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The role of natural dietary antioxidants in animals under oxidative stress

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Abstract: Among the main environmental stressors the most toxic are heavy metals including cadmium (Cd). Stress conditions caused by Cd are associated with overproduction of free radicals following a disturbance in prooxidant-antioxidant balance and tissue damage. The protective action of nutrients with natural antioxidative activities against Cd-induced oxidative stress in chickens was investigated. The experimental analyses demonstrated enhanced production of reactive oxidation species and immunosuppressive effect in Cd-exposed chickens. As antioxidant dietary supplements the salts of zinc ($ZnCl_2$) and selenium (Na_2SeO_2), and vitamin C (ascorbic acid) were used in the experiments with chickens administered orally 100 mcg of Cd (water solution of $CdCl_2$). The antioxidative effect of developed new natural innovative product from red beetroot (fractionated juice) was studied in additional experiment, when chickens exposed to Cd with diet (50 mg/kg). Experimental results demonstrated the prospect of preventive role of Zn, Se, ascorbic acid, and beetroot fractionated juice in the improvement of Cd-induced disorders in the body in case of environmental pollution with heavy metals.

Key words: cadmium, zinc, selenium, ascorbic acid, red beetroot juice, pro/antioxidative action

Introduction

The harmful effect of the most dangerous environmental heavy metal cadmium (Cd) is accompanied by an antioxidant-prooxidant imbalance in animal organs and tissues. Cd catalyzes the formation of reactive oxygen species such as superoxide anions, hydroxyl radicals, and hydrogen peroxide in cell membranes following the oxidative stress and the risk of developing metabolic disorders and diseases (Bull, 2010). Oxidative processes are believed to be leading causes of the detrimental consequences of stress in biological systems and resulted in disturbance of antioxidant defense system (Surai, 2003). Natural antioxidants including minerals and vitamins play a vital role in the maintenance of antioxidant status in biological systems

(Surai, 2019). The protective effect of dietary zinc (Zn) and selenium (Se) against Cd toxicity was studied in broilers (Zoidis et al, 2020). Zn is one of the most important essential trace elements and forms an integral component of several cellular enzymes. Moreover, the structural similarities of the Zn and Cd atoms ensure their competitive interaction and leads to their physiological antagonism. The protective effect of Se against Cd-induced hematological disturbances, immunosuppressive, oxidative stress and hepatorenal damage was revealed in rats (El-Boshy et al., 2015). Vitamin C or ascorbic acid (AA) belongs to antioxidant vitamins and is capable of scavenging both superoxide and hydrogen peroxide (Elias A. & Deo, 2013). Dietary natural antioxidant supplements are used to protect the body from the oxidative stress consequences. A special interest for these purposes is the use of the local natural sources – such as beetroot juice and its innovative products. The technology of fractionated red beetroot juice was developed and obtained in the Institute of Innovative Biomedical Technology (Latvia).

The aim of the present study was to investigate the protective action of natural antioxidants as additional dietary support for the antioxidant defence system in Cd-exposed chickens.

Materials and methods

Ethics Statement

All experimental procedures were approved by the animal Ethics Committee of the Food and Veterinary Service (Riga, Latvia, authorization reference number 53 (April 10, 2012).

Material

For the experimental dietary additions, used for two different experiments, the following supplements were used:

1. Chemicals: Cd (cadmium chloride, $CdCl_2$,), Zn (zinc chloride, $ZnCl_2$), Se (sodium selenite, Na_2SeO_2), AA (ascorbic acid) obtained from Sigma Chemical Co, EU.
2. Fermented fractionated (FF) beet root juice obtained from squeezed and centrifuged red beet root juice by the fermentation during 48 h (at 25 °C) using activated culture of *Saccharomyces cerevisiae* (2 $g{\cdot}L^{-1}$). (Babarykin et al., 2018)

Experimental design and animals

Two different experiments on Cd-exposed male Lohmann brown chickens were undertaken.

