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Full Length Article

Hepatic Injury Induced by Hexavalent Chromium in Chickens

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Abstract

The aim of this study was to determine the effect of different doses of chromium on liver in chickens. For this purpose, a total of 200 chickens were chosen and randomly divided into four equal groups: control group administered with normal diet; chromium groups (three equal groups) were treated with basal diet supplemented with chromium @ 50, 100 and 200 mg/kg. The mortality rate, liver pathological changes, the contents of serum antioxidant capacity and biochemical were examined on day 14, 21, 28 and 35 from each group. The results showed that chromium can increase the number of deaths of chickens and induce hepatotoxicity significantly ($P<0.05$). Moreover, the high dose of chromium increased the contents of AST, GGT and ALT in serum along with increased the oxidative stress via decreasing the level of SOD, CTA and GSH-Px. These results suggest that irrational use of chromium can cause toxic affect in chickens. Thus, our study provides the experimental basis for the practical applications of chromium as feed additive in chickens. © 2018 Friends Science Publishers

Keywords: Hexavalent chromium; Hepatotoxicity; Oxidative stress; Chickens

Introduction

Chromium element is one of the important chemical raw materials with atomic number 24 and molecular weight 51.996, and it plays an important role in the printing, dyeing, leather making and metallurgy industry (Shahid *et al.*, 2017; Wang *et al.*, 2017). Chromium improves the production performance, carcass quality, reproductive performance and immunity in the animals, so its trace amount is essential element in the diet as one of the indispensable trace elements in animals (Zha *et al.*, 2009; Gładysz-Płaska *et al.*, 2012; Shahid *et al.*, 2017). In the normal metabolism of chromium, it is always combined with albumin and transported to the blood circulation system (Zhang *et al.*, 2012). Chromium is commonly present in two oxidized forms: the most common and stable valences of chromium are chromium Cr (VI) and trivalent chromium Cr (III) (Sarkar *et al.*, 2013; Fang *et al.*, 2014). It is generally believed that hexavalent chromium is more easily absorbed and stored by the body, but it is more toxic (Yang *et al.*, 2013). Whereas, Cr (VI) is a soluble oxidizing agent that is easily absorbed and reduced intracellularly to Cr 5C and reacts with nucleic acids and other cellular components to inhibit RNA synthesis that create irritating, carcinogenic, antigenic, and corrosive properties in biological systems (Yang *et al.*, 2013; Rahman *et al.*, 2015; Shahid *et al.*, 2017). Hence hexavalent chromium Cr (VI) is classified as a priority pollutant, therefore, remediation of Cr(VI) is essential to protect animal health. The chromium mainly distributed in heart, liver, lung, spleen, kidney and other tissues. Many diseases are associated with chromium deficiency (Yang *et al.*, 2013; Rahman *et al.*, 2015). Chromium is a potential immunomodulator for some specific immune responses that can improve immunity of animal (Zha *et al.*, 2009).

Underwood found that the symptom of chromium deficiency is abnormal of glucose intolerance; however, chromium can improve glucose tolerance and increase blood glucose levels in rats (Underwood, 1987). Chromium supplementation can reduce blood fat and prevent fatty deposits; moreover chromium has a protective effect on the vascular endothelial cells by reducing plasma lipids to enter into the blood vessel walls (Togna *et al.*, 1988). Thereby, chromium supplementation can reduce endothelial cell permeability to reduce the incidence of atherosclerosis (Vinson *et al.*, 2002). Recent studies have shown that as an auxiliary factor for insulin, chromium plays an important role in the protective effect on myocardial cell injury via

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maintaining the stability of the membrane and enhancing the antioxidant capacity that may be an important mechanism for the resistance of chromium to myocardial injury (Peters *et al.*, 1982).

In recent years, chromium and its compounds has been widely used in animal husbandry, often appear as feed additive in feed, whereas some studies have shown that a large amount of chromium can lead to acute toxicity due to its irritating, carcinogenic, antigenic and corrosive effects when it is absorbed by the intestinal tract and skin (Yang *et al.*, 2013). This study aimed to evaluate the toxic effect of chromium supplementation on chickens that overuse of chromium can suffer toxic affect. Thus, this study provides the experimental basis for the practical applications of chromium as feed additive in chickens.

Materials and Methods

Ethics Approval

This study was conducted according institutional Animal Welfare and Research, Ethics Committee guideline of Huazhong Agricultural University, Wuhan, China.

