitamin E (ppm)	Bentonite (%)
1.	-	-	-
2.	270	-	-
3.	-	150	-
4.	-	-	5
5.	270	150	-
6.	270	-	5
7.	270	150	5

Results

All the birds of group 2 (Cr) showed more prominent signs (++++) of toxicity as compared to control. Clinical signs observed in the present study were reduced feed and water intake, decreased body weight gain. Birds were dull and depressed (Table 2).

Feed intake and live body weight decreased significantly ($p \leq 0.05$) in Cr-treated birds (Group 2) at experimental days 21 and 42. However, groups 5, 6 and 7 treated with vitamin E and bentonite along with Cr, showed significant ($p \leq 0.05$) increase in feed utilization and live body weight. Increased FCR in Cr treated birds (Group 2) as compared to control birds was observed on both experimental days. Groups 5, 6 and 7 treated with vitamin E and bentonite along with Cr showed significant ($p \leq 0.05$) decrease in FCR (Table 3).

Hematological parameters (RBC, WBC, Hb, PCV, ESR) showed significant ($p \leq 0.05$) decrease in Cr-treated group (group 2) as compared to control (group 1) at experimental day 21 and 42 of experiment (Table 4). At day 21 and 42, a significant ($p \leq 0.05$) decrease in ALT, AST and serum total proteins values were recorded in Cr (group 2) treated birds as compared to control (group 1). At experimental days 21 and 42 significantly ($p \leq 0.05$) increased urea and creatinine values were recorded in Cr (group 2) treated birds as compared to control (Table 5).

Oxidation stress parameters (TAC, CAT) showed significant ($p \leq 0.05$) decreasing trend in Cr treated group (group 2) as compare to control (group 1) at experimental day 21 and 42 of experiment, while TOS, MDA values in group 2 at experimental days 21 and 42 significantly increased than control (Table 4). Highest Cr concentration was observed in birds of group 2, followed by in groups 6, 5, and 7 at experimental days 21 and 42 (Table 5).

Discussion

Clinical signs observed in the present study were reduced feed and water intake and decrease in body weight gain. Birds were dull, depressed in Cr-treated birds compared to control group. Previous studies carried out in broiler chicks (Mohammed *et al*., 2014) and rats (Scibior *et al.,* 2006) noted a significant reduction in body weight. Decrease in feed intake could be due to inhibition of satiety center by Cr resulting in loss of interest in the feed (Asmatullah *et al*., 1999). Decrease in body weight could be due to decrease internal activity which damage health leading to impaired growth and weight gain (Mishra and Mohanty, 2009). Another reason of decreased body weight could be due to irregularities in metabolism mediated by Cr-induced liver damage (Saxena and Tripathi, 2007).

Hematological parameters including TEC, Hb, and PCV were significantly ($P<0.05$) decreased while ESR was significantly increased in Cr-treated birds compared to control group in the present study. Previous studies noted decreases in hematological parameters following Cr-administration i.e. in broiler (Kumari *et al*., 2013), rats (Kim *et al*., 2004; Balakrishnan *et al*., 2013) and fish (Shaheen and Akhtar, 2012). Decrease in TEC, Hb and PCV level indicate anemia resulting due to iron deficiency and its decreased utilization for Hb synthesis so Hb concentration also decreases. Another possible reason could be that the Cr can cross the red blood cell membrane easily and accumulate in it, thereby leading to DNA protein crosslinking and thus occurrence of anemia. Another reason could be that Cr has ability to bind to beta chain of hemoglobin so no hemoglobin available for heme synthesis leading to anemia (Adjroud, 2010).

In the present study, TLC was significantly ($p \leq 0.05$) decreased in Cr treated birds compared to control group. Previous studies report decreases in TLC following Cr administration in the rats (Balakrishnan *et al*., 2013). Decrease in TLC could be due to in activation of immune response by Cr. Another opinion could be that leucopenia could be due to ability of Cr to cross membrane through active transport and remain there till the life of cell leading to depletion of leukocytes. Cr-administration leads to apoptosis of leukocytes leading to leucopenia. Cr when comes in contact with leukocytes causes peroxidation and inhibits trans membrane potential of mitochondria of lymphocytes (Adjroud, 2010).

