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Biochemical Effects of Copper Sulfate, after Chronic Treatment in Quail

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Abstract: The present study was designed to investigate the biochemical toxicity and influence of antioxidant defense. Experiments were undertaken with different doses (100, 250, 500, 750 and 1000 ppm) of CuSO₄-supplemented casein diet (CSCD) in commercial Quail. The following parameters were studied. (1) CuSO₄ concentration in liver; (2) effect on body weight; (3) estimation of the serum levels of ALT, AST, alkaline phosphatase, uric acid, total proteins and blood glucose; (4) analysis of the plasma levels of total lipids, cholesterol, HDL and LDL and (5) estimation of malonaldehyde (MDA), glutathione peroxidase (GSH-PX) and superoxide dismutase (SOD) levels in the plasma. The results demonstrated increase in the Cu contents of liver and caused significant loss of body weight. Although the treatment enhanced antioxidant defense enzymes (SOD and GSH-PX), there was no reversal observed in the induced biochemical toxicity, including the increase of MDA. This study revealed that pro-oxidant activity of CuSO₄ superceded the endogenous antioxidant defense, which might be a major factor in the pathogenesis of several drastic diseases, both in birds and humans. The beneficial effects of Cu may be utilized by reducing the levels of supplementation. Further experiments are suggested on characterization of dose-response relationship by mathematical modeling. This might require the use of a sufficient number of lower doses to determine a threshold dose below which there would be no toxicity.

Key words: Quail, copper, biochemical changes, oxidant activity, antioxidant defense system

INTRODUCTION

By virtue of its significance in most of the enzymatic reactions and cellular metabolism, Cu is an essential component of human nutrition and the diet of birds, including poultry. It is very much essential to maintain the physiological activities and the serum Cu concentration ranges, approximately, up to 1.5 mg L⁻¹ in healthy persons. The feed of birds, including poultry, is supplemented with Cu to regulate growth performance, including increase in body weight gain and feed intake. It is also used to reduce the microbes in intestine, in addition to improving the activities of total protease, amylase and lipase (Xia *et al.*, 2004). Lien *et al.* (2004) found supplemental Cu to reduce VLDL-cholesterol and enhance HDL-cholesterol in egg yolk of laying hens. CuSO₄ is reported to reduce the saturated fatty acid proportion in abdominal fat and increase the PUFA:SFA ratio. It also reduces the cholesterol content in breast muscles and eggs (Pesti and Bakalli, 1998; Skrivan *et al.*, 2000). Supplementation of Cu as CuSO₄ and copper acetate in the diet of the leghorn hen was found to lower the plasma and yolk cholesterol, triglycerides (Al-Ankari *et al.*, 1998).

Nevertheless, Cu is a very toxic metal, as revealed by a large number of papers published on toxicity and cases

of fatal poisoning in human beings (Gulliver, 1991; Kurisaki *et al.*, 1988; Ahasan *et al.*, 1994). If the levels exceed the range of human tolerance, it would cause toxic effects such as hemolysis, jaundice and even death. A number of studies have indicated that the overload of Cu *in vivo* can induce a set of toxicological activities such as hepato-cirrhosis, anemia, neutropenia, osteoporosis, edema, apnoea and gastrointestinal symptoms (Sutton *et al.*, 1985; Takeuchi *et al.*, 1993; Barceloux, 1999; Bjorn *et al.*, 2003). Increased levels of Cu are also known to induce serious toxic implications such as nausea, vomiting, hemolysis, methemoglobinemia, hepatorenal failure, chronic tubulo-interstitial nephritis, metabolic acidosis, septicemia, shock, carcinogenic effects and death in human beings (Pizarro *et al.*, 2001; Bhowmik *et al.*, 2001; Borisenkova *et al.*, 2002; Yang *et al.*, 2004). Ahasan *et al.* (1994) reported a large number of fatal cases (24.9%) of copper sulfate poisoning from southern region of Bangladesh. Most of the toxicity implications are related with free radicals and disorders of the antioxidant defense system, which have a pathogenic impact on human tissues and hence are seen as important factors in the development of various diseases (Ersan *et al.*, 2006).

Thus, there are many reports on the toxicity of Cu in humans, however; there is a paucity of literature on

toxicity of Cu in birds, especially Quail. Since, Quail is a consumable bird, health care of these palatable birds is of vital significance. The present study was designed to investigate the influence of endogenous antioxidant defense on Cu-induced-biochemical toxicity in Quail.