The first treatment was performed on 1 to 34 days old cockerels. For the first 20 days, all chickens were fed a standard starter fool-fed basal diet (BD-1). Then the birds were divided into 5 groups of 25 heads each. Group 1 (Control-1) continued

to consume BD-1 without any supplements. Chickens of the other 4 groups during next 14 days daily were administered *per os* 100 mg of cadmium (water solution of $CdCl_2$) per 100 g of body mass and also provided the same diet but differed by dietary supplements: Group 2 (+Cd) – fed the BD-1 without any supplements. Group 3 (+Cd+Zn) received BD-1, calculated to contain zinc 500 mg kg^{-1} (as $ZnCl_2$). Group 4 (+Cd+Se) consumed BD-1, calculated to contain 1 mg selenium kg^{-1} food (as Na_2SeO_2). The same BD-1 of Group 5 (+Cd+AA) was supplemented by 100 mg AA kg^{-1}.

For the second treatment 35-day-old male Lohman Brown chickens were obtained and used for 10 days of investigation. The chickens were divided into 4 groups of 7 heads each. Group 1 (Control-2) fed corresponding for the birds of this age standard grower fool-fed basal diet (BD-2) without any supplements. Group 2 (+Cd) was given the same BD-2 but supplemented by Cd 50 mg kg^{-1} ($CdCl_2$). Group 3 (+FF) consumed the BD-2 and each chicken was administered by 1 ml of FF *per os* daily. Chickens of Group 4 (+Cd+FF) were fed the same diet as Group 2 but administered *per os* by 1 ml of FF daily.

At the end of experiments, chickens were weighed and sacrificed by decapitation in accordance with the Recommendation for Experimental Animals of the European Convention (Close et al., 1997). Whole blood, blood serum and liver of chickens were collected and used for analyses.

Biochemical and immunological assays

The content of cadmium and zinc in blood serum and liver was determined by atomic absorption spectrophotometer Perkin-Elmer (model AAnalyst 700) according to the procedures of the AOAC (1999). The content of selenium in whole blood and liver was estimated by fluorometry applying 2,3-diaminonaphthalene reagent (AOAC, 1997). The antioxidant status was evaluated by measuring the level of lipid peroxidation product malodialdehyde (MDA) in liver homogenate by the thiobarbituric acid reaction (Surai et al., 1996). Activity of glutathionperoxidase (GSH-P_x) in liver homogenate was measured based by modified the method described by Pinto and Bartley (1969).

Immunological analyses

The parameters of humoral immunity were investigated using the complex of methods presented in Vasilyeva et al., (2001). Serum lysozyme content was evaluated by a modified nephelometric assay by determining the decrease in turbidity of a suspension of *Micrococcus lysodeicticus*. Nonspecific circulating immune complexes (CIC) in blood serum were estimated spectrophotometrically using precipitation with polyethylene glycol.

Statistical analyses

All statistics were performed using the software *Statistica* 7. Results of Cd, Zn, Se content and biochemical parameters are presented as mean ± SE. Multiple group comparison was done using *one-way* ANOVA and *Post-hoc* Tukey HSD test.

Results and discussion

Cd-exposed chickens during the experimental periods of both presented treatments showed no evidence of clinical toxicity. However, a slight tendency of chicken growth suppression was observed in both Cd exposure experiments.

Administration of 100 mg of cadmium for 14 days *per os* caused a non-significant decrease of chicken body mass by 3.0% ($P > 0.05$) compared to the Control-1: 421.6 ± 20.4 g *vs* 434.8 ± 22.2 g. After consumption of the diet supplemented by Cd 50 mg kg^{-1} for 10 days chicken body mass was very close to the Control-2: 473.4 ± 19.8 g *vs* 480,8 ± 23.6 g and tended to decrease only by 1.6% ($P > 0.05$).

Amelioration of the adverse effect of Cd had observed when antioxidants Zn, Se and AA were added to the diet and when beet juice FF was given *per os* to Cd-treated chickens.

