From the Research Laboratories
В изследователските лаборатории

EFFECT OF DIFFERENT CADMIUM CONCENTRATION ON SOME BIOCHEMICAL PARAMETERS IN 'ISA BROWN' HYBRID CHICKEN

[1)]Imer Haziri, [1)]Adem Rama, [1]Fatgzim Latifi, [2)]Dorjana Beqiraj-Kalamishi, [3)]Ibrahim Mehmeti, [1)]Arben Haziri
[1)]University of Prishtina (Kosovo)
[2)]Agriculture University in Tirana (Albania)
[3)]Norwegian University of Life Sciences

Abstract. The objective of this study was to verify an impact of different Cd ion concentrations on metabolic (biochemical) parameters in blood of egg chickens. Taking in to consideration that change of these values reflects the level of damage of the vital organs like liver, kidneys, hart etc. These vital organs determine the health status, growth and development of organisms. In the total of four groups of chickens have been included in the experiment. Three groups were treated with different concentrations of cadmium (2, 4 and 6 mg/kg) while one group was set as a control group without treatment. Eleven different biochemical parameters have been measured and analyzed such as: total proteins, albumins, globulins, aspartate transaminase, alanine transaminase, lactate dehydrogenase, gamma-glutamyl transferase, alkaline phosphatase, blood urea nitrogen, creatinine and glucose. The results indicate the significant changes on the level of biochemical parameters in blood, by increasing the cadmium concentration. The level of albumins, alanine transaminase, lactate dehydrogenase, gamma-glytamyl transferase, and creatinine has been decreased significantly compared to control group while other parameters were increased. The biochemical parameters between treatments were changed significantly. Total levels of proteins, globulins, aspartate transaminase, alanine transaminase, gamma-glutamyl transferase, creatinine and glucose has been increase by increased the cadmium concentration. While in another hand, biochemical parameters such as lactate dehydrogenase and alkaline phosphatase has been decreased significantly when the Cadmium was increased. Changes on biochemical parameters are an indication showing that, cadmium effects are negatively oriented on metabolic processes of feed on egg-layer chickens.

Keywords: cadmium; biochemical analyses; body weight; chickens

Introduction

Cadmium (Cd), is considered as a toxic element, nonessential transition metal that causes a hazard to animal and human health due to its toxicological effects

(Doust & Glasziou, 2013; El-Demerdash et al., 2004). The Cadmium can be found on different organs of animal and human body which in high doses will cause toxic effects. Cd has possibility to induce membrane damage (Girault et al., 1998). In several organs, cell damage is followed by release of a number of cytoplasmic enzymes in the blood; this provides the basis for clinical diagnosis (Sundberg et al., 1994). In particular, cadmium accumulation on the body of different animals and human has been linked to the damage of the organ structure (Begum et al., 2015), different changes on blood cells (Hussein et al., 2013), and changes on various biochemical parameters especially glucose, and osmotic regulation (Fu et al., 1990), also modification in enzyme activity (Lionetto et al., 2000). Cadmium sulfate and cadmium chloride are considered most toxic type of cadmium (Pari & Murugavel, 2005; Hawkins & Dugaiczyk, 1982) and the doses are different from different types of animals, concentrations and type of organs (Uyanik et al., 2001). The toxic effects are manifested differently on organisms when present in excessive amount (Novelli et al., 1999), including damage of essential biochemical and physiological functions (Heath, 1995). In the egg-laying breed chickens the likelihood of cadmium toxicity can be assessed (as normal, high and toxic range). The cadmium may cause different complications on metabolic processes such as osteomalacia and remodeling of osseous tissue (Hamad et al., 2011). Previously has been shown that, biochemical blood tests have great importance in the diagnosis of different disease (Wang & Du, 2013), monitoring the health conditions (Doust & Glasziou, 2013) and study of the effects of various pharmaceutical substances on human and veterinary medicine. In the same time the changes caused by Cd ion in the animal and human organs can cause decrease in reproductive processes, renal dysfunction, hepatic toxicity, osteomalacia, neurological impairment, pancreatic activity changes (Hooser, 2007; Wang & Du, 2013). Cd toxicity causes an oxidative stress throughout lipid peroxidation and consumption of some antioxidant systems in broilers (Cinar et al., 2010). It also affects various structures and metabolic processes, such as nucleic acid, carbohydrates, energy metabolism, protein synthesis and enzyme systems (Cinar, 2003). Biochemical parameters in different animals are also sensitive for detecting potential adverse effects of various heavy metals accumulations.