ALT and AST were significantly ($p \leq 0.05$) increased while plasma protein was significantly decreased in Cr-treated birds compared to control group in the present study. Previous studies noted an elevation in the level of ALT and AST i.e. in fish (Shaheen and Akhtar, 2012) and rats (Kim *et al.,* 2004; Mehany *et al*., 2013). Cr-toxicity cause elevation of serum hepatic enzymes due to leakage of theses enzymes or increase in their production. Elevated ALT and AST could be due to biotransformation of heavy metals in liver leading to hepatic injury.

In the present study, creatinine and urea were significantly ($p \leq 0.05$) increased in Cr-treated birds compared to control group. Previous studied noted increase in creatinine and urea i.e. in rats (Pedraza-Chaverri *et al.,* 2008; Mehany *et al*., 2013). Increase in creatinine and urea level indicated renal injury. Increase in urea and creatinine level could be due to interaction of Cr with cell membrane of kidneys resulting in alteration of permeability ultimately functional impairment and loss of integrity (Parveen *et al*.,

Table 2: Clinical signs and behavioral alterations in broiler birds of different groups administered chromium, vitamin E and bentonite in different combinations

Groups	Dullness	Depression	Reduced feed and water intake
G1 (Control)	-	-	-
G2 (Chromium)	++++	++++	++++
G3 (Vitamin E)	-	-	-
G4 (Bentonite)	-	-	-
G5 (Chromium+Vitamin E)	++	++	++
G6 (Chromium + Bentonite)	+++	+++	+++
G7 (Chromium+Vitamin E+ Bentonite)	+	+	+

No sign (-), mild (+), moderate (++), severe (+++), highly severe (++++)

Table 3: Mean values of feed intake (g/day), live body weight (g) and feed conversion ratio on day 21 and 42 experiment in broiler birds of different groups administered chromium, vitamin E and bentonite in different combinations

Groups	Experimental Day 21			Experimental Day 42		
	Feed consumed (g/day)	Live body weight (g)	FCR	Feed consumed (g/day)	Live body weight (g)	FCR
G1 (Control)	975±13.7a	765±24.2a	1.27	3366±24.9a	2045±28.2a	1.64
G2 (Chromium)	842±10.2b	535±14.3b	1.57	3201±13.3b	1610±15.2b	1.98
G3 (Vitamin E)	977±12.9a	769±20.1a	1.27	3332±25.2a	2025±21.7a	1.64
G4 (Bentonite)	965±12.3a	747±19.6a	1.29	3325±24.2a	2001±20.9a	1.66
G5 (Chromium+Vitamin E)	899±11.7c	621±17.9c	1.44	3287±13.9c	1855±19.7c	1.77
G6 (Chromium + Bentonite)	885±11.2c	615±17.25c	1.43	3276±13.1c	1825±17.2c	1.79
G7 (Chromium+Vitamin E+Bentonite)	970±13.2a	760±21.2a	1.27	3335±24.2a	2017±25.2a	1.65

Values (mean±SE) with different letters in a column differ significantly ($p \leq 0.05$)

Table 4: Mean values of different hematological parameters on day 21 and 42 experiment in broiler birds of different groups administered chromium, vitamin E and bentonite in different combinations