At the first treatment body weight of Cd-co-administered chickens with Zn, Se and AA on average approached the Control-1, and by 3–6% ($P > 0.05$) non-significant exceeded +Cd group. At the second experiment administration of FF to the Cd-treated birds resulted in a non-significant (about 2%, $P > 0.05$) increase in body mass.

It is possible that the small negative effect of Cd on chicken growth was due to the short period of Cd exposure experiments.

A more noticeable effect of Cd was manifested in the blood serum of chickens. Both when cadmium was administered to chickens *per os,* and when it supplemented in the diet, the concentration of Cd in the blood serum significantly increased by 40% and 134% correspondingly (Table 1 and Table 3). Analysis of biochemical parameters after Cd exposure at the first treatment showed a tendency to decrease blood Zn and Se levels in chickens of +Cd group.

Table 1. Effect of antioxidative dietary supplements on the concentration of trace elements in blood of Cd-exposed chickens

Group	Cd in blood serum, $\mu g \cdot dL^{-1}$	Zn in blood serum, $\mu g \cdot dL^{-1}$	Se in whole blood, $\mu g \cdot dL^{-1}$
Control-1	$5.0 \pm 0.6^{a*}$	$300.0 \pm 10.3^{a\,b}$	14.3 ± 1.5^{a}
+Cd	7.0 ± 0.5^{b}	276.0 ± 17.9^{a}	12.2 ± 2.0^{a}
+Cd+Zn	4.3 ± 0.4^{a}	353.0 ± 30.0^{b}	11.6 ± 2.1^{a}
+Cd+Se	4.7 ± 0.5^{a}	$293.0 \pm 24.0^{a\,b}$	25.3 ± 2.2^{b}
+Cd+AA	4.3 ± 0.3^{a}	$310.0 \pm 16.9^{a\,b}$	–
* Statistically different or similar within column according to Post-hoc Tukey HSD test ($P < 0.05$) Cd exposure: 100 mg of cadmium administered per one chicken *per os* daily			

The observed trace element imbalance accompanied by enhanced production of reactive oxidation species (Table 2). Very often overproduction of free radicals, a major cellular source of oxidative stress in biological systems, compromise antioxidant defense in the cell/whole body (Surai et al., 2019).

The results represented in Table 2 Cd-induced decrease of antioxidant enzyme GSH-P_x activity in blood and growing extent of lipid peroxidation production (increase of MDA level in liver) indicated the development of oxidative stress in chickens.

Table 2. Parameters of lipid peroxidation in liver and blood in chickens induced by Cd and effect of antioxidative dietary supplements

Group	Malondialdehyde, μmol·g^{-1} liver	Glutathionperoxidase, μmol GSH·min^{-1}·ml^{-1} blood
Control-1	16.70 ± 0.55^{a*}	2.75 ± 0.07^{b}
+Cd	18.10 ± 0.30^{b}	2.33 ± 0.03^{a}
+Cd+Zn	15.10 ± 0.51^{a}	2.95 ± 0.11^{b}
+Cd+Se	15.57 ± 0.64^{a}	3.97 ± 0.12^{c}
+Cd+AA	15.83 ± 0.48^{a}	2.69 ± 0.09^{b}
* Statistically different or similar within column according to Post-hoc Tukey HSD test ($P < 0.05$) Cd exposure: 100 mcg of cadmium administered per one chicken *per os* daily		

All the experimental antioxidative nutrients demonstrated preventive effect against the harmful impact of Cd. In the first treatment, the most pronounced protective result manifested in the case of Zn supplement. This effect may be due to synergistic and antagonistic interactions between Cd and Zn at the molecular level (Markovs et al., 1997). The physiological role of Se in the body relates to activity of glutathionperoxidase (GSH-Px). Se is incorporated in the active site of this enzyme which acts as a free radical scavenger in the body protecting cellular membranes against oxidative stress products (Zoidis et al., 2020). The data of Table 2 showed that intake of diet supplemented with Se prevented Cd-induced disturbance of antioxidant-prooxidant balance in chickens. Consumption of the diet supplemented with AA also provided an improvement of Cd-induced damage.