MATERIALS AND METHODS

The present study on the biochemical effects of CuSO₄ in commercial Quail (*Coturnix coturnix*) was conducted in the Department of Zoology, College of Science, King Saud University. The experimental part was undertaken during the period January to April 2005.

Tested chemicals: Casein diet was supplemented with CuSO₄ (CSCD) as a source of Cu. Casein was used in the diet of birds, because, it is reported to bind with Cu (O'Neill and Tanner, 1989). Pure CuSO₄ was purchased from Reanal (Budapest, Hungary).

Experimental animals: Birds (Quail), weighing 190-202 g, aged 8 weeks, were obtained from commercial sources. They were maintained in bird cages in the normal environmental conditions of temperature, humidity and light (12 h, light/12 dark cycle) in the Animal facilities of Zoology Department, College of Science, King Saud University, before commencing the experiments. The birds were maintained on normal and/or experimental diet and water *ad libitum*. The conduct of experiments and the procedure of sacrifice (using ether) were approved by the Ethics Committee of the Experimental Animal Care Society, College of Science, King Saud University, Riyadh, Saudi Arabia.

Experimental groups: A total of 48 Quail were used in the entire experiment. Each group constituted 8 birds. The experimental groups of birds consisted of the following: group 1, control (normal casein diet); group 2, 100 ppm of CuSO₄ in CSCD; group 3, 250 ppm of CuSO₄ in CSCD; group 4, 500 ppm of CuSO₄ in CSCD; group 5, 750 CuSO₄ in CSCD and group 6, 1000 ppm CuSO₄ in CSCD. The birds were treated for a period of 30 days.

Sampling for liver tissue, plasma and serum: Twenty four hour following the treatment, the birds were sacrificed, using ether. Blood were withdrawn from the heart and then divided into two tubes with and without Na₂ EDTA (1 mg mL⁻¹) to collect plasma and serum samples after centrifugation (2000 rev/min; 20 min; 4°C). The serum samples were used for the analysis of biochemical indices, including ALT, AST, ALP, proteins, blood glucose, total lipids, cholesterol, LDL and HDL. The estimation of malondialdehyde (MDA), glutathione

peroxidase(GSH-PX) and superoxide dismutase (SOD) was done in the plasma. Samples of liver tissue were collected to study the estimation of Cu levels.

Determination of gain and/or loss of body weight: The initial and final body weights of birds were measured in control and treated birds. The difference was statistically computed for loss and/or gain in the weight.

Estimation of biochemical indices: The analysis of different biochemical indices (ALT, AST, ALP, uric acid and blood glucose) was done in the serum by using specific commercial kits (Randox diagnostic reagents, Randox Laboratories, USA). The lipid analyses (total lipids, cholesterol, LDL and HDL) in the serum were done by enzymatic method (test Kit CELM, Modern Laboratory Equipment Company, Sao Paulo, Brazil). The measurements were done with a UV-visible spectrophotometer, Ultrospec III (LKB).

Analysis of Proteins: Total proteins were estimated in the plasma by the modified Lowry method of Schacterle and Pollack (1973). Bovine serum albumin was used as standard.

Estimation of malondialdehyde concentration: The method described by Ohkawa *et al.* (1979) was followed. The samples (Plasma) were mixed in TCA, vortexed and suspended in thiobarbituric acid. After centrifugation the optical density of the clear pink supernatant was read at 532 nm. Malondialdehyde bis (dimethyl acetal) was used as an external standard (Buege and Aust, 1978; Ohkawa *et al.*, 1979).

Determination of glutathione peroxidase (GSH-PX): The GSH-PX activity was determined with the DTNB photometric method (Deng *et al.*, 2000). Briefly, the plasma was mixed with GSH. The mixture was incubated followed by addition of H₂O₂. The mixture was incubated again and 5% trichloroacetic acid was added to the mixture. After centrifugation, the supernatant was collected and mixed with 2.5 mL disodium hydrogen phosphate, NaOH and DTNB. The absorbance value of the sample was recorded against the blank at 422 nm using a spectrophotometer.

Estimation of superoxide dismutase (SOD): SOD activity was assayed by the inhibition of pyrogallol autoxidation at 25°C and was followed kinetically at 420 nm (Marklund and Marklund, 1974). One unit of SOD activity was defined as the amount of enzyme that caused 50% inhibition of pyrogallol autoxidation.