Different enzyme parameters in animals such as aspartate transaminase, Alanine transaminase and Lactate dehydrogenase are considered as a useful biomarker to determine pollution levels during chronic exposure (Basaglia, 2000; El Saidy et al., 2015). Increasing the cadmium on the body weight has been shown by reflecting on the state of the health of the organism and reducing manufacturing capability (Satarug et al., 2010; 2011). A study performed by Cinar et al. (2010) shows that from all heavy metals studies on animal body the cadmium becomes highly toxic even when the level of the cadmium which has entered body is very low. Cadmium is deposited in several organs like mostly in liver (Mani et al., 2007), pancreas (Ohta et al., 1995), bone, nervous system, reproductive system and can cause disorder of

several metabolic pathways (Ohta et al., 1995; Min et al., 1992). The aim of this study was to investigate the effects of exposure to three different concentrations of cadmium and its accumulation in the blood of the egg-layer chickens and analyzing the biochemical parameters.

Material and methods

Birds: egg-laying chicken breed

Four groups of chickens ("*Isa Brown*" hybrid) have been used in experiment. In brief, each group contains six egg-laying breed chickens and was placed two by two per one cage. Chickens were treated for a month in the preparatory period with the same food portion. The first group was selected as a control group; the second group was treated with cadmium chloride supplement of 2 mg/kg of live weight; the third and four groups were treated with 4 mg/kg, 6 mg/kg, respectively. Finally, the blood from egg-laying chicken was collected and used for analysis at Faculty of Agriculture and Veterinary, University of Prishtina.

Biochemical analyses

On the 55 day of the experimental period, we have analyzed the biochemical parameters from sera by using commercial Diagnostic kits for determination of total proteins (EP56-660); Albumins (EP03-570); aspartate transaminase (EP15-500); alanine transaminase (EP07-500); alkaline phosphatase (EP04L-660); blood urea nitrogen, (EP20-420); Creatinine (EP33K-660); Glucose (EP37L-660), from ELIPSE, United diagnostic industry, UDI, Rome, Italy). Concentration of the biochemical constituents was calculated according to the manufacturer instructions. In the same time total protein concentration for each sample was measured using the colorimetric assay RC DC protein assay reagent (Bio-Rad), using bovine serum albumin (BSA) as a standard as described previously (Mehmeti et al., 2011).

Sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) electrophoresis

Samples of different treatments of egg layer chickens were separated and the serum has been used for further processes. The treatment has been as follow: 3 µl of loading buffer (containing β-mercaptoethanol, SDS, glycerol, Tris-HCl and bromophenol blue) were added to 7µL of sera and the mixture was heated for 5 minute at 95 ^{0}C. Samples were loaded on 10% polyacrylamide gel and the gel was stained by commasie blue staining protocol as has been described previously (Mehmeti et al., 2011).

Statistical data analyses

ANOVA Test was used for data analyzes and the medium and standard error of the mean (SEM) was presented. In same time the coefficient of correlation between groups has been analyzed.

Results and discussion

Eleven biochemical parameters have been analyzed using serum derived from egg-laying breed chickens under a growth when the feed was supplemented with cadmium chloride. The egg-laying chickens have been divided on four different groups included a control group and three other groups with different concentration of cadmium chloride (2, 4, and 6mg/mL respectively). The results are presented on Table 1.

