



Bulgarian Journal of Veterinary Medicine, 2018, **21**, No 4, 470–477

ISSN 1311-1477; DOI: 10.15547/bjvm.1095

Original article

EFFECTS OF TRIVALENT AND HEXAVALENT DIETARY CHROMIUM ON BLOOD BIOCHEMICAL PROFILE IN JAPANESE QUAILS

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Summary

Alijagic, A., E. Islamagic, M. Focac & D. Suljevic, 2018. Effects of trivalent and hexavalent dietary chromium on blood biochemical profile in Japanese quails. *Bulg. J. Vet. Med.*, **21**, No 4, 470–477.

Trivalent chromium is an essential trace element for humans and animals and it is the final product of hexavalent chromium reduction in the cell. It is an integral part of the glucose-tolerance factor, which increases the systemic impact of insulin. The aim of this study was to analyse the effect of trivalent and hexavalent chromium on selected biochemical parameters in Japanese quails. Birds were divided into three groups: group I (control group, n=12), group II (treated with trivalent chromium, n=12) and group III (treated with hexavalent chromium, n=12). During 20 days of follow-up, control and experimental groups of quails had identical diet; chromium was added daily into the food (5 mg/kg) and water (5 mg/L) of the experimental groups. Glucose concentrations were the highest in Group III, while lowest glucose concentrations were obtained in Group I. There were higher differences regarding blood biochemical parameters between control group and quails treated with Cr(VI). Our results showed that hexavalent chromium had negative effects on biochemical parameters in quails, while treatment with Cr(III) showed minor fluctuations in concentrations or activities of blood biochemical parameters.

Key words: biochemical parameters, birds, diet, metals, serum, toxicity

INTRODUCTION

Extracellular, water-soluble, hexavalent chromium exists in form of chromium oxyanion. The reduction of hexavalent chromium is usually mediated by non-enzymatic reductants, such as: ascorbate (vitamin C), low molecular weight thiols, GSH and cysteine (Shati, 2014). Trivalent chromium is the final product of hexava-

lent chromium reduction in the cell (Zhitkovich, 2011).

To date, a lot of useful information related to identification and the positive effect of derivatives of chromium with a different valence was collected. Chromium chloride is most important mineral form of chromium used as a dietary supplement (Uyanik *et al.*, 2002). Large

number of studies showed that organic chromium in form of chromium picolinate and chromium nicotinate might be used in diets because of their positive influence on reduction of serum glucose and cholesterol concentrations in laying quails (Yildiz *et al.*, 2004). Additionally, inorganic compounds containing trivalent chromium, such as chromium chloride, also, to a lesser extent, had a positive effects on biochemical parameters as well as physiological and anatomical characteristics of the animals and humans (Boudjouk & Ho So, 2007). Earlier studies showed that Cr(III) in its inorganic form does not affect the cholesterol concentration in the serum of quails and other experimental animals (Thomas & Gropper, 1996) as well as in the human serum. Therefore, the World Health Organization recommended limited intake of Cr(III) as antihyperglycaemic and antihyperlipidaemic agent, with emphasis on the necessity of using organic Cr(III) compounds, due to their harmlessness, higher solubility and lack of its precipitation affinity in the organism. Otherwise, Cr(VI) and its derivatives, showed extremely toxic effect which results in various point mutations in DNA and to chromosomal damage, as well as to oxidative changes in proteins and adducts formation (Dayan & Paine, 2001).

Alanine aminotransferase (ALAT) and succinate dehydrogenase increased severalfold, especially ALAT, whose serum concentration was elevated up to four times in broiler chickens (Króliczewska *et al.*, 2004). The concentration of bile acids was also twice higher regardless of the gender. Results regarding cholesterol concentration were not uniform. A study (Króliczewska *et al.*, 2004) reported decreased cholesterol in broiler chickens along with lower concentration of triglycerides. Concentration of creatine kinase

increased twice after chromium application in chickens (Cupo & Donaldson, 1987). Trivalent chromium is an essential trace element in humans and animals (McDonald *et al.*, 1992). Expressed interest in monitoring the utilisation of chromium supplemented to broiler chickens diet has been shown (Króliczewska *et al.*, 2004). As in the case of triglycerides, studies showed that short-term application of Cr(VI) (up to a month) decreased the concentration of total proteins in chickens.