Estimation of Cu in the hepatic tissue: Dried tissue samples were transferred to acid-washed 10 mL plastic tubes, dissolved in 0.5 mL concentrated nitric acid at room temperature for 1 h and placed in a pre-heated block at 65°C for 4 h. Subsequently, the tube was vortexed, diluted with 2.5 mL of dionized water, vortexed again and centrifuged for 5 min at 1500 rpm. The supernatant was transferred to a fresh 10 mL tube and analysed by direct aspiration into the flame of an atomic absorption spectrophotometer (VARIAN SpectrAA-880). Copper levels are expressed as $\mu\text{g g}^{-1}$ tissue (White *et al.*, 1999).

Statistical analysis: The readings shown are mean±standard error of means. The mean determination of treatment groups was compared statistically with that of control by one way ANOVA and Post hoc Tukey-Kramer multiple comparison test.

RESULTS

The treatment with CSCD for 30 days was found to inhibit growth, as revealed by loss of body weight, with an increase in the hepatic levels of Cu (Table 1). The levels of Cu in the hepatic tissue were significantly increased at 100 ppm ($p<0.01$) and 250 to 1000 ppm ($p<0.001$) of CSCD., as compared to the values obtained in the control group. Body weight was found to increase at the lower doses (250 and 500 ppm) ($p<0.01$), while there was a significant ($p<0.001$) reduction of body weight at the high dose (1000 ppm) as compared to the values obtained in the control group.

There was a significant ($p<0.01$) increase of ALT concentrations at 250 ppm of CSCD and ($p<0.001$) at the

higher doses (500, 750 and 1000 ppm). The AST also showed an increase ($p<0.05$) at 250 and ($p<0.001$) at 500, 750 and 1000 ppm. The concentrations of Alkaline phosphatase were significantly ($p<0.05$) increased at the higher concentrations of CSCD (750 and 1000 ppm). The levels of uric acid were also increased ($p<0.01$) increased at 500, 750 and 1000 ppm. There was a significant ($p<0.01$) increase observed in the protein levels at the high dose. Blood glucose levels were significantly ($p<0.05$) increased at the higher concentrations (750 and 1000 ppm) (Table 2).

The study on lipid profile, after the treatment with CSCD revealed significant increase of the levels of total lipids, cholesterol and LDL, as compared to the values obtained in the control (Table 3). There was an increase of total lipids at 750 ($p<0.05$) and 1000 ($p<0.01$) ppm of CSCD. The levels of cholesterol were also found to increase ($p<0.05$) at 500 and ($p<0.01$) at 750 and 1000 ppm. The LDL levels were increased at 500 ($p<0.05$), 750 and 1000 ($p<0.001$) ppm, while there was no change observed in the levels of HDL.

The levels of MDA were found to significantly increase ($p<0.01$) at 250 and ($p<0.001$) at 500, 750 and 1000 ppm of CSCD. The GSH-PX levels were increased ($p<0.05$) at 100, ($p<0.01$) at 250 and ($p<0.001$) at 500, 750 and 1000 ppm. There was a significant ($p<0.001$) increase in the levels of SOD at 500, 750 and 1000 ppm (Table 4).

DISCUSSION

Results of the present study clearly demonstrate that CuSO_4 supplementation in the casein diet of quail cause increase in the Cu levels of hepatic tissue at all the different doses of CSCD. There was an increase of

Table 1: Effect of CuSO_4 on the hepatic levels of Cu and body weight in quail

Treatment and dose (ppm/casein diet) (CuSO_4)	Hepatic levels of Cu	Average body weight (g)		Net gain in body weight (g)
		Initial weight (g)	Final weight (g)	
Control (normal diet)	51.36±2.61	199.15±10.85	239.42±13.05	40.27±2.87
CuSO_4 (100)	63.79±1.97*	196.20±7.13	242.20±11.13	46.25±1.89
CuSO_4 (250)	120.32±7.21**	190.01±12.10	246.87±8.17	56.36±2.01*
CuSO_4 (500)	460.17±23.47**	202.17±9.19	266.71±16.01	64.54±4.11*
CuSO_4 (750)	651.82±42.01**	194.23±15.77	229.96±9.23	35.73±2.31
CuSO_4 (1000)	790.00±39.11**	201.33±12.06	226.65±17.11	25.32±1.01**