Table 1. Biochemical parameters exposed to different concentrations of цadmium

Parameters	Different groups of egg-layer breed chickens treated with Cd			
	1	2	3	4
Total proteins (gr/l)	29.65±0.8	30.5±1.2	34.07±1.2	35.82±4.4
Albumins (gr/l)	17.95±0.2	14.68±0.2	14.58±0.7	15.03±0.3
Globulins (gr/l)	11.7±0.7	15.82 ±0.8	21.24±1.8	19.04±1.3
Aspartate transaminase (U/l)	205.5±0.6	264.2±14	300±5.2	329.5±10
Alanine transaminase (U/l)	3.77±0.53	3.56±0.28	4.28±0.38	4.66±0.32
Lactate dehydrogenase (U/l)	3481±198	2728±334	2686±162	2210±502
Gamma-glutamyl transferase (U/l)	21.48±4.9	11.27±4.5	14.43±1.5	15.12±4.0
Alkaline phosphatase (ALP) (U/l)	418±31	499.8±31	247±32	181±54
Blood urea nitrogen (mmol/l)	0.95±0.06	1.12±0.03	0.99±0.08	1.09±0.09
Creatinine (mmol/l)	20.73±3.7	9.13±1.9	13.5±1.1	15.73±4.1
Glucosa (mmol/l)	8.8±0.56	8.00±0.52	9.22±46	9.66±1.2

Our result shows that by increasing the level of cadmium chloride the level of all biochemical parameters were changed. Similar changes have been shown on globulin level (Table 1). To confirm these results we have run the SDS-PAGE of protein from different treatments and control groups (Fig. 1).

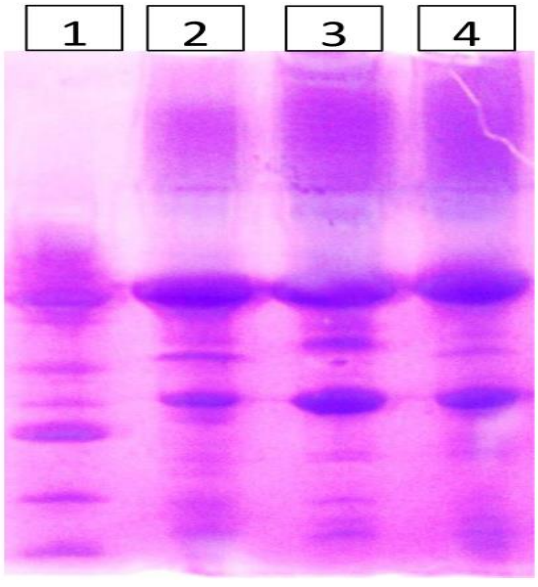

Figure 1. Proteins fraction of samples performed by different Cd treatment concentration: (1) control sample; (2) first treatment 2 mg/kg; (3) second treatment 4 mg/kg and 4) third treatment 6 mg/kg

The data shows increased level of protein by increasing the Cd concentration. Albumin shows significant decrease between control group and treated group. While no significant differences were seen on other treated groups. When we have studied the correlation between albumin and globulin, the level between treated groups and control groups shows significant differences. Relation between albumin and globulin was 1.57 g/L. While, relation between group I was 0.95 g/L, group II 0.69 g/L and group three 0.8 g/L. These results show significant differences which can be seen also between treated groups and control group and that the level has been decreased significantly. Significant differences have been shown also on aspartate transaminase, alanine transaminase, gamma-glutamyl transferase, creatinine and glucose, parameters between control group and treated groups has been significantly increased (Table 1). By increasing the level of cadmium the biochemical parameters were increased significantly. In opposite with that, the level of lactate dehydrogenase and alkaline phosphatase has been decreased by adding the Cd. From different treatments, by increasing the level of Cd on egg layer breed chicken the Cd level has been decreased significantly. The differences between groups (control group and different treatments groups) are shown on Table 2.

Table 2. Different variation between groups of egg-layer breeds chicken

Parameters	Different variation between groups					
	1:2	1:3	1:4	2:3	2:4	3:4
Total proteins (gr/l)	0.27	4.40**	3.01*	4.12*	4.34**	0.64
Albumins (gr/l)	7.00***	4.77**	8.07***	0.12	0.67	0.6
Globulins (gr/l)	3.31*	4.98**	4.94**	3.0*	4.39**	1.0
Aspartate transaminase (U/l)	4.21*	4.29**	5.20**	4.5**	3.82**	4.52*
Alanine transaminase (U/l)	4.28*	0.33	1.77	4.74*	0.77	4.11
Lactate dehydrogenase (U/l)	3.11*	1.94	4.35**	0.12	0.9	0.86
Gamma-glutamyl transferase (U/l)	4.67*	4.72*	1.81	0.4	1.2	1.07
Alkaline phosphatase (ALP) (U/l)	1.65	3.78**	3.82**	4.8**	5.07**	1.07
Blood urea nitrogen (mmol/l)	4.53*	0.45	1.32	1.52	0.24	0.85
Creatinine (mmol/l)	4.79*	1.88	1.17	4.0*	4.3**	0.93
Glucose (mmol/l)	0.59	1.04	0.65	1.75	0.34	1.26

Statistical analysis shows that between control group and different treated groups a significant difference exists. The values from Table 2 represent the data of variation between control groups and treated groups. On protein level significant differences between treated groups has been shown ($P < 0.05$ and $P < 0.01$). While there are no differences between group three and four (Table 2). Similar significant differences were seen on albumin and globulin levels (Table 2).