Parameters	Experimental Days	Groups						
		G1 (Control)	G2 (Cr)	G3 (Vitamin E)	G4 (Bentonite)	G5 (Cr + Vitamin E)	G6 (Chromium +Bentonite)	G7 (Cr +Vitamin E+ Bentonite)
TEC ($\times 10^6$/µL)	21	3.49±0.09a	2.85±0.01d	3.29±0.01c	3.22±0.05b	3.21±0.07b	3.25±0.02c	3.37±0.02ab
	42	3.57±0.03a	2.92±0.05d	3.42±0.07c	3.39±0.06b	3.35±0.09b	3.32±0.05c	3.49±0.08ab
Hb(g/dl)	21	11.85±0.06a	7.05±0.07d	10.64±0.02ab	9.39±0.08b	9.43±0.03b	9.05±0.07c	10.56±0.09ab
	42	12.02±0.08a	7.37±0.03d	10.87±0.03ab	10.75±0.01ab	10.34±0.01ab	9.25±0.09b	11.74±0.03ab
PCV (%)	21	33.45±0.02a	23.11±0.09c	31.74±0.06ab	29.67±0.03ab	29.09±0.02ab	26.97±0.03b	31±0.07ab
	42	31.97±0.01a	24.42±0.02c	30.01±0.01ab	30.95±0.03ab	28.73±0.09b	27.95±0.05b	31±0.05ab
ESR (mm/h)	21	22.05±0.06a	17.57±0.08c	20.63±0.05ab	17.95±0.07b	18.52±0.07bc	17.59±0.01b	21.63±0.02bc
	42	26.19±0.09a	18.73±0.05c	21.07±0.07c	20.79±0.05b	20.77±0.05b	19.53±0.09b	21.75±0.03a
TLC	21	7.35±0.03a	4.53±0.06d	6.75±0.08c	5.65±0.06bc	5.43±0.03b	5.35±0.02b	6.47±0.08bc
($\times 10^3$/µL)	42	7.99±0.03a	5.43±0.03d	7.89±0.01ab	6.57±0.09b	7.45±0.06ab	6.54±0.08b	7.75±0.03ab

Values (mean ± SE) with different letters in a row differ significantly ($p \leq 0.05$)

2009). Acceleration in Cr-induced nephrotoxicity could be due to over production of ROS, which damage the membrane components leading to necrosis (Liu and Shi, 2001).

The level of catalase was significantly ($p \leq 0.05$) decreased in Cr-treated birds compared to control group in the present study. Previous studies noted depression in catalase following Cr-administration in rats (Molina-Jijon *et al*., 2011) and fish (Shaheen and Akhtar, 2012). The first line of defense of antioxidant enzyme system is superoxide dismutase then comes the catalase. Both work in combination (Atawodi *et al.*, 2014; Li *et al*., 2014). Oxidative stress parameters i.e. catalase activity significantly decreased following 10 ppm Cr^{3+}-administration to goldfish (Lushchak *et al*., 2009).

In the present study, level MDA was significantly ($p \leq 0.05$) increased in Cr-treated birds compared to control group. Previous studies noted increase in MDA in rats (Balakrishnan *et al*., 2013; Shati, 2014). MDA is well-known marker of oxidation stress. MDA level increases because heavy metals such as Cr produces ROS, which interact with redox metals/biomolecules forming MDA and DNA adducts (Valko *et al*., 2005).

High concentration of Cr was found in the serum of birds of Cr-treated group compared to control group as determined by atomic absorption spectroscopy in the present study. Higher concentration of Cr in liver (36.79 ppm) as compared to control (21.8 ppm) determined through atomic absorption spectroscopy after administration of potassium dichromate (Shati, 2014). Previous studies noted an elevation following Cr-administration in fish (Batool *et al*., 2014).

Table 5: Mean values of different biochemical parameters on day 21 and 42 experiment in broiler birds of different groups administered chromium, vitamin E and bentonite in different combinations

Parameters	Experimental Days	Groups						
		G1 (Control)	G2 (Chromium)	G3 (Vitamin E)	G4 (Bentonite)	G5 (Chromium +Vitamin E)	G6 (Chromium+ Bentonite)	G7 (Chromium +Vitamin E + Bentonite)
Total Protein (g/dl)	21	4.17±0.06a	3.67±0.01d	3.87±0.07b	3.82±0.03b	3.79±0.05c	3.72±0.02c	3.95±0.05b
	42	3.84±0.07a	3.29±0.02d	3.48±0.09c	3.43±0.04c	3.41±0.09b	3.35±0.01b	3.57±0.07c
ALT (IU/L)	21	10.29±0.08a	13.97±0.04c	11.19±0.01ab	12.37±0.07b	11.31±0.07a	12.3±0.05b	12.3±0.05b
	42	15.04±0.09a	17.27±0.06c	15.72±0.04ab	16.03±0.09b	16.52±0.05b	16.75±0.06b	15.07±0.04b
AST (IU/L)	21	4.61±0.03a	5.39±0.07c	4.85±0.03b	4.97±0.04b	5.03±0.04bc	5.11±0.07bc	5.29±0.03bc
	42	4.81±0.09a	6.37±0.09c	5.85±0.05b	5.92±0.04b	6.04±0.06bc	6.09±0.09bc	5.77±0.03b
Creatinine (g/dl)	21	0.42±0.06a	1.05±0.05c	0.59±0.06b	0.71±0.05bc	0.62±0.05bc	0.75±0.04bc	0.52±0.05b
	42	0.32±0.04a	1.09±0.08c	0.42±0.07ab	0.77±0.08bc	0.52±0.09ab	0.82±0.05bc	0.39±0.03b
Urea (g/dl)	21	18.92±0.02a	30.11±0.04d	20.62±0.09b	22.14±0.06ab	27.14±0.06bc	28.31±0.07c	20.09±0.02ab
	42	21.19±0.09a	35.25±0.03d	28.17±0.03c	22.52±0.05 ab	23.45±0.07ab	25.15±0.05bc	26.57±0.05bc