Animal Treatment and Experimental Groups

Two hundred (n = 200) day old Arbor Acres (AA) broiler chickens of weighing were provided by Chia Tai Animal Husbandry Co. Ltd., Wuhan, China. The chickens were raised according the standard temperature and hygienic environment. After 7 days, the chickens were equally divided into the control group (CG, n = 50), low dose chromium group (LG, n = 50), middle dose chromium group (MG, n = 50) and the high dose chromium group (HG, n = 50). The CG was fed a standard normal diet ad libitum as suggested by the National Research Council, while the LG, MG and HG were fed a normal diet supplemented with chromium @ 18.56, 37.12 and 74.24 mg/kg of feed, and then the mortality in each group were recorded. Ten chickens (n=10) on days 14, 21, 28 and 35 from each group were sacrificed by cervical dislocation and the livers were photographed and stored at -80°C for future use. Some livers from different groups were collected and fixed in 4% paraformaldehyde (hematoxylin & eosin staining). Blood samples were collected by anterior vena cava, and samples were centrifuged at 3000×g for 20 min for separation of serum and stored at -20°C.

Histological Assessment of the Liver

The livers were cleaned in PBS and fixed in 4% paraformaldehyde by immersion according to previous studies (Zhang *et al.,* 2017a). Fixed pieces were washed with 70% ethanol, then dehydrated and embedded in paraffin, 5-μm-thick histological sections were cut and stained with hematoxylin and eosin (H&E) according to the method of Zhang (Zhang *et al.,* 2017b). The samples were observed with the light sensitive microscope.

Contents of Serum Antioxidant Capacity and Biochemical

The SOD, GSH-Px and CAT activity in liver were assessed using assay kits (Nanjing Institute of Biological Engineering, Inc. Jiangsu, China) by following manufacturer's instructions, while the AST, ALT and GGT activities were presented using commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to previous studies (Zhang *et al.,* 2017c).

Statistical Analysis

The results were analyzed with one way ANOVA by student t-test to compare the differences among control and chromium groups using SPSS 18.0 software. The results are presented as means ± SD, the values of $P < 0.05$ were considered as statistically significant.

Results

Toxic Effect of Chromium on Mortality of Chickens

The chicken mortality rate assay indicated that there was considerable mortality in chromium group compared with control group chickens as shown in Fig. 1. The number of deaths was increased with the increasing dose of chromium, but the low dose chromium did not have significant toxic effect to chickens.

Morphological and Pathological Examination of Live

Liver samples were subjected to gross lesions and histopathological examination by H&E straining. Fig. 2 showed that compared with the normal groups, chromium poisoning groups had poor liver condition with irregular surface, nodularity and pale color. The microscopic morphology of the liver showed the diapedesis, hemolysis, degeneration, necrosis and inflammation, nucleus pycnosis and central phlebectasia with the increased dose of chromium. However, there is no significant difference between control groups and low dose of chromium (Fig. 3).

Biochemical Criterion Analysis of Serum

A significant increase in AST, GGT and ALT contents in the serum were observed during the entire experiment period (from day 14 to day 35) in chromium groups as compared to control group, but near to control group in LG. Moreover, the contents of AST, GGT and ALT increased significantly (P<0.05) in MG and HG as compared with LG (Fig. 4A, B and C).

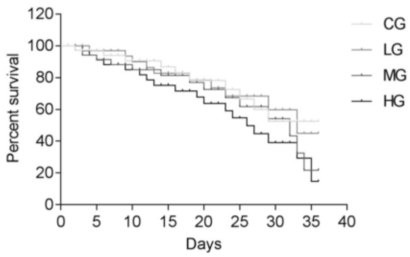

Fig. 1: The percentage of survival among control groups (CG), low chromium groups (LG), middle chromium group (MG) and high chromium groups (HG) of chickens during the whole experiment period

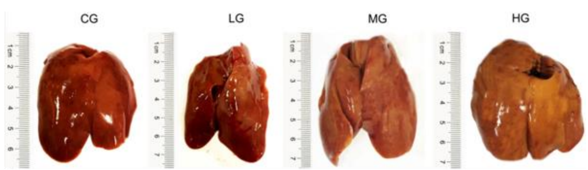

Fig. 2: Gross photographs of liver analysis among different groups with the toxic effects of chromiun

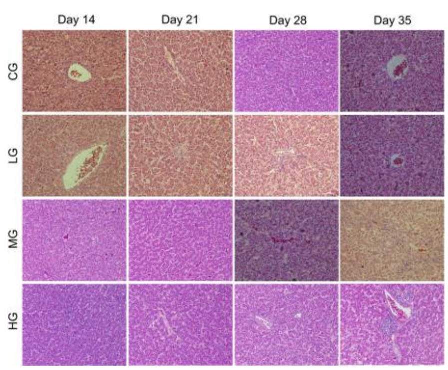

Fig. 3: The pathological observation of liver with the used of different concentration of chromium as compared with normal groups

Determination of Oxidative Stress in Liver

The hepatic toxicity and oxidative stress in liver samples was measured to evaluate the toxic affect of chromium in broiler chickens. The level of SOD, CTA and GSH-Px were significantly decreased (P<0.05) in chromium administration broiler chickens as compared to control group. Meanwhile, the level of SOD, CTA and GSH-Px were significantly decreased with the increased of chromium administration in chickens (Fig. 5D, E and F).