The present investigation was undertaken to assess the effects of addition in water of different concentration of Cd (2, 4 and 6 mg/kg and control group) and its accumulation in the blood of the egg-layer chickens and impact on the biochemical parameters. Total level of proteins, albumin, aspartate transaminase, alanine transaminase, alkaline phosphatase, blood urea nitrogen, creatinine and glucose has been analyzed by using ELISA. To find out if there are any differences between samples, we have analyzed also protein data by using SDS-PAGE between control sample and treated samples.

Many studies have investigated Cd accumulation and distribution among organs. By increasing the cadmium concentration, the body weight of egg-layer breed chickens decreased significantly (Haziri et al., 2017). Moreover, in some other studies was shown that, level of mortality was increased by increasing the Cd concentrations

(Lane et al., 2015). The present data shows, significant changes on biochemical parameters by increasing the level of cadmium. These results were in agreement with a data obtained by Erdogan et al. (2005) and Cinar et al. (2010). This probably has to do on negative effect of Cd on liver and kidney (Hooser, 2007). In other hand the changes of total protein have been manifest Cd on the albumin and globulin level. This is an indication showing that organs of animals especially kidneys were damaged when the Cd was present (Thévenod & Lee, 2013). Our data are in agreement with previous work, performed by several authors, showing that, high Cd concentration on diet increases, the level of different biochemical parameters such as albumins, aspartate transaminase, alanine transaminase, and gamma-glutamyl transferase activities which were reported by Uyanik et al. (2001) Pari & Murugavel (2005) in broilers and Tandon et al. (2001) in rats who have found these enzymes elevated.

The level of aspartate transaminase and alanine transaminase is increased by adding the Cd supplement on diet of egg layer chickens. Interestingly, by increasing the level of Cd, level of aspartate transaminase and alanine transaminase was increased significantly.

In the present study the level of glucose and creatinine activity was greatly increased ($p<0.05$) in egg-layer chicken treated compared to control group. Even the level is increased by increasing the Cd concentration. This is an indication for poor liver functions or impaired synthesis, either liver cells damage or diminished protein intake and reduced absorption of amino acids caused by a malabsorption syndromes or malnutrition (Al-Fartosi et al., 2012). On the other hand, a significant increase in concentration of creatinine can be seen. This is an indication that, cadmium has effect to stop the creatinine phospokinase which will not make possible the synthesis of creatinine (Borchel et al., 2014). By increasing the level of creatinine may be demaged the liver. When the other chemicals are present the level of serum creatinine decrease or no significant changes (Ozdogan et al., 2002; Khan & Alzohairy, 2011) could be found. This is an indication that, Cd increases the creatinine level.

Conclusion

Based on our data we come to conclusion that, Cd accumulation on egg layer chickens depends on Cd concentration. Proteins, aspartate transaminase, alanine transaminase, and Alkaline phosphatase has been increased by increasing the cadmium concentrations. While the other analysed parameters such as; lactate dehydrogenase, creatine and glucose are lower than in control group and has been decreased. This differences are shown also on gel electroforesis. Biochemical parameters indicates that cadmium acts as a stressor leading to changes in some blood parameters and accumulation in important tissues.

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✉ **Dr. Arben Haziri (corresponding author)**
Department of Chemistry
Faculty of Natural Sciences
University of Pristina
George Bush St., p.n.
10000 Pristina, Kosovo
E-mail: arben.haziri@uni-pr.edu

✉ **Dr. Fatgzim Latifi (corresponding author)**
Department of Veterinary Medicine
Faculty of Agriculture and Veterinary
University of Pristina
George Bush St., p.n.
10000 Pristina, Kosovo
E-mail: fatgzim.latifi@uni-pr.edu