* $p<0.01$; ** $p<0.001$ (One way ANOVA and post hoc Tukey-Kramer multiple comparison test was done individually for different parameters)

Table 2: Effect of CuSO_4 on some markers of biochemical toxicity in blood serum of quail

Treatment and dose (ppm/casein diet) (CuSO_4)	Biochemical Indices (Mean±SE)					
	ALT ($\text{I } \mu\text{L}^{-1}$)	AST ($\text{I } \mu\text{L}^{-1}$)	Alkaline phosphatase ($\text{I } \mu\text{L}^{-1}$)	Uric acid (mg dL^{-1})	Total proteins (mg dL^{-1})	Blood glucose (mg dL^{-1})
Control	41.87±2.97	26.72±1.63	217.34±15.20	6.98±0.31	6.92±0.41	217.34±15.20
CuSO_4 (100)	44.23±2.30	29.65±1.31	220.20±19.10	7.01±0.32	7.22±0.51	200.00±21.01
CuSO_4 (250)	66.19±5.21**	33.61±1.97*	228.70±21.01	7.19±0.51	7.12±0.32	228.70±21.01
CuSO_4 (500)	71.98±5.21***	39.11±1.21***	272.40±23.60	8.63±0.27**	6.21±0.19	272.00±23.60
CuSO_4 (750)	87.32±7.81***	42.14±2.12***	300.11±27.10*	9.37±0.62**	5.68±0.41	300.611±27.10*
CuSO_4 (1000)	96.78±8.21***	45.78±3.15***	321.61±29.71*	10.64±0.89**	4.90±0.31**	321.61±29.71*

* $p<0.05$; ** $p<0.01$; *** $p<0.001$ (One way ANOVA and Post hoc Tukey-Kramer multiple comparison test was done individually for different parameters)

Table 3: Effect of CuSO₄ on serum levels of total lipids, cholesterol, HDL and LDL in quail

Treatment and dose (ppm/casein diet) (CuSO ₄)	Total lipids (mg dL ⁻¹)	Total cholesterol (mg dL ⁻¹)	HDL (g dL ⁻¹)	LDL (g dL ⁻¹)
Control	560.10±49.12	160.24±11.20	78.36±4.28	80.78±5.31
CuSO ₄ (100)	550.20±39.10	164.11±5.10	80.21±3.12	82.63±3.28
CuSO ₄ (250)	580.00±28.19	168.74±9.10	75.46±4.01	88.96±7.11
CuSO ₄ (500)	620.00±52.06	202.36±13.21*	90.61±7.21	106.37±6.91*
CuSO ₄ (750)	780.00±49.20*	250.61±21.13**	89.62±3.21	169.61±11.08***
CuSO ₄ (1000)	890.00±67.11**	278.31±23.10**	80.21±5.67	188.72±17.37***

*p<0.05; **p<0.01; ***p<0.001 (One way ANOVA and Post hoc Tukey-Kramer multiple comparison test was done individually for different parameters)

Table 4: Effect of CuSO₄ on plasma levels of glutathione peroxidase, superoxide dismutase and malonaldehyde in quail

Treatment and dose (ppm/casein diet) (CuSO ₄)	Plasma levels (Mean±SE)		
	Malonaldehyde	Glutathione peroxidase	Superoxide dismutase
Control	26.41±1.79	30.14±0.99	145.86±6.05
CuSO ₄ (100)	26.61±1.01	34.28±1.03*	159.39±7.12
CuSO ₄ (250)	33.82±1.27**	41.05±2.13**	169.86±9.31
CuSO ₄ (500)	39.83±2.01***	51.35±1.47***	214.73±8.99***
CuSO ₄ (750)	49.83±2.91***	63.25±4.05***	257.95±14.01***
CuSO ₄ (1000)	62.61±3.13***	79.42±3.71***	301.75±23.27***

*p<0.05; **p<0.01; ***p<0.001 (One way ANOVA and Post hoc Tukey-Kramer multiple comparison test was done individually for different parameters)

body weight observed at the lower doses and loss of body weight at the high dose of CSCD. These results clearly show that the loss of weight at the higher concentrations of Cu might have influenced the balance between the oxidant and antioxidant systems in favor of the former (Nishiyama *et al.*, 1998). The overload of Cu *in vivo* is reported to induce oxidative stress and changes in lipid profile, in addition to several pathological conditions (Bjorn *et al.*, 2003; Galhardi *et al.*, 2004). These changes might be responsible for the observed loss in weight of the birds.