The positive effect of chromium on human health is related to fact that chromium is an integral part of the glucose-tolerance factor (GTF) that increases the impact of insulin, one of the most important anabolic hormones (Anderson & Polansky, 1995; Cefalu & Hu, 2004). GTF consists of one trivalent chromium atom linked with several molecules of niacin (vitamin B₃) and amino acids found in glutathione (glutamic acid, glycine and cysteine) (Cefalu & Hu, 2004). However, several studies have investigated the association between chromium valence and its carcinogenicity (Langard, 1990; Zhitkovich, 2011).

Hexavalent chromium passes easily through biological membranes and reacts with proteins and nucleic acids inside the cell, which potentially had negative impact on cell metabolism and concentration of blood biochemical parameters.

The aim of this study was to analyse the effects of trivalent and hexavalent chromium on selected biochemical parameters in Japanese quails (*Coturnix japonica* Temminck & Schlegel, 1849) to determine if trivalent chromium as a convenient dietary supplement was safe for use. Also we aimed to evaluate whether hexavalent chromium should be definitely characterised as toxic or should we start to re-consider its pleiotropic effects.

MATERIALS AND METHODS

Breeding

All individuals used in the study were females and they come from a private farm in Vogošća, Bosnia and Herzegovina. Growing of quails was carried out according to standardised procedures and optimal conditions for this species (Randall & Bolla, 2008). All animals were treated in accordance with the Declaration on the Rights of Animals and Universal Declaration on Animal Welfare.

Experimental design

Quails used in this experiment were 37 days old. Experimental design included three groups: group I (control group, n=12), group II (quails treated with trivalent chromium, n=12) and group III (quails treated with hexavalent chromium, n=12). All birds had identical diet consisting of corn grits, however chromium supplements were added in food and water to the experimental group of animals (5 mg of chromium supplements per liter of water and 5 mg per kilogram of food). During the 20 days of chromium treatment, supplementation was repeated daily.

Chromium derivatives

Chromium (III) chloride (CrCl_3) is a derivative of trivalent chromium, stable in two forms: hydrated and anhydrous. During the experiment the anhydrous chromium (III) chloride (Semikem, Bosnia and Herzegovina), a solid purple coloured compound was used. Chromium (VI) oxide (CrO_3) is a derivative of hexavalent chromium, (IUPAC name chromium trioxide). Chromium (VI) oxide (Semikem, Bosnia and Herzegovina) was used during the experiment.

Sampling and biochemical analysis

Blood samples were collected by jugular vein venipuncture and rapid decapitation. Venipuncture is performed by using a sterile insulin syringe. Collected blood samples were transferred to test tubes without anticoagulants for serum analysis. The next step was centrifugation (Heraeus-Sepatech, Germany) at 2000 rpm for 10 minutes. Analyses of biochemical parameters: triglycerides, cholesterol, proteins, lactate dehydrogenase (LDH), creatine kinase (CK), creatinine and urea were performed immediately after blood collection and by spectrophotometric methods (Zuzi 4110ED, (Auxilab, Spain). Glucose concentration were measured by using glucometer Accu-Check Code 268 (Roche, Switzerland).

Statistical analysis

Results were analysed by using SPSS (v. 20; SPSS, Inc., Chicago, IL, SAD) software for estimation: mean values of different biochemical parameters and statistically significant difference in all above mentioned parameters with regard to factors supposed to cause variation. ANOVA was used to test statistical differences between groups I, II and III. *Post hoc* multiple tests (Tukey HSD) were also done, for the detection of pairs of groups between which the differences are significant. P values lower than 0.05 ($P < 0.05$) considered significant and p values lower than 0.01 ($P < 0.01$) considered highly significant.