Discussion

Chromium is one of the important heavy metals which has been employed in numerous industry and manufacturing. However, there is less data is available about its uses associated with health risks (Yang *et al.*, 2013). Hexavalent chromium is a stable form of Cr which is widely used in metal processing, electroplating, leather industry, and other industrial processes. It has been associated with oxidative stress, cytotoxicity, and carcinogenicity via damage to the respiratory system, digestive system and skin as result of constant exposure to chromium through the respiratory tract, alimentary canal and skin in human (Yang *et al.*, 2013). Moreover, chromium (Cr) is one of the most important feed additives, and it has been widely used in animal husbandry (Underwood, 1987). The irrational use of chromium can suffer the occurrence of chromium poisoning in animal husbandry. Though studies declared that chromium is not an essential element for mammals or humans, there are studies found that Cr (III) is a micronutrient benefiting animals by using it as a feed additive to favors animal growth. Liver is known as a toxic target organ of Cr (VI) and other heavy metals, and liver also aids in the metabolism and detoxification of heavy metals and other foreign substance (Permenter *et al.*, 2011; Pablack *et al.*, 2014).

Our study was aimed to check the toxic effect of Cr (VI) on chickens. The death rates revealed adding more than a certain proportion of chromium would result in increased mortality in broilers, whereas less than that proportion would have no obvious influence. Histopathology and the observation of liver results showed that though there was some fat in the control group and low dose group, they were normal throughout the growth phase on a whole. However, necrosis could be found in the pathological sections, and yellowing could be found in the whole liver, which revealed injury/disease in livers. ALT, AST, and GGT are indexes for hepatic pathological changes, and ALT is more sensitive among the three indexes. Biochemistry results suggested an increase in ALT, AST, GGT, which is in line with previous study (Soudani *et al.*, 2011). It showed the changes in livers and indication for injury and diseased. SOD can transform the harmful superoxide radicals into hydrogen peroxide, and CAT turn hydrogen peroxide into water, and they can respond to the body's antioxidant capacity. GSH-Px is a vital enzyme that catalyzes the decomposition of hydrogen peroxide, and it can specifically catalyze reduction of glutathione (GSH) to hydrogen peroxide, which protects the structure and functions of membrane. Soudani *et al.* (2011) declared that exposure of chromium to rats lead to a decrease in GSH-Px in rat liver. Our results also showed a marked reduction of GSH-Px in chicken liver. However, a decrease of SOD and CAT is in our study is different from previous study. Our results were in line with Hao *et al.* (2017) who suggested that chronic exposure to Cr (VI) can induce oxidative stress and histological changes in chicken brains. So, our study declares that Cr (VI) causes hepatic injury in chickens.

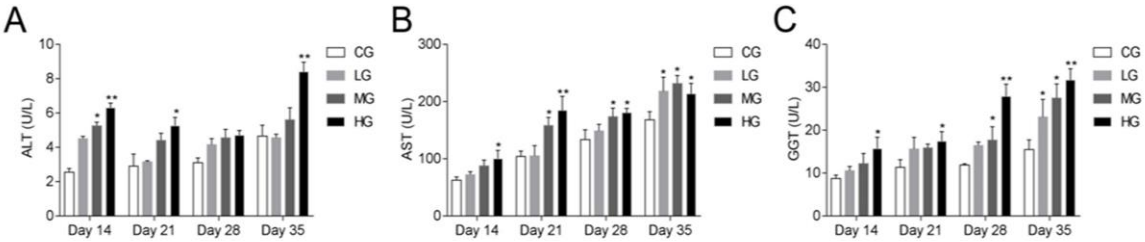

Fig. 4: The serum biochemical analysis in chicken at 14, 21, 28 and 35 d among different groups. The data are expressed as the mean ± SD, **P*<0.05, ***P*<0.01

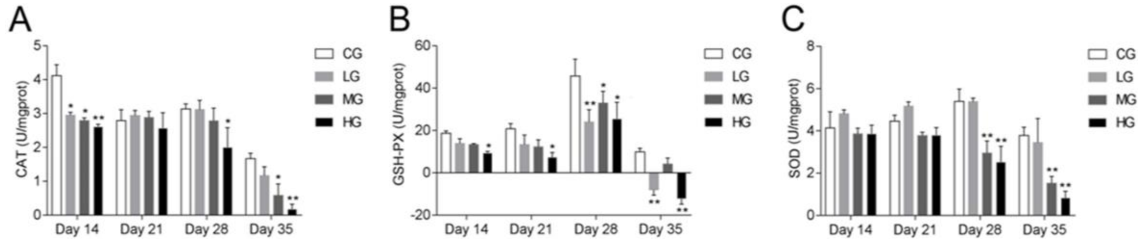

Fig. 5: The antioxidant activities analysis of liver in chicken at 14, 21, 28 and 35 d among different group. The data are expressed as the mean ± SD, **P*<0.05, ***P*<0.01

Conclusion

We can say that irrational use of chromium can cause toxic affect in chickens. This study provides important information of chromium as a feed additive and scientific basis for hepatic injury in chickens.

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