Values (mean ± SE) with different letters in each row differ significantly from each other ($p \leq 0.05$)

Table 6: Mean values of different oxidation stress parameters on day 21 and 42 experiment in broiler birds of different groups administered chromium, vitamin E and bentonite in different combinations

Parameters	Experimental days	Groups						
		G1 (Control)	G2 (Chromium)	G3 (Vitamin E)	G4 (Bentonite)	G5 (Chromium +Vitamin E)	G6 (Chromium Bentonite)	G7 (Chromium + +Vitamin E+ Bentonite)
TAC (mmol/L)	21	1.43±0.01a	1.12±0.05c	1.31±0.03ab	1.29±0.07ab	1.27±0.02b	1.15±0.03bc	1.35±0.01ab
	42	1.45±0.05a	1.19±0.02c	1.29±0.07ab	1.25±0.02ab	1.21±0.01b	1.23±0.05ab	1.32±0.02ab
TOS (uMol/L)	21	1.21±0.09a	1.51±0.06c	1.30±0.02ab	1.32±0.09ab	1.37±0.07b	1.39±0.09b	1.35±0.05b
	42	1.43±0.07a	1.87±0.08c	1.62±0.05ab	1.65±0.03ab	1.71±0.04b	1.77±0.01b	1.68±0.07b
MDA (nmol/L)	21	9.34±0.02a	13.92±0.04c	10.42±0.09ab	12.57±0.02b	11.75±0.09b	13.27±0.02bc	10.09±0.03ab
	42	9.15±0.03a	14.07±0.07c	9.62±0.08ab	11.54±0.06b	10.41±0.01ab	13.72±0.05bc	9.57±0.09ab
Catalase (Kilo U/L)	21	62.9±5.2a	32.1±3.4b	61.2±4.1a	57.4±3.4a	49.3±4.3c	45.2±3.1c	62.7±5.4a
	42	72.7±7.4a	39.7±4.3b	70.3±7.3a	67.3±5.2a	56.2±2.3c	54.3±2.7c	70.5±5.3a

TAC = total antioxidant capacity; TOS = Total oxidant stress; MDA = malondialdehyde; Values (mean±SE) with different letters in a row differ significantly ($p \leq 0.05$)

Table 7: Mean values of residue analysis of chromium (ppm) on day 21 and 42 experiment in broiler birds of different groups administered chromium, vitamin E and bentonite in different combinations

Groups	Days of experiment	
	21	42
G1 (Control)	0	0
G2 (Chromium)	1.14±0.05a	1.24±0.07a
G3 (Vitamin E)	0	0
G4 (Bentonite)	0	0
G5 (Chromium+Vitamin E)	1.11±0.04a	1.12±0.03b
G6 (Chromium+Bentonite)	1.13±0.02a	1.19±0.05b
G7 (Chromium+Vitamin E+Bentonite)	1.02±0.03b	0.99±0.04b

Values (mean±SE) with different letters in a column differ significantly ($p \leq 0.05$)

Conclusion

Chromium is a strong oxidative stress inducer. Vitamin E administration ameliorated Cr-induced toxicity efficiently. Bentonite partially ameliorated Cr-induced toxicity. Vitamin E and Bentonite combination is most efficient in alleviating toxicity.

Acknowledgements

This work was supported by research grant from Higher Education Commission, Islamabad, Pakistan.

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(Received 21 April 2016; Accepted 08 June 2016)