The levels of AST and ALT levels were significantly increased, which indicated gross hepatic damage. Both the amino transferases are widely distributed in body but are particularly rich in the liver. Hepatocytes are virtually the only cells with high ALT content. Hence, increase of ALT in the serum has a high specificity for liver damage. Although, both AST and ALT are excellent indicators of damage to the liver, the increase of ALP is also linked with liver damage and cause hepatitis, bile duct obstruction and biliary cirrhosis (Sacher and McPherson, 1991). The increase of these enzymes clearly demonstrated that the treatment with CuSO₄ disrupts the liver function, more intensely at the higher doses. The elevation of ALP and uric acid levels in the serum revealed that the treatment with CuSO₄ in birds, also interfere with kidney function. Sacher and McPherson (1991) showed that elevation of ALP and accumulation of uric acid in kidney cause renal failure. Furthermore, our study showed an increase in the serum levels of blood glucose, which might be attributed to hepato-cellular damage leading to loss of the islets of Langerhan amidst glands of exocrine pancreas. Our observation on the elevation of ALT, AST, ALP, proteins and Blood glucose clearly demonstrate that CuSO₄ is toxic

to liver, kidney and pancreas. These results confirm the earlier observation that CuSO₄ might cause liver and kidney dysfunction, such as, hepato cirrhosis, jaundice (Bjorn *et al.*, 2003), hepato-renal failure (Yang *et al.*, 2004) and tubulo-interstitial nephritis (Galhardi *et al.*, 2004). These changes are attributed to hemolysis and methemoglobinemia caused by Cu through oxidative stress (Yang *et al.*, 2004). Free radicals and disorders of the antioxidant defense system are known to have a pathogenic impact on tissues and hence are seen as important factors in the development of various pathological conditions and diseases (Mahadik *et al.*, 2001; McCord, 2000).

The experiments on lipid profile revealed increase of total lipids at the higher doses. This corresponds to an increase of total cholesterol and LDL, observed in the present study. However, there was no change in the concentrations of HDL. The absence of any significant change in the HDL concentrations might be due to difference in the cholesterol content of KDL and HDL. The significant impact of CuSO₄ on LDL, cholesterol and total lipids is attributed to Cu-induced oxidative modification of LDL (Ide *et al.*, 1997). Furthermore, the genesis of Cu-linked free radical species, as evidenced by the increase of the plasma levels of MDA observed in the present study, might also be responsible for changes in the lipid profile. The results obtained in the present study, confirms a recent study (Becaria *et al.*, 2006), which showed Cu to be a redox active metal and can mediate the formation of reactive oxygen species.

The present study showed that oxidative stress, as measured by the thiobarbituric acid reaction for MDA, might be an important mechanism by which Cu exerts the changes in different biochemical indices, such as ALT, AST, ALP, uric acid, blood glucose, LDL, LDH and

cholesterol. Nevertheless, living cells have evolved numerous defense mechanisms to neutralize the harmful effects of free radicals. The antioxidant defense system includes enzymes, such as GSH-PX and SOD, which protect against the toxicity of oxidants (Huang *et al.*, 1996; Frei, 1999). In the present study, the plasma levels of GSH-PX and SOD were significantly increased after treatment with CSCD, which demonstrated an increase of antioxidant defense. However, despite the increase of the antioxidant defense enzymes, there was no difference in the toxicity observed in different biochemical indices. Taken together, results obtained in the present study revealed that the treatment with CuSO₄ might influence the balance between the oxidant and antioxidant systems in favor of the former, which results in weakening of the antioxidant defense. Nevertheless, the beneficial effects of CuSO₄ in poultry may be utilized by reducing the Cu-supplementation levels. Further experiments are suggested on characterization of dose-response relationship by mathematical modeling. This might require the use of a sufficient number of lower doses of CuSO₄ to determine a threshold dose below which there would be no toxicity.

CONCLUSIONS

Cu causes weakening of the antioxidant defense system, which is a threat for many pathological disorders. Hence, the supplementation of Cu in feed of birds and poultry may be reduced to a threshold dose below which there would be no toxicity.

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