RESULTS

Values (triglycerides, cholesterol, proteins, creatinine and urea) and activity (lactate dehydrogenase and creatine kina-

Table 1. Mean± SD (n=12) values of observed blood biochemical parameters of Japanese quails

Parameter	Group I (Control, n=12)	Group II (Cr ³⁺ , n=12)	Group III (Cr ⁶⁺ , n=12)	Level of significance
Triglycerides (mmol/L)	1.19±0.31	1.16±0.36	1.85±0.13	P _{I-III} <0.001; P _{II-III} <0.001
Cholesterol (mmol/L)	6.91±0.89	5.52±0.36	7.85±1.34	P _{I-II} <0.01; P _{I-III} <0.05; P _{II-III} <0.05
Proteins (g/L)	47.22±7.24	39.73±3.03	32.37±3.94	P _{I-II} <0.05; P _{I-III} <0.001; P _{II-III} <0.05
LDH (U/L)	745.27±10.79	776.09±11.84	795.20±10.42	P _{I-II} <0.001; P _{I-III} <0.001; P _{II-III} <0.001
CK (U/L)	204.98±26.78	416.33±21.68	744.16±24.70	P _{I-II} <0.01; P _{I-III} <0.05; P _{II-III} <0.05
Creatinine (µmol/L)	34.44±5.47	32.03±3.78	29.39±1.62	P _{I-III} <0.01
Urea (mmol/L)	5.26±0.87	5.09±0.86	7.14±1.01	P _{I-III} <0.001; P _{II-III} <0.001
Glucose (mmol/L)	13.32±1.95	16.56±2.08	17.48±1.52	P _{I-II} <0.001; P _{I-III} <0.001;

se) of biochemical parameters are presented in Table 1.

Comparing the quails from the control group and quails treated with Cr(III) (I vs II) we found that differences regarding triglyceride, creatinine and urea concentrations were not statistically significant; however all other biochemical parameters showed statistically significant difference. Otherwise, when we analysed differences between control group and the quails treated with Cr(VI) (I vs III), we found that all parameters were statistically different. Concentrations of the creatinine and the glucose were not significantly different when we compared quails treated with Cr(III) and Cr(VI) (II vs III).

DISCUSSION

Until today, large number of studies showed that organic chromium in form of chromium picolinate and chromium nicotinate might be used in diets because of their positive influence on reduction of serum glucose and cholesterol concentrations (Yildiz *et al.*, 2004). Data from few animal experiments indicate that with equal solubility, Cr(VI) compounds are absorbed more readily than Cr(III) compounds, probably because Cr(VI) readily penetrates cell membranes. Chromium had no effect in reducing body and liver weights as well as liver and serum cholesterol concentrations.

An explanation for low triglyceride values in all individuals analysed in our

study, probably lies in the fact that all birds were very young and that they were fed exclusively corn grits which have low content of lipid compounds. Cr(III) did not show high impact on values of triglyceride concentration in serum (Group II) and were not statistically different compared to control group ($P>0.05$). After its absorption in the digestive tract, Cr (III) is "trapped" by the erythrocytes and plasma proteins, so chromium in trivalent form could not convey a significant change in triglyceride serum concentrations (Yildiz *et al.*, 2004). Cr(VI) shows cytotoxic irreversible effects on hepatic tissue, thereby inhibits the synthesis of triglycerides (Cuppe & Donaldson, 1987), which in turn leads to a reduction of triglyceride concentration in serum. Different results in our study may be related to the significantly shorter period of exposure to Cr(VI), compared to the exposure periods in the cited study. Also, other studies showed that long-term effects of the introduced Cr(VI) are correlated with the selection of the method for nutrient intake and period of Cr(VI) elimination from the body (Kitagawa *et al.*, 1988). In this study we used Cr(VI) oxide that may cause lipid peroxidation, which could explain high level of triglycerides. High urea levels may be consequent to renal disfunction, due to toxic effects of Cr(VI).

The concentration of cholesterol in Group III was increased ($P<0.05$), which is in accordance with previous researches on the effects of Cr(VI). The reason for higher cholesterol concentration probably lies in the fact that Cr(VI) has a large oxidation potential. Cr(VI) is referred as the remarkable pro-oxidant that causes a severe form of cellular stress that subsequently leads to lipid peroxidation of the plasma membrane, which is rich in cholesterol, and hence its significantly increased

concentration in serum of individuals in Group III ($P<0.05$) (Tonks, 2003).