An increased Cd accumulation in chicken blood after Cd exposure in the second experiment is accompanied by enhance of oxidative processes activity in organs (Table 3).

The results of this treatment showed that administration of FF to Cd-exposed chickens prevented prooxidative impact of this heavy metal. The data of MDA level and GSH activity in chickens after FF administration approached to the Control-2 group.

Table 3. The effect of red beetroot juice fermented fraction (FF) on cadmium (Cd) concentration in blood serum and indices of oxidative stress in liver of Cd-exposed chickens

Group	Cd, μg·dL^{-1} blood serum	Malondialdehyde, μmol·g^{-1} liver	Glutathionperoxidase, mM GSH·min^{-1} g^{-1} liver
Control-2	4.4 ± 0.2^{a*}	16.90 ± 0.76^{a}	9.60 ± 0.23^{b}
+Cd	10.3 ± 0.7^{c}	18.30 ± 0.37^{b}	8.20 ± 0.28^{a}
+FF	4.6 ± 0.7^{a}	16.60 ± 0.52^{a}	9.51 ± 0.19^{b}
+Cd+FF	8.0 ± 0.3^{b}	17.10 ± 0.40^{a}	8.90 ± 0.27$^{a, b}$
* Statistically different or similar within column according to Post-hoc Tukey HSD test ($P < 0.05$). Cd exposure: diet supplemented by Cd 50 mg kg^{-1}			

In addition to Cd accumulation in blood and oxidative imbalance in liver a suppression of humoral immunity parameters was observed (Table 4). The index of nonspecific humoral resistance is characterized by a decrease of lysozyme enzymatic activity in blood serum of Cd-exposed chickens. This enzyme plays an important role in natural defense reactions, participating in regulation of immune responses by creating the host antibacterial barrier (Ragland & Criss, 2010). A suppressive effect of low concentration of cadmium on serum lysozyme content was observed in common carp serum after 30 days (Ghiasi et al., 2010). The noticed increase of antigen – antibody nonspecific CIC level in Cd-administered chickens' serum also indicated a disturbance of immune response (Table 4). Normally, CIC formed in the bloodstream, phagocytized, and destroyed. Pathological reactions to CIC may be due to an increase in the rate of their formation over the rate of elimination (Cacheiro-Haguno et al., 2021). FF administration in experimental Cd-exposed birds provided antistress defense at the level of nonspecific humoral immunity.

The preventive action of FF may relate to its components – pigments of betalain group. Betalains act as a free radical scavenger and an inductor of defense mechanism in cells (Esatbeyoglu et al., 2014). It determines their antioxidative capacities.

Table 4. The influence of red beetroot juice fermented fraction (FF) on humoral immune parameters in blood serum of Cd-exposed chickens

Group	Lysozyme, μg·mL^{-1}	Circulating immune complexes (CIC), extinction x 100
Control -2	6.9 ± 1.10^{b*}	2.9 ± 0.11^{a}
+Cd	4.4 ± 0.91^{a}	3.4 ± 0.08^{b}
+FF	6.6 ± 0.90$^{a, b}$	3.0 ± 0.12$^{a, b}$
+Cd+FF	5.0 ± 0.88$^{a, b}$	3.3 ± 0.09^{b}
* Statistically different or similar within column according to Post-hoc Tukey HSD test ($P < 0.05$). Cd exposure: diet supplemented by Cd 50 mg kg^{-1}		

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The present investigations had demonstrated that Cd is an environmental stressor for chickens. It may cause an imbalance of mineral nutrients and oxidative stress development. The adverse effect of Cd is manifested regardless of the method of this heavy metal administration to the chickens. It resulted in the development of oxidative stress and suppressed parameters of innate humoral immunity in Cd-exposed chickens. Supplementation of natural antioxidants Zn, Se and ascorbic acid to the diet prevented Cd-induced oxidative damage. The protective role of red beetroot juice fermented fraction against Cd danger was to support the antioxidant and humoral immunity defence systems.

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