The activity of lactate dehydrogenase (LDH) was significantly changed in the experimental groups ($P<0.05$), especially in quails treated with Cr(VI). LDH activity in quails treated with Cr(III) was significantly changed. Interestingly, even in the control group, the activity of LDH was not within the reference range (Sakas, 2002; Scholtz *et al.*, 2009; Sokoll *et al.*, 2015). This increased LDH activity in the control group might be related with growing of quails under stress conditions (cage breeding). A recent study showed that birds in stressful conditions develop more diseases due to cellular stress and lipid peroxidation, accordingly they should be bred with a certain amount of dietary antioxidants (Çiftçi *et al.*, 2016). The trend of the increased LDH activity, due to application of chromium (III) chloride, would certainly continue if individuals are exposed to long-term intake of Cr(III), probably because of the increased accumulation of chromium ions and their irreversible binding to the cellular proteins. LDH activity was also increased in Group III of quails which is a result of the destruction of parenchymal tissues containing LDH due to extremely toxic effects of Cr(VI) on cell membranes. Large amounts of LDH enter the circulation as a result of cell death and increased cell membrane permeability (Lewandowski *et al.*, 1986; Binev *et al.*, 2014). Destruction of parenchymal tissues containing LDH (heart, kidney, liver, muscle and other tissues) appears as the consequence of the hydrolytic lysosomal enzymes activity, released due to reduced membrane integrity (Anderson & Polansky, 1995).

The values of the urea and creatinine serum concentrations were significantly changed only in Group III ($P<0.05$); how-

ever in quails treated with Cr(III) statistically significant difference was not obtained ($P>0.05$). Values of both parameters changed, but remained within the reference range in all three analysed groups (Ukashatu *et al.*, 2014). Cr(III) caused a slight decrease of the urea and creatinine concentrations, which is similar compared to recent studies concerning the effects of Cr(III) (Shinde & Goyal, 2003). Urea is found in the plasma of birds in very small quantities, therefore, its role in the assessment of renal efficiency is still questionable. Creatinine is quite "unadequate" parameter for monitoring of renal efficiency, because the birds excrete creatine before its conversion to creatinine (Lewandowski *et al.*, 1986; Lumeij & Remple, 1991). However, values of these two parameters indicated that Cr(VI) decreased glomerular filtration and function of renal tubules, especially in Group III.

Serum glucose levels in Groups II and III were significantly different compared to control group. However, many studies showed decrease in serum glucose level due to the effects of Cr(III) on pancreas chromium-sensitive tissue, which indirectly via LMWCr and GTF modulates glucose metabolism, primarily by enhanced activation of the insulin receptor and stimulation of insulin signalling pathway (Kleefstra *et al.*, 2004). Elevated levels of glucose obtained in Group II resulted from several factors: stress during breeding (Çiftci *et al.*, 2016), insolubility of chromium chloride, and development of oxidative stress or development of endoplasmic reticulum stress (Hu *et al.*, 2011). Effects of Cr(VI) were arguably stronger and mainly the result of generating numerous biomarkers of oxidative stress.

Higher differences regarding biochemical parameters were noted between control group and quails treated with

Cr(VI). In general, Cr(VI) had toxic effect on quail organism which is manifested with increased values of biochemical parameters except for protein and creatinine concentrations. It could be suggested that Cr(VI) had harmful influence on functioning and physiological processes of kidneys and muscles. Minor fluctuations regarding concentration or activity of biochemical parameters were observed in quails when they were treated with Cr(III). It would be necessary to do additional analysis regarding the effect of chromium on the biochemical parameters which will confirm or reject the results obtained in this study. Follow-up period in this study was relatively short to make definitive conclusions regarding the effects of trivalent chromium, therefore future research should examine the long-term effects of trivalent chromium as dietary supplement

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Paper received 03.12.2016; accepted for publication 06.02